Final Draft

Proposal to Introduce Whiteleg Shrimp (*Litopenaeus vannamei*) to the Kingdom of Saudi Arabia for Aquaculture Development

Prepared for

Saudi Aquaculture Society

by

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Acronyms and Abbreviations

ALOP	Appropriate level of protection
BBC	Biosecure breeding center
BFT	Bio floc technology
BMN	Baculoviral midgut gland necrosis
BMNV	Baculoviral midgut gland necrosis virus
BMPs	Better management practices
BP	Baculovirus penaei
CCRF	Code of Conduct for Responsible Fisheries (of the FAO)
CSR	Corporate social responsibility
FAO	Food and Agriculture Organization of the United Nations
FCR	Food conversion ratio
F1	First generation following broodstock (F0)
GAPs	Good aquaculture practices
GATT	General Agreement on Tariffs and Trade
GAV	Gill associated virus
GSP	Generalized system of preference
HACCP	Hazard analysis and critical control point
HH	High health
HPV	Hepatopancreatic parvovirus
ICES	International Council for the Exploration of the Sea
IHHNV	Infectious hypodermal and hematopoietic necrosis virus
IMNV	Infectious myonecrosis virus
IRA	Import risk analysis
ISO	International Organization for Standardization
KSA	Kingdom of Saudi Arabia
LOV	Lymphoid organ virus
MAFF	Ministry of Agriculture, Fisheries and Forestry (Australia)
MBV	Monodon baculovirus
MC	Multiplication Center
NBC	Nucleus breeding center
MrNV	Macrobrachium rosenbergii nodavirus
NHP	Necrotizing hepatopancreatitis
NPC	National Prawn Company
OIE	World Organisation for Animal Health
PL	Postlarvae
PvNV	Penaeus vannamei nodavirus
RDS	Runt deformity syndrome
RLB-MHD	Rickettsia-like bacteria-Milky hemolymph disease
RNA	Ribonucleic acid
SAS	Saudi Aquaculture Society

SB	Spherical baculovirosis
SFC	Saudi Fisheries Company
SOPs	Standard operating procedures
SPF	Specific pathogen free
SPR	Specific pathogen resistant
SPS Agreement	Sanitary and Phytosanitary Agreement
SPT	Specific pathogen tolerant
TS	Taura syndrome
TSV	Taura syndrome virus
USMSFP	United States Marine Shrimp Farming Program
WSD	Whitespot disease
WSSV	White spot syndrome virus
WTD	Whitetail disease
WTO	World Trade Organization
XSV	Extra small virus
YHD	Yellowhead disease
YHV	Yellowhead virus

1. Introduction

1.1 Background

This proposal for the introduction of whiteleg shrimp (*Litopenaeus vannamei*)[2] to the Kingdom of Saudi Arabia for aquaculture development was prepared by an international team of experts at the behest of the Saudi Aquaculture Society (SAS). The proposal is the result of the urgent need of the Saudi shrimp culture industry to address current production difficulties resulting from problems in the sustainable culture of the currently used Indian white prawn (*Fenneropenaeus indicus*). These difficulties are having major impacts on the ability of the sector to achieve production growth and to remain competitive in global markets. The Saudi Aquaculture Society believes that there is an urgent need to shift the industry from the currently cultured Indian white prawn to the culture of *L. vannamei*, a species that offers many advantages to the industry and whose culture has expanded rapidly during the past decade such that it is now the predominant species being raised globally (FAO, 2012).

This proposal outlines a rigorous process for the introduction of whiteleg shrimp that conforms to best international practice, addressing concerns due to possible pathogen, genetic and ecologic/environmental risks via the commissioning of expert risk analyses and, in the case of possible pathogen risks, the implementation of a series of risk management measures, the most important being the use of specific pathogen free (SPF) shrimp broodstocks derived from a list of Approved Suppliers.

1.2 Current Constraints to the Development of Shrimp Aquaculture in KSA

The first outbreak of whitespot disease (WSD, caused by the whitespot syndrome virus, WSSV) in the Kingdom of Saudi Arabia occurred in November 2010. The incident, however, was not reported promptly. The excellent overall sanitary situation at the time can best be characterized by the fact that Biosecurity Australia, Department of the Ministry Agriculture, Fisheries and Forestry (MAFF), was about to recommend the project site of the country's largest producer, the National Prawn Company (NPC), as a disease-free zone.

Implementing a strategy focused on surveillance and eradication, Saudi shrimp cultivation projects carried out strict disinfection and dry out programs for all elements of their operations during the first half of 2011. A few projects stocked again in mid 2011 with subsequent harvest in late 2011, followed by another stocking cycle in early 2012. However, when outbreaks of WSSV occurred again around April 2012, it became obvious that the virus is prevalent in the

[2] The genus *Penaeus* was revised by Pérez Farfante and Kensley (1997), who raised the Subgenus *Litopenaeus* (and other subgenera) to generic rank. Although some authors are reluctant to accept this change (e.g. Flegel, 2007), their arguments do not appear to hold taxonomic validity (see McLaughlin *et al.*, 2008). We thus encourage readers to become accustomed to using these new taxonomic combinations (some of which are listed in Annex 1).

surrounding environment, including in the Red Sea, and that WSSV had become established in the previously clean broodstock shrimp via infected zooplankton that were entering the operations through intake water. As there is evidence that WSSV is endemic in culture systems in KSA and is also present in wild crustaceans in the vicinity of the farms, a strategy of viral exclusion has been initiated.

Recent extensive disease screening throughout KSA has indicated that there are no proven “clean” stocks of Indian white pawn available. Even sites that were considered to be biosecure have shown the virus to be prevelant. This includes analysis of zooplankton samples in the Al Lith area, which have routinely tested positive for the virus. The combination of potentially infected broodstock and infected zooplankton has limited the ability of operators to quantitatively test methods for eradication and exclusion, and a thus significant portion of the industry has been unable to produce shrimp reliably.

It is estimated that full rehabilitation of broodstocks of *Fenneropenaeus indicus* could take two years or more, without any guarantee of successfully creating a specific pathogen free (SPF) broodstock.

The limitations imposed on the industry by the culture of *F. indicus* can only be seen by comparison; it is expected that *L. vannamei* will provide substantially better performance with respect to fecundity, leading to lower operating costs for breeding centers and hatcheries and lower cost of production due to higher growth raes and a lower food conversion ratio (FCR).

In addition to the sanitary issues associated with KSA stocks of *F. indicus*, the fecundity levels of these broodstocks are a significant limitation to production and increase the risk of disease introduction due to the large number of broodstock required. Fecundity is limited in two aspects:

- the number of nauplii produced per female (per spawn) is significantly lower compared to that of other commercial species (nauplii production ranges from 25,000 – 75,000 per female); and
- commercial spawning of *F. indicus* is generally limited to a single spawn per female.

As a comparison, *L. vannamei* is capable of producing some 200,000 nauplii per spawn and is able to be spawned multiple times (up to 8 spawns have been reported). Table 1 provides some comparisons:

Table 1. Comparison of spawning parameters in *Fenneropenaeus indicus* and *Litopenaeus vannamei.*

	Fennerpenaeus indicus	***Litopenaeus vannamei***
Nauplii per spawn	30,000	200,000
Number of spawns	1	8 (maximum)
Nauplii per female	30,000	1,600,000
Females required per billion nauplii (70% spawn rate)	47,619	892

From an economic and social perspective, it is obvious that none of the affected shrimp cultivation projects can afford an extended period an operation requiring large infrastructure and assets in place without generating income from shrimp production.

The real social impact of failure of the shrimp farming industry in KSA would be the significant loss of employment opportunities for Saudi nationals, particularly in rural areas where jobs are scarce anyway. In addition to loss of direct income, the effect of reduced or eliminated Corporate Social Responsibility (CSR) projects and reduced or eliminated supplier business would further aggravate the situation.

2.0 Status and Trends in Shrimp Production from Aquaculture

2.1 Global trends

World aquaculture production of crustaceans in 2010 was comprised mainly of marine species, which made up 70.6 percent of the global total (FAO, 2012). Whiteleg shrimp (*Litopenaeus vannamei*) is the most successful internationally introduced species, and dominates the marine shrimp culture sector. At the same time, global production of the giant tiger prawn (*Penaeus monodon*) has declined significantly during the last decade. In 2010, whiteleg shrimp accounted for 71.8 percent of world production of all farmed marine shrimp species, of which 77.9 percent was produced in Asia and the remainder in the Americas, where this species originates.

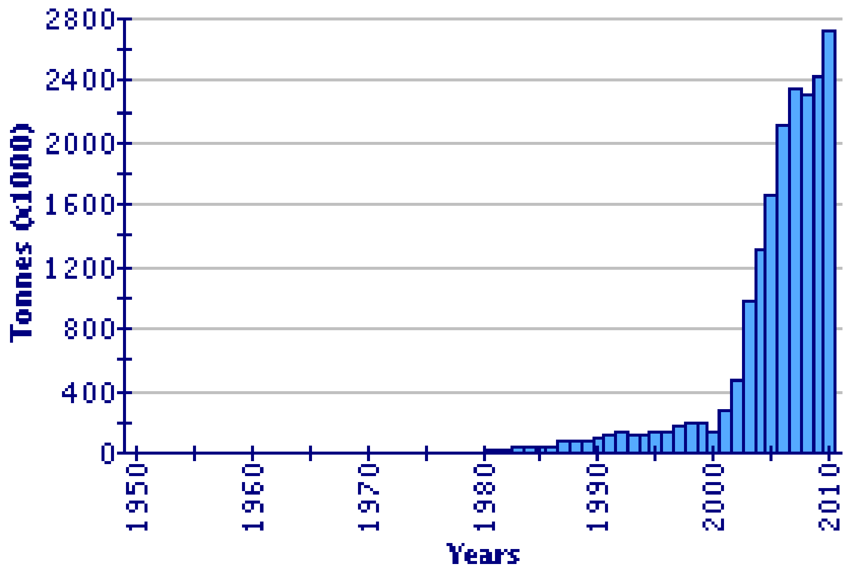
The first spawning of whiteleg shrimp was achieved in Florida in 1973. Following good pond results and the discovery of unilateral ablation (and adequate nutrition) to promote maturation in Panama in 1976, commercial culture of *L. vannamei* began in South and Central America. Subsequent development of intensive breeding and rearing techniques led to its culture in Hawaii, mainland United States of America, and much of Central and South America by the early 1980s.

Food and Agriculture Organization of the United Nations (FAO) statistics (see Figure 1) show the phenomenal rise in global production of whiteleg shrimp that has occurred during the last decade as most shrimp-producing countries have shifted to production of this species. Production of *L. vannamei* by aquaculture increased steadily from only 8,000 tonnes in 1980 to 194,000 tonnes in 1998. After a small decline in 1999 and a more significant decline in 2000 due to the arrival of WSSV in Latin America, there was a rapid increase in production to over 1,386,000 tonnes in 2004, due to the rapid adoption of this species by Asian shrimp farmers. Global production of whiteleg shrimp continued to grow such that it had doubled again by 2010 when it was estimated at almost 2,720,929 tonnes having a value of more than more than US$ 11 billion.

In the Americas, only two penaeid species contribute significantly to aquaculture production (Lightner, 2011). Of these, whiteleg shrimp currently accounts for more than 95% of the total regional production, while blue shrimp (*L. stylirostris*) accounts for the remaining 5%. The latter species formerly accounted for nearly 20% of regional production; however, its high susceptibility to WSSV and to new strains of Taura syndrome virus (TSV) led to its near abandonment in 1999–2000 (Lightner, 2011).

The countries culturing *L. vannamei* include China, Thailand, Indonesia, Brazil, Ecuador, Mexico, Venezuela, Honduras, Guatemala, Nicaragua, Belize, Viet Nam, Malaysia, Taiwan POC, Pacific Islands, Peru, Colombia, Costa Rica, Panama, El Salvador, the United States of America, India, Philippines, Cambodia, Suriname, Saint Kitts, Jamaica, Cuba, Dominican Republic and Bahamas. In the Red Sea area, it has been introduced for culture by Iran (Matinfar *et al*. undated; Afshar Nasab *et al*., 2007), Egypt and Eritrea.

Figure 1. Global aquaculture production of *Litopenaeus vannamei* (FAO Fishery Statistic) (source: http://www.fao.org/fishery/culturedspecies/Litopenaeus_vannamei/en)



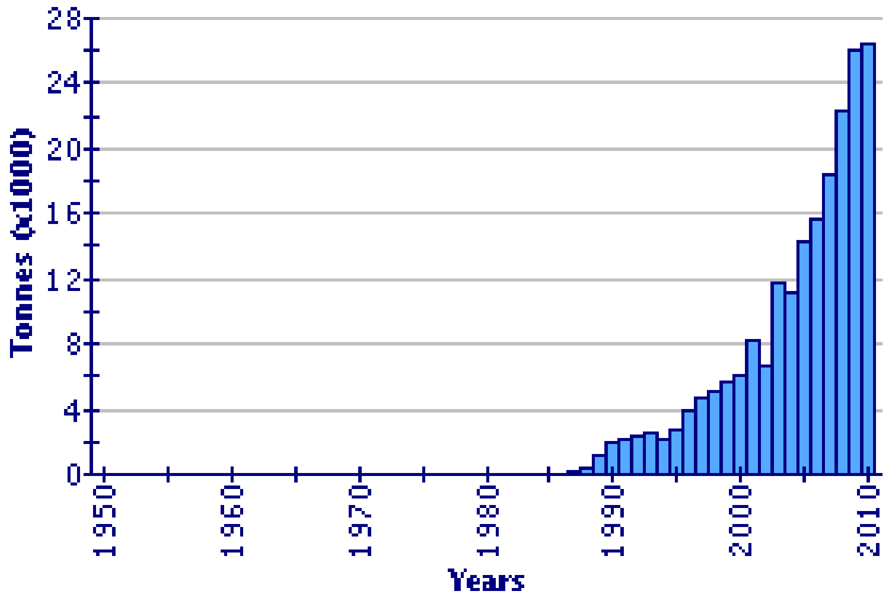
2.2 Shrimp Aquaculture in the Kingdom of Saudi Arabia

Aquaculture in the Kingdom of Saudi Arabia (KSA) began during the early 1980s when some farmers started culturing tilapia in freshwater bodies in inland areas. Nile tilapia (*Oreochromis niloticus*) was the main species cultured up to 2000, when shrimp began to be produced in large quantities. Initially, shrimp culture focused mainly on giant tiger prawn (*Penaeus monodon*), using breeding and culture technologies developed in Southeast Asia. However, because of the highly saline waters around KSA, *P. monodon* culture (as well as *P. semisucatus*) was not successful. Instead, it was replaced by the Indian white prawn (*Fenneropenaeus indicus*), a native species that grows well in high saline conditions.

Shrimp aquaculture in KSA basically evolved out of two initiatives; an initiative of Saudi Fisheries Company which led to the operation of a shrimp cultivation project with a pilot phase as early as 1995, and an initiative of the Al-Ballaa family which led to the establishment of the National Prawn Company (NPC).

Figure 2 shows the reported aquaculture production for KSA from 1950 to 2010, when total volume and value reached 26,374 tonnes and US$ 273 million. (source: http://www.fao.org/fishery/countrysector/naso_saudiarabia/en).

Figure 2. Reported aquaculture production in Saudi Arabia (from 1950)
(FAO Fishery Statistic).



2.2.1 Saudi Shrimp Cultivation Projects

All six Saudi shrimp cultivation projects grow *F. indicus*, and all are located on the Red Sea coast. The projects are described below:

- *Arabian Shrimp Company* is an integrated shrimp cultivation located North of Jizan. The total project area is about 6,200 ha, with about 3,000 ha allocated to production ponds.
- *Island Prawn* is based about 150 km south of Jeddah and has 35 ponds of 2.2 ha size, giving a total pond surface area of 77 ha.
- *Jazadco Development Company* is located in the Jizan area and has been in shrimp production operation since 2003. It reached a full development of 440 ha of water surface area in 2007-2008.
- *National Prawn Company* (NPC) is based about 180 km south of Jeddah. This project started with a research project in 1982, progressing through a number of pilot stages

during which various aspects such as species selection, domestication, hatcheries, grow out, harvesting, processing and engineering have been worked through. These activities resulted in a technically and economically feasible 1,000 ha operation out of which the current National Prawn Company LLC evolved. Its current set up as a fully vertically integrated operation, from a breeding center to cold storage and farm construction has been developed since 1999. Once fully developed and having a pond size of 10 ha and a total pond surface area of about 4,200 ha, the technical production capacity of the project will be between 25,000 and 28,000 tonnes annually.

- *Red Sea Aquaculture,* situated on the Red Sea coast 15 km south of Al-lith, was started in 2008 with an overall focus on developing a totally integrated marine aquaculture project. As a startup, the company has developed a 200 ha water spread area with a total of 1,350 ha of land dedicated to shrimp aquaculture. The farm has fully developed infrastructure, including pumping, aeration and associated facilities such as a workshop, technician & other farm workers' accommodation, etc. The operation began stocking in 2010, with fry and feed being sourced from NPC. As a backward integration, the company is also in the advanced stage of completing its ice plant. The company is also in the process of constructing the multispecies hatchery. The company’s long-term objective is to produce 2,000 tonnes of shrimp per year.
- *Saudi Fisheries Company* (SFC) reached commercial production scale in 1995. It is located about 500 km south of Jeddah. SFC is currently operating its shrimp cultivation project on area of 750 ha with 108 ponds and an annual production of 1,500 tonnes. Expansion plans have been completed that will increase the production capacity to 3,000 tonnes annually. The operation includes two hatcheries.

2.2.2 History of Previous Introductions

There have been no previous introductions of penaeid shrimps to the Kingdom of Saudi Arabia. Current shrimp culture is based entirely upon domesticated stocks of *Fenneropenaeus indicus* that were originally sourced from the Red Sea.

2.2.3 Penaeid spp. occurring in the Red Sea

At least 15 species of penaeid shrimp are native to the Red Sea (Table 2). Of these, four species have importance to global aquaculture (*F. indicus*, *P. japonicus*, *P. monodon* and *P. semisulcatus*) and three have been tested for aquaculture in KSA (*P. monodon*, *P. semisulcatus and F. indicus*).

Table 2. Penaeid Shrimp of the Red Sea[1]

Scientific name	Common name	Habitat	Economic importance
Fenneropenaeus indicus	Indian white prawn	demersal	aquaculture; fishery (major importance in some parts of its range)
Marsupenaeus japonicus	Kuruma prawn	demersal	aquaculture; fishery (minor to major importance - minor in Red Sea)
Penaeus monodon	Giant tiger prawn	demersal	aquaculture; fishery (minor to major importance across range)
Penaeus semisulcatus	Green tiger prawn	demersal	aquaculture, fisheries (minor to moderate importance)
Melicertus latisulcatus	Western king prawn	benthic	fishery (minor, secondary importance in Red Sea)
Melicertus hathor		demersal	none?
Metapenaeopsis aegyptia		demersal	none?
Metapenaeopsis erythraea		demersal	none?
Metapenaeopsis mogiensis	Mogi velvet shrimp	demersal	fishery (minor) (India)
Metapenaeopsis vaillanti		demersal	none?
Metapenaeus monoceros	Speckled shrimp	demersal	fishery (throughout range)
Metapenaeus stebbingi	Peregrine shrimp	demersal	fishery
Parapenaeopsis acclivirostris	Hawknose shrimp	demersal	none?
Parapenaeus fissuroides		demersal	none?
Trachysalambria curvirostris	Southern rough shrimp	demersal	fishery (usually minor due to small size)
Trachysalambria longipes		demersal	none?

[1]Extracted from Holthuis (1980) and Palomares and Pauly (2012); note that all species present in the Red Sea are native species.

3.0 Benefits to be Derived from the Introduction of *Litopenaeus vannamei*

Litopenaeus vannamei offers significant advantages over the presently cultured *F. indicus* that include:

- immediate availability of broodstock of known health history from SPF facilities that are certified to be free from certain major pathogens;
- a reliable supply of postlarvae (PL) free from serious pathogens;
- much higher nauplii production per spawning;
- ability to use broodstock for more than a single reproductive cycle;

- wide acceptability in international markets;
- possibility of better and faster growth than *P. indicus*; and
- existence of some white spot syndrome virus (WSSV) tolerant stocks, allowing the possibility for improved survival in grow-out ponds with existing WSSV infections.

Should pilot testing of whiteleg shrimp in KSA prove successful, the long-term goal of the Saudi Aquaculture Society is to develop specific pathogen free (SPF) lines of hatchery broodstock within the context of breeding and genetic improvement programs. This will minimize the need to further importations of broodstock or postlarvae (PL) from abroad with the inherent risks of pathogen translocation.

A summary of the advantages and disadvantages of culturing *L. vannamei* is given in Table 3 (also see Table 1).

Table 3 . Summary of advantages and disadvantages of the culture of *Litopenaeus vannamei*.

Characteristic	Advantages	Disadvantages
Breeding and Domestication	• Closed lifecycle permits breeding and genetic selection programs to be readily established, eliminating problems associated with use of wild broodstock and/or PL collection. • Can have good spawnings up to 8 times • Females produce extremely high numbers of nauplii, greatly reducing the number of broodstock needed as compared to *F. indicus.*	• SPF animals often have high mortality in disease-laden environments.
Growth rate	• Fast growth up to 20 g	• Growth rate slows after reaching 20 g, making production of large-sized shrimp slower.
Stocking density	• Easier to culture in very high densities (60-150/m^2, but up to 400/m^2) • Not as aggressive as *P. monodon* or *L. stylirostris*.	• Very high stocking densities require high control over pond/tank management practices and are high-risk strategies.
Salinity tolerance	• Tolerant of a wide range of salinities (0.5-45 ppt) and more amenable to inland culture sites than *P. monodon* or *L. stylirostris*.	• None
Temperature tolerance	• Highly tolerant of low temperatures (to 15 °C), enabling culture in cold season.	• None
Dietary	• Requires lower protein feed (20-35%)	• None

protein requirements	than *P. monodon* or *P. stylirostris* (36-42%), resulting in reduced operational costs and amenability for closed, heterotrophic systems.	
Disease tolerance	• Some WSSV-tolerant SPF stocks available.	• Highly susceptible to and a carrier of TSV, WSSV, IHHNV and YHV/LOVV.
Larval Rearing	• Higher survival rates in hatchery (50-60%) as compared to *P. monodon* (20-30%).	• None
Post-harvest characteristics	• If treated with ice, are resistant to melanosis.	
Marketing	• Generally preferred in US due to taste. Strong demand in Asia. • Meat yield is higher (66-68%) than for *P. monodon* (62%)	• *P. monodon* and *L. stylirostris* can grow to larger size, commanding higher price than *P. vannamei*. • High competition on international markets for *L. vannamei* as production is world-wide.
Origin	• SPF, SPR and SPT stocks are readily available, greatly reducing the likelihood of pathogen introduction.	• Exotic to KSA, and thus risks due to pathogen, genetic and ecological/environmental impacts must be considered.

A species profile for *L. vannamei* is given in Annex 1.

4.0 Alternatives to Introduction of Whiteleg Shrimp

The primary alternate strategy available is to continue culture of *Fenneropenaeus indicus.* However, the fact that broodstocks of *F. indicus* are now infected with WSSV would necessitate "cleaning" of existing broodstocks or their complete re-establishment from wild populations, a costly and time-consuming undertaking that would require a minimum of two years to achieve. At the same time, this would not address the current problems of high susceptibility to WSSV, inability to spawn broodstock more than once, and continued vulnerability of breeding programs to WSSV infection from enzootic infections in grow-out ponds and the natural environment.

Another alternate strategy would be the use of blue shrimp (*Litopenaeus stylirostris*), another, less widely cultured exotic species for which broodstock from SPF facilities are available. However, such SPF facilities are more limited in number than for *L. vannamei*, stocks generally have less history under SPF conditions, and health guarantees may therefore be less reliable.

The use of giant tiger prawn (*P. monodon*), a species native to the Red Sea and previously widely cultured in the Asia-Pacific, is also possible. However, this would also involve importations of SPF stocks (again, with a more limited number of potential suppliers) and would be more difficult to implement, as a closed life cycle for *P. monodon* is more difficult to achieve than for *L. vannamei* or *L. stylirostris*. Attempts were made during the early years of aquaculture development in KSA to culture both *P. monodon* and *P. semisulcatus* (green tiger prawn), but were abandoned due the generally higher salinities found in the country (source: http://www.fao.org/fishery/countrysector/naso_saudiarabia/en).

5.0 The Responsible Introduction of New Species for Aquaculture Development

The introduction of an exotic species can have major ecological, environmental and social impacts on the receiving country, and is not to be undertaken lightly. The importing country must be reasonably certain that the economic benefits will be significant and that the possibility of adverse impacts are minimized. It is now generally accepted that appropriate risk analyses should be commissioned before the introduction of a new species is approved. In the case of introduction of an exotic species of aquatic animal, this includes undertaking pathogen, genetic and ecologic/environmental risk analyses.

5.1 The ICES Code of Practice

Although not obligatory to KSA, the International Council for the Exploration of the Sea's (ICES) *Code of Practice for the Introduction and Transfer of Marine Organisms* (ICES 2005, 2012) is widely accepted globally as the key framework for assessing proposals to introduce exotic aquatic species to new environments outside their native range. Among others, the ICES Code addresses the evaluation of potential genetic, ecologic and pathogen risks associated with the translocation of aquatic organisms. Conformation with the recommendations of the ICES Code can thus be considered best practice when introducing new species for aquaculture development.

With regard to the introduction of an exotic aquatic species (including those for aquaculture development), the ICES Code (ICES, 2005) outlines[3]:

A recommended procedure for all species prior to reaching a decision regarding new introductions:

- A detailed prospectus should be prepared that includes the purpose and objectives of the introduction, the stage(s) in the life cycle proposed for introduction, the native range, the donor location, and the target area(s) of release. The prospectus should also include a review of the biology and ecology of the species as these pertain to the introduction (such as the physical, chemical, and biological requirements for reproduction and growth, and natural and human-mediated dispersal mechanisms) and information on the receiving environment. The prospectus should also provide a detailed analysis of the potential

[3] Points relevant only to ICES member countries have been removed.

impacts on the aquatic ecosystem of the proposed introduction. This should include, wherever possible, assessments from previous introductions.

- This analysis should include a thorough review of:
  - the ecological, genetic, and disease impacts and relationships of the proposed introduction in its natural range and donor location;
  - the expected ecological, genetic, and disease impacts and relationships of the introduction in the proposed release site and projected range, as well as vectors for further distribution;
  - an economic assessment, where appropriate.
- The prospectus should conclude with an overall assessment of the issues, problems and benefits associated with the proposed introduction. An evaluation of risks should be included.

If the decision is taken to proceed with the introduction:

- Using internationally recognized protocols, such as the World Organisation for Animal Health (OIE, formerly the Office International des Épizooties,), or any other appropriate protocols available at the time, to review the health records of the donor location and surrounding area of the organisms to be introduced.
- The introduced organisms should be used to establish a broodstock for the production of progeny. The organisms should be transferred into a quarantine facility in the recipient country or other location agreed to by the recipient country.
- The imported consignment(s) is not to be released to the wild, and should be separated from subsequent progeny.
- Only progeny of the introduced species may be transplanted into the natural environment, provided that:
  - a risk assessment indicates that the likelihood of negative genetic and environmental impacts is minimal;
  - no disease agents, parasites, or other non-target species become evident in the progeny to be transplanted; and
  - no unacceptable economic impact is to be expected.
- During the pilot phase, the progeny, or other suitable life stages, should be placed into open waters on a limited scale to assess ecological interactions with native species, and especially to test risk assessment assumptions.
- Contingency plans, including the removal of the introduced species from the environment, should be ready for immediate implementation.
- A monitoring programme addressing specific issues of the introduced species in its new environment should be undertaken.
- Annual progress reports should be submitted for review.

The proposed introduction protocol to be followed by the SAS closely follows best international practice as recommended by ICES (2005, 2012), as well as the FAO *Code of Conduct for Responsible Fisheries* (FAO, 1995) and the *Technical Guidelines for Responsible Fisheries, Aquaculture development 2. Health management for responsible movement of live aquatic animals* (FAO, 2007).

5.2 Risk Analysis for the Introduction of Aquatic Species

While methodology for genetic and ecologic/environmental risk assessments are not codified, a standardized framework for pathogen risk analysis (import risk analysis, IRA) for live aquatic animals and their products is laid out in the World Organization for Animal Health's (OIE) Aquatic Animal Health Code (OIE, 2012a).

With the liberalization of international trade through the General Agreement on Tariffs and Trade (GATT), the establishment of the World Trade Organization (WTO) and its *Agreement on the Application of Sanitary and Phytosanitary Measures* (SPS Agreement), WTO member countries are now required to use the risk analysis process as a means to justify any restrictions on international trade beyond those specified by the Aquatic Animal Health Code (OIE, 2012a) based on risks to human, animal or plant health (see WTO 1994, Rodgers 2004). Risk analysis has thus become an internationally accepted standard method for assessing whether trade in a particular commodity (e.g., a live aquatic animal or its product) poses a significant risk to human, animal or plant health, and if so, what measures could be adopted to reduce that risk to an acceptable level. As a member of both the OIE and the WTO, the Kingdom of Saudi Arabia is obligated to follow OIE and WTO procedures.

5.3 Dealing with Disease

5.3.1 Impacts of Disease on Shrimp Culture

Aquaculture remains highly vulnerable to adverse impacts of disease. Disease outbreaks in recent years have affected marine shrimp farming in several countries in Asia, South America and Africa, resulting in partial or sometimes total loss of production. In 2010, aquaculture in China suffered production losses of 1.7 million tonnes caused by natural disasters, diseases and pollution. Disease outbreaks virtually wiped out marine shrimp farming production in Mozambique in 2011 (FAO, 2012).

In the early days of shrimp aquaculture, pathogens were largely limited to specific geographic locations. However, the rapid growth of the industry and the associated globalization of trade led to the emergence of serious diseases in both the Americas (e.g. baculovirus penaei (BP), necrotizing hepatopancreatitis (NHP), Taura syndrome (TS), infectious myonecrosis (MN)) and in Asia (spherical baculovirus (SB), yellowhead disease (YHD), white spot disease (WSD)). The majority of these diseases have caused significant production issues in shrimp farming regions distant from their original site of emergence.

Disease plays a major role in limiting shrimp production from aquaculture. Approximately 40% of potential penaeid shrimp production is estimated to be lost to infectious diseases each year (see Stentiford *et al*., 2012). Approximately 60% of disease-associated losses in shrimp aquaculture are attributed to viruses, with bacteria accounting for a further 20%.

As noted by Stentiford *et al.* (2012), the emergence and rapid spread of serious diseases of penaeid shrimps on a regional and global basis has resulted primarily from poor industry practices, including the careless transboundary movement of broodstock and PL of unknown or poorly known health status and the common practice of siting shrimp production facilities near natural waterbodies where transfer of pathogens between cultured stocks and wild decapod crustaceans continues to contribute to the emergence of new diseases, even in originally SPF stocks of penaeid shrimp. In the future, improved siting of farms in biosecure settings is expected to contribute to a reduced emergence rate of significant pathogens in penaeid shrimp (Flegel, 2012).

As noted by Stentiford *et al*. (2012), domestication of *L. vannamei* was a major step forward for the industry, in that it led to increased yields and better disease control at the farm and country level; however, the focus of the shrimp culture industry on a single species has facilitated the translocation of important pathogens to distant regions. The naivety of domesticated stocks of *L. vannamei* to pathogens present in local species and environments also increases the potential for disease emergence in this translocated species, particularly where stocks are farmed in ponds with direct contact to natural waters and their associated fauna.

Cumulative global production losses due to WSSV have been estimated as being at least US$8 billion since the early 1990s; however, actual losses may be closer to US$15 billion (Lightner *et al.*, 2012). Losses due to other important crustacean pathogens have been estimated as $1 billion for IHHNV $0.5 billion for YHD, $3 billion for TSV and $1 billion for IMNV. Moss *et al.* (2012) noted that if estimated production losses of 15% volume occur, the top five viruses reduce global shrimp production by almost 500,000 tonnes per annum, an amount equivalent to the total importation of shrimp products by the European Union or the USA in a year.

5.3.2 National Aquatic Animal Disease Status

Knowledge about the national aquatic animal disease status of KSA is very limited. Regular sanitary reports have been produced by the government only since November 2011. In 2010 and 2011, WSSV and TSV, two viral diseases of penaeid shrimp listed by the World Organisation for Animal Health (OIE, 2012a) were detected in cultured Indian white prawn (*F. indicus*) (CEFAS, 2012; *Tang et al.,* 2012a,b).

Based on molecular genetic studies, Tang *et al.* (2012a,b) concluded that both viruses were likely to have become established in aquaculture facilities in KSA through the use of infected broodstock of *F. indicus* originating from the Red Sea, and not introduced via the importation of exotic penaeid species, as has often been the case in other shrimp-growing countries. The above observation does not, however, explain how these two exotic pathogens could have become established in wild populations of *F. indicus*.

Other countries bordering the Red Sea include Israel (Sinai Peninsula), Djibouti, Egypt, Eritrea, Ethiopia, Sudan and Yemen. The only crustacean disease listed by the International Aquatic Animal Disease Database (CEFAS, 2012) for these countries is hepatopancreatic parvo-like virus (HPV), which is listed as occurring in Israel based on OIE data. However, the distribution of HPV within Israel is unknown (OIE, 2007), and thus this report may not pertain to the Red Sea.

5.3.3 Advantages of use of Specific Pathogen Free Broodstocks

The use of shrimp sourced from SPF facilities, improvements in selective breeding, and the adoption of strict on-farm biosecurity practices are essential elements for the future expansion and long-term sustainability of global shrimp aquaculture (Moss *et al.,* 2012). Currently, only SPF populations of *P. vannamei* are commercially available on a large scale, a factor that has played a major role in this species becoming the most widely cultured penaeid globally (Stentiford *et al*., 2012).

As summarized by Flegel (2012), the paramount need for SPF domesticated shrimp stocks for sustainable aquaculture is based on major physiological differences between shrimp (and other crustaceans) and vertebrate species. Vertebrates are often capable of eliminating viral pathogens from their systems during suitable periods of quarantine; however, crustaceans often carry viral pathogens as persistent infections for long periods (often for life), without showing any gross signs of disease. Although these viruses are often present in low levels, they are not latent but active, and can be passed on to naïve shrimp or crustaceans to cause mortality. They can also be passed vertically from broodstock to their grossly normal larvae and PL, and this may lead to subsequent disease outbreaks in rearing ponds stocked with the infected PL. This propensity of grossly normal crustaceans to carry viral pathogens means that special precautions are needed whenever live crustaceans destined for aquaculture are translocated over large distances, and especially to areas outside their natural range.

In developing their national shrimp culture industries, Flegel (2006, 2012) suggested that countries should not import exotic crustaceans of any kind without including provisions for recommended quarantine procedures and testing for unknown viruses that may be a danger to local species. This process should be applied even when importing exotic domesticated stocks that are sourced from SPF facilities. To further minimize pathogen risks, countries that import exotic stocks for aquaculture development should establish local nuclear breeding centers (NBCs) that can be used for ongoing supply of broodstock to multiplication centers (MCs) for production of PL to stock grow-out ponds or alternatively, following an initial period of quarantine and diagnostics testing, they should contain imported SPF broodstocks in high-security Biosecure Breeding Centers (BBCs), with release only of PL (F1 generation) to production ponds.

As an example of the possible benefits to be derived from the use of SPF shrimp, broodstock of *L. vannamei* sourced from SPF suppliers in the USA were imported to Indonesia in early 2002, resulting in a sharp increase in shrimp aquaculture production. In Indonesia, as in many other Asian countries (e.g. China, Thailand, Viet Nam, Malaysia, Philippines), the species has replaced the traditionally cultivated *P. monodon* as the dominant cultivated species. Unfortunately, irresponsible importation of non-SPF stocks of *P. vannamei* for aquaculture probably resulted in the importation of infectious myonecrosis virus (IMNV) to Indonesia via smuggled shrimp stocks in 2006 (see Untari *et al*., 2012).

The recent phenomenal expansion of the shrimp culture industry in India and Viet Nam has been largely due to the shift to culture of whiteleg shrimp, along with use of SPF broodstocks and improved biosecurity at aquaculture facilities. India is predicted to produce some 50,000 tonnes in 2012.

Although stocks of penaeid shrimp originating from SPF facilities are free from specifically listed pathogens, they are not necessarily free of all possible pathogens, nor do they have innate resistance, tolerance or susceptibility to their listed pathogens. Although these traits can be bred into a line of shrimp through genetic selection, these characteristics have no bearing on SPF status (Moss *et al*., 2012).

After vertical transmission from infected broodstock to PL, influent water is the most important route of pathogen entry into shrimp farming systems, and is a particular issue where farms are sited close to the coast. Moss *et al.* (2012) have thus suggested that an increased focus on integrated management practices (e.g. stocking of high health (HH) fry originating from SPF shrimp into farms using bio floc technology (BFT)) will allow breeders to focus selection pressures on growth and grow-out survival, rather than on disease resistance, and that this may lead to increased production and profitability.

6.0 Commodity Description

The commodity description (Table 4) is the starting point for risk analysis. It defines precisely what commodity (live aquatic animal or aquatic animal product) is to be introduced and the details of the proposed introduction.

Table 4. Commodity description for the proposed introduction of whiteleg shrimp (*Litopenaeus vannamei*) to the Kingdom of Saudi Arabia.
Species to be introduced*: Litopenaeus vannamei* (whiteleg shrimp)
Proposed date of importation: beginning January 2013, for a period of 3 years
Life cycle stage to be imported: Broodstock only
Importers: Participating members of the Saudi Aquaculture Society, Jeddah, Saudi Arabia (List of Approved Importers)
Exporter: Approved SPF facilities (list of Approved Suppliers to be developed)
Source*:* High security SPF culture facilities (List of Approved Suppliers)
Proposed number of shipments: as required
Volume**:** as required
Proposed destination: participating shrimp farms along the Red Sea coast, KSA

7.0 Summary of Risk Management Measures Proposed by the Saudi Aquaculture Society

The proposed importation is characterized by a high level of risk management that is designed to ensure that (i) serious pathogens are not present in imported broodstocks of *L. vannamei*; and (ii) that in the unlikely event a serious pathogen does enter the country with imported broodstock, it will not gain access to aquaculture grow-out ponds or the natural environment where it could possibly establish in wild crustaceans. The risk mitigation measures to be applied are described in detail below.

7.1 Use of Specific Pathogen Free Shrimp

Only broodstock sourced from a list of Approved Suppliers of SPF shrimp will be used. Table 5 presents a summary of changes in the United States Marine Shrimp Farming Program (USMSFP) working list of "specific" and excludable pathogens for penaeid shrimp for the period 1990-2010 .[4] Note that the listing presented in Table 5 is indicative of pathogens which meet the requirements of exclusion from SPF stocks, i.e.: (i) they are biological agents, (ii) they cause serious disease in penaeid shrimp; and (iii) reliable diagnostics methods are available for screening of broodstock and other life cycle stages. In addition to the 15 pathogens/pathogen groups listed in Table 5, one additional disease, whitetail disease (WTD), caused by Macrobrachium rosenbergii nodavirus (MrNV) and extra small virus (XSV) meets these criteria. In practice, due to the emerging nature of some pathogens or their restricted geographic distributions, in conjunction with the known histories of their SPF stocks, SPF suppliers do not routinely screen for all of these 16 pathogens/pathogen groups (the Oceanic Institute, for example screens for 15 pathogens/pathogen groups, all those listed in Table 5, but not for MrNV (see Annex 2)).

Table 5. United States Marine Shrimp Farming Program (USMSFP) working list of ‘‘specific’’ and excludable pathogens for penaeid shrimp for 1990, 2000 and 2010 (modified from Moss *et al.*, 2012).

Pathogen	**1990**	**2000**	**2010**
Viruses			
Infectious hypodermal and hematopoietic necrosis virus (IHHNV)	X	X	X
White spot syndrome virus (WSSV)		X	X
Yellow head virus complex (YHV, GAV, LOV)		X	X
Taura syndrome virus (TSV)		X	X
Baculovirus penaei (BP)	X	X	X
Monodon baculovirus (MBV)		X	X
Baculoviral midgut gland necrosis (BMN)		X	X
Hepatopancreatic parvovirus (HPV)	X	X	X
Infectious myonecrosis virus (IMNV)		X	X
Penaeus vannamei nodavirus (PvNV)			X
Prokaryotes			
Necrotizing hepatopancreatitis (NHP)		X	X
Rickettsia-like bacteria-Milky hemolymph disease (RLB-MHD)			X
Protozoans			
Microsporidians	X	X	X

[4] Funding to USMSFP was discontinued in 2011 (S. Moss, Oceanic Institute, Hawaii, pers. comm.).

Haplosporidians	X	X	X
Gregarines	X	X	X
Number of pathogens/pathogen groups	**6**	**13**	**15**

7.2 Establishment of a List of Approved Suppliers of SPF Broodstock

Importations of broodstock will only be permitted through a list of Approved Suppliers. The following criteria must be met for a company to be listed as an Approved Supplier:

- Current stock has been held under SPF conditions for at least two years.
- During this period, no outbreaks of serious disease have occurred.
- During this period, diagnostics testing for listed pathogens has been conducted by an independent laboratory at least four times (at six-month intervals or more frequently) with no positive results (testing methods and results of screening for all diseases are submitted; testing for OIE-listed diseases has been done to specifications given in the OIE Manual (OIE, 2012b)).
- Supplier attests that no purchaser of SPF stocks originating from his facility has complained of receiving diseased animals or has initiated legal action against the supplier for this reason.
- Supplier agrees in principle that his facility may be inspected by experts designated by the SAS to verify statements regarding stock history and biosecurity.
- The stock(s) must be certified as free from the following 12 pathogens/pathogen groups:
    - Infectious hypodermal and haematopoietic necrosis virus (IHHNV)
    - White spot syndrome virus (WSSV)
    - Yellow head virus (YHV)
    - Taura syndrome virus (TSV)
    - *Baculovirus penaei* (BP)
    - Monodon baculovirus (MBV)
    - Hepatopancreatic parvo-like virus (HPV)
    - Infectious myonecrosis virus (IMNV)
    - Necrotising hepatopancreatitis (NHP)
    - Microsporidians
    - Haplosporidians
    - Gregarines
- Additionally, freedom from the following four additional pathogens/pathogen groups must be demonstrated based on (i) SPF status for these diseases or (ii) stock history and production records, supplemented by additional diagnostics testing as specified:
    - Whitetail disease (WTD)
    - Baculovirus midgut gland necrosis virus (BMNV)
    - Penaeus vannamei nodavirus (PvNV)
    - Rickettsia-like bacteria-Milky hemolymph disease (RLB-MHD)

An initial listing of known suppliers *L. vannamei* broodstock produced in SPF facilities that can be screened as potential Approved Suppliers is presented in Annex 2. This list will need to be further developed and screened using the criteria listed above and by on-site inspection (see below).

7.3 On-site Inspection of Suppliers

Prospective suppliers must agree in principle to on-site inspection of their facilities by a team of experts appointed by SAS to confirm that the required biosecurity measures are in place.

7.4 List of Approved Importers

The SAS will establish a list of Approved Importers who will agree to meet all specified standards for risk management measures and to allow independent verification of same.

7.5 Limited Time Period During which Importation will be Permitted

To facilitate pilot testing of *L. vannamei* to local culture conditions and to reduce the risk of pathogen entry with translocation of broodstock, importations from approved suppliers will be terminated once sufficient broodstocks have been established. SAS will conduct an annual assessment of the program and future needs to determine if importations can be terminated.

7.6 High Security Quarantine of Imported Broodstock

Upon entry into KSA, imported broodstock will be held in high security quarantine facilities that will prevent the escape of broodstock and any larval (F1) stages and any pathogens that may be present. Quarantine facilities will meet minimum standards of construction and standard operating procedures (SOPs) appropriate to such high containment facilities (i.e. as outlined in Section 4 of Arthur *et al.*, 2007 and in Annex 6 of ICES, 2012; See Annex 3 of this proposal). Construction and operating standards will also minimize the possibility of diseases present in the external environment gaining entry to the facility. Upon satisfactory completion of diagnostics testing, broodstock will be moved to Biosecure Breeding Centers (BBCs) having a similar level of biosecurity.

7.7 Monitoring and Diagnostics Testing of Broodstock While in Quarantine and BBCs

Broodstock in quarantine facilities or BBCs will be monitored for health on a daily basis and will be tested for specified pathogens upon arrival, before leaving quarantine and at termination, so that if pilot testing is successful, BBCs may eventually achieve SPF status. Diagnostics testing will also be conducted should any unexplained mortalities occur.

7.8 Release of only F1 Postlarvae to Grow-out Ponds

Only postlarvae (F1 generation) will be released from BBCs. Imported broodstock will be destroyed and disposed of in a sanitary manner once they are no longer useful for breeding.

7.9 Monitoring and Diagnostics Testing of Shrimp in Grow-out Ponds

Postlarvae (F1) stocked in grow-out ponds (commodity shrimp) will be monitored daily and prior to harvest samples taken for diagnostics testing. In the event of any unusual condition or mortality, disease investigations will also be conducted.

7.10 Contingency Planning in case of Disease caused by an Exotic Pathogen

Each participating farm will develop a contingency plan to deal with a disease emergency due to an exotic pathogen. Emergency preparedness will allow rapid response, restricting pathogen spread and increasing the possibility that the pathogen can be contained and eradicated. Contingency planning will follow the recommendations given in Arthur *et al.* (2005), OIE (2012a) and ICES (2005, 2012).

8.0 Results of the Risk Analyses

As an additional risk management measure, the Saudi Aquaculture Society has commissioned ecologic/environmental, genetic, and pathogen risk analyses. Each risk analysis is conducted by an international expert/expert team in the specific area being considered. A summary of the conclusions of each of the risk analyses is presented below. The general findings resulting from the risk analyses examining the risks due to the introduction of whiteleg shrimp are that (i) the direct and indirect genetic risks are extremely low; the ecologic/environmental risks are low; and that (iii) if the risk management measures outlined in this proposal are fully implemented, there are no potential hazards and thus risk of pathogen introduction is negligible.

8.1 Summary of the Genetic Risk Analysis

The genetic risk assessment was conducted by Dr Rodger W. Doyle, Genetic Computation Ltd., Victoria, Canada. Dr Doyle is an internationally recognized scientist in the field of aquatic animal genetics. Dr Doyle's risk analysis has been independently reviewed by Dr Eric

Hallerman, an internationally renowned aquatic geneticist at Virginia Polytechnic Institute and State University, Blacksburg, Virginia. The final genetic risk assessment as presented in Annex 4 has addressed all comments and suggestions made by Dr Hallerman (review comments are attached as part of Annex 4).

Dr. Doyle concluded that:

> *In summary, the risk of any direct genetic impact on native populations of* L. vannamei, *or on populations of other species in the same genus, is extremely low.*

- The genetic risk assessment follows the structure and content guidelines recommended by the ICES Working Group on Introduction and Transfers of Marine Organisms as well as FAO documents that deal with genetic risks associated with marine introductions.

- The risks assessed include both direct and indirect genetic risks to wild organisms in the Red Sea, as well as adjacent areas that might exchange genetic material with the Red Sea (Persian Gulf, Arabian Sea and western Indian Ocean).

- Farmed *Litopenaeus vannamei* has escaped and established local populations on both coasts of the Americas, and possibly in the Middle East and Asia. Escaped *L. vannamei* have had no reported genetic impact of any sort on natural *L. vannamei*, anywhere in the world.

- There are no wild *L. vannamei* in the Red Sea or anywhere within dispersal range. There is therefore very low risk of direct genetic impact on natural conspecific populations.

- Non-native (feral populations) of *L. vannamei* may already exist in the Persian Gulf and oceanic regions within range of the Red Sea. By definition, non-native species have no conservation value and are not relevant to the genetic risk assessment (2012 Report of the ICES Working Group on Introduction and Transfers of Marine Organisms).

- Experimental hybridization within the genus *Penaeus* (as constituted before the revision of Pérez Farfante and Kensley, 1997) has had little success, even with species that appear morphologically compatible (now usually grouped in the genus *Litopenaeus*). There are no species in this group in or near the Red Sea. The risk of genetic impact on congeneric species is therefore very low.

- Shrimp species in the Red Sea and areas within dispersal range have a gonadal structure that renders natural hybridization with open-thelycum species in the genus *Litopenaeus* virtually impossible. The risk of direct genetic impact through hybridization or introgression with other shrimp species is very low.

- Possible indirect impacts might stem from any evolutionary increase in the invasiveness of *L. vannamei;* by competing with other shrimps, the latter populations may decline, losing adaptive genetic variability or by stimulation of the emergence of recombinant pathogens in feral *L. vannamei* (which would be a novel host for RNA viruses in the Red Sea). The risk of high-impact indirect genetic effects are non-calculable, but expected to be low.

- The risk of high-impact indirect genetic effects are non-calculable. Mitigation of indirect genetic effects will involve monitoring, so that any unexpected evolutionary change can be recognized early and its genetic basis understood in a timely way.

8.2 Summary of the ecologic/environmental risk assessment

The ecologic/environmental risk assessment was undertaken by a group of international experts in crustacean biology at the Queensland University of Technology, Brisbane, Australia, comprised of Drs Peter M. Mather, David Hurwood and Satya Nandlal. The risk analysis was subjected to independent review by Prof. Raymond T. Bauer, University of Louisiana, Lafayette, LA. The final ecologic/environmental risk analysis as presented in Annex 5 has addressed all comments and suggestions made by Dr. Bauer (review comments are attached as part of Annex 5).

The ecological risk assessment focuses on the invasiveness and “pest potential” of the species to be translocated and considers the likelihood of its escape and/or release into the natural marine environment of KSA and the nature and extent of any potential ecological impacts such escape or release may entail. Dr. Mather and his colleagues concluded that:

> *The ecological risk analysis is characterized by a high level of certainty, and the estimated risk potential is low based on information available, for example, on impacts of L. vannamei escapees into the wild that include follow-up studies from previous introductions of this species and its subsequent escape into the wild, as seen in Thailand.*

- Mitigation measures are not identified, since the overall estimated risk potential is low; however, additional recommendations have been made to be aware of the meteorological conditions of the farm site and the climatic conditions of the region to reduce the likelihood of escapes from farms and hatcheries.
- In addition, establishment of a monitoring program for the presence of *L. vannamei* in the wild to allow detection of the geographical spread of escapees (should this occur) and to assess their impacts on wild species is also recommended.
- The ecological risk analysis suggests that the benefits of introduction outweigh any potential negative effects.
- However it is emphasized that the results should not be taken as a sole basis for a decision by the Kingdom of Saudi Arabia and Saudi Aquaculture Society to approve or not approve a request for the proposed species translocation. Such a decision may require additional consideration by the Kingdom of policy, legislation, etc. and should include extensive stakeholder consultation.

8.3 Summary of the pathogen risk analysis

The pathogen risk analysis (import risk analysis) was conducted by Drs Richard Arthur, Barriere, Canada and Dr Victoria Alday-Sanz, Barcelona, Spain. Dr Arthur is an independent consultant and expert in aquatic animal health and risk analysis who is frequently contracted by the United Nations Food and Agriculture Organization (FAO), Rome, to conduct international training to national fishery and veterinary staff in aquatic animal risk analysis; Dr Alday-Sanz is an international expert in shrimp aquaculture and disease diagnostics. The risk analysis was subjected to independent review by Dr. Ben Diggles, DigsFish Services Pty Ltd, an expert international consultant in pathogen risk analysis who resides in Queensland, Australia. The final pathogen risk analysis as presented in Annex 6 has addressed all comments and suggestions made by Dr. Diggles (review comments are attached as part of Annex 6).

Drs Arthur and Alday-Sanz concluded that:

> *In summary, the proposal to introduce whiteleg shrimp to KSA is characterized by the high level of risk management measures that are to be implemented by the SAS. While there are many serious pathogens of penaeid shrimp, the proposed risk management measures are considered to be sufficient to remove all of these pathogens from consideration as potential hazards.*

- The proposal is characterized by the high level of risk management measures that are proposed by the SAS. These include: (i) use of specific pathogen free (SPF) shrimp; (ii) establishment of a list of approved suppliers of SPF broodstock *P vannamei* to KSA; (iii) provision for the possible on-site inspection of suppliers; (iv) list of approved importers; (v) limited time period during which importation will be permitted (3 years); (vi) high security quarantine of imported broodstock; (vii) monitoring and diagnostics testing of broodstock while in quarantine and Biosecure Breeding Centers (BBCs); (viii) release of only F1 postlarvae to grow-out ponds; (ix) monitoring and diagnostics testing of shrimp in grow-out ponds and (xi) contingency planning in case of disease caused by an exotic pathogen.

- The risk analysis considers 30 pathogens/pathogen groups (possible hazards) that have been reported globally from *L. vannamei* or other penaeid shrimp. While many of these possible hazards are serious pathogens of penaeid shrimp, the risk management measures proposed by SAS are considered to be sufficient to remove all of these pathogens from consideration as potential hazards.

- In addition, Dr Arthur made the following recommendations:
  - The Government of KSA should confirm that the suggestion that the national appropriate level of protection (ALOP) should be "high" or "very high" and with an acceptable level of risk that is "low" or "very low".
  - As the risk assessment is highly dependent upon the risk management measures proposed by the proponents, monitoring systems must be established to ensure that all risk management measures are fully and effectively implemented.
  - To minimize the risk of WSSV, TSV and other pathogens already present in KSA gaining entry, it is recommended that the initial high security quarantine facility and the BBCs be located as far away as possible from existing shrimp farms.

- Saudi shrimp growers should strive to become self-sufficient in broodstock and postlarval production as soon as possible by setting up a breeding and genetic improvement program for *L. vannamei*, as this will further reduce the risk of pathogen introduction.
- To better understand the potential for pathogen transfer between cultured and wild stocks, baseline studies of diseases of decapod crustaceans in the vicinity of aquaculture facilities should be conducted. Such monitoring will also help to detect any transfer of introduced exotic pathogens from *L. vannamei* to wild crustacean populations.
- The SAS should conduct susceptibility testing of local penaeids to check for the presence of cryptic or unknown pathogens in the imported broodstocks.

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Utari, H.B., Senapin, S., Jaengsanong, C. Flegel, T.E. & Kruatrachue, M. 2012. A haplosporidian parasite associated with high mortality and slow growth in *Penaeus* (*Litopenaeus*) *vannamei* cultured in Indonesia. *Aquaculture*, 85-89.

WTO. 1994. Agreement on the Application of Sanitary and Phytosanitary Measures. p. 69-84. *In* The results of the Uruguay Round of multilateral trade negotiations: the legal texts. General Agreement on Tariffs and Trade (GATT), World Trade Organization, Geneva.

Annex 1

Litopenaeus vannamei Species Profile[1]

Litopenaeus vannamei is a decapod crustacean belonging to the family Penaeidae. Important taxonomic features (Figure 1) include the presence of teeth on both the upper and lower margins of the rostrum and by a lack of setae on the body. The rostrum is moderately long with 7-10 dorsal and 2-4 ventral teeth. In mature males, the petasma is symmetrical and semi-open. Spermatophores are complex, consisting of sperm mass encapsulated by a sheath. Mature females have an open thelycum. Colouration is normally white but can change depending on substratum, feed and water turbidity. Females commonly grow faster and to larger size than males.

Taxonomy

The taxonomic position of *Litopenaeus vannamei* is as follows:

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Crustacea
Class: Malacostraca
Subclass: Eumalacostraca
Superorder: Eucarida
Order: Decapoda
Suborder: Dendrobranchiata
Superfamily: Penaeoidea
Family: Penaeidae
Genus and species: *Litopenaeus vannamei* (Boone, 1931)
Syn.: *Penaeus* (*Litopenaeus*) vannamei Boone, 1931

[1] Information on *L. vannamei* is obtained from mainly from http://www.fao.org/fishery/culturedspecies/Litopenaeus_vannamei/en. Additional information can be found in the main body of the proposal and in the genetic, ecologic/environmental and pathogen risk analyses attached as Annexes 4-6.

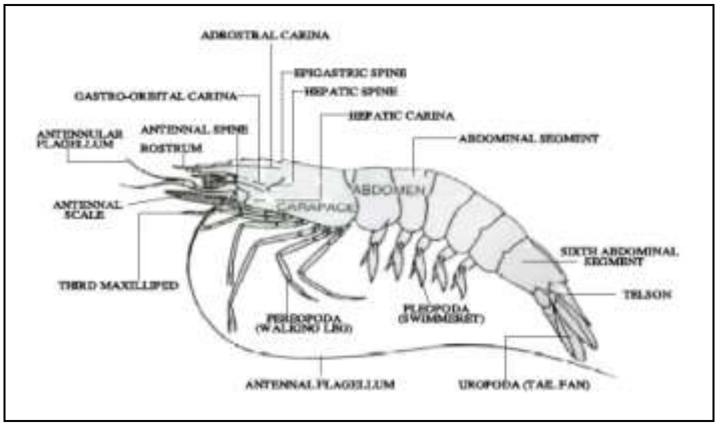

Figure 1. External anatomy of an adult *Litopenaeus vannamei*.

Distribution

Litopenaeus vannamei is native to the western Pacific coast of Latin America, occurring from southern Mexico in the north to northern Peru in the south, between latitudes 32°N and 23°S, (Figure 2) in areas where water temperatures are usually above 20 °C across the year. This species is highly abundant along the coast of Ecuador to Esmeraldas (the border province of Columbia) and is fished commercially in the Gulf of California and Gulf of Tehuantepec.

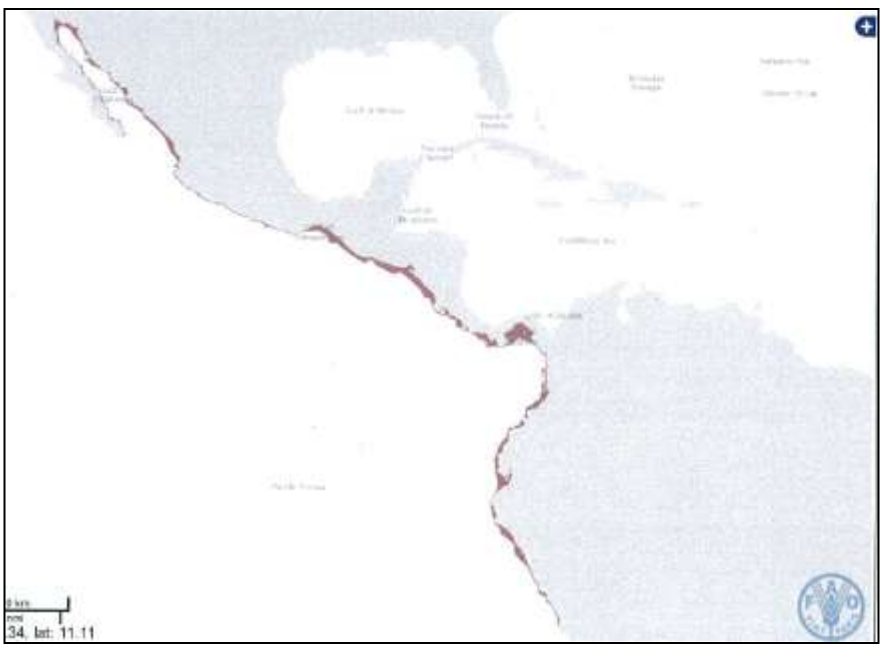

Figure 2. Map showing the natural range of *Litopenaeus vannamei*.

Life cycle

In general, prawns in the genus *Litopenaeus* mate and spawn in deeper near-shore waters at a temperature of 26-28 °C and a salinity of approximately 35‰. Males become mature from 20 g and females from 28 g onwards at the age of 6-7 months. *Litopenaeus vannamei* weighing 30-45 g will spawn 100,000-250,000 eggs of approximately 0.22 mm in dia. Hatching occurs about 16 hours after spawning and fertilization. The life cycle with various development stages is shown in Figure 3. In general, the first-stage larvae (the nauplii), swim intermittently and are positively phototactic. The next larval stages (protozoea, mysis and early postlarvae, respectively) remain planktonic for some time and are carried towards the shore by tidal currents. The postlarvae (PL) change their planktonic habit approximately 5 days after moulting into PL and move inshore and settle to the bottom, where they begin feeding on detritus, worms, bivalves and crustaceans. After several months in an estuary, juvenile shrimp return offshore, where sexual maturation, mating and spawning occurs.

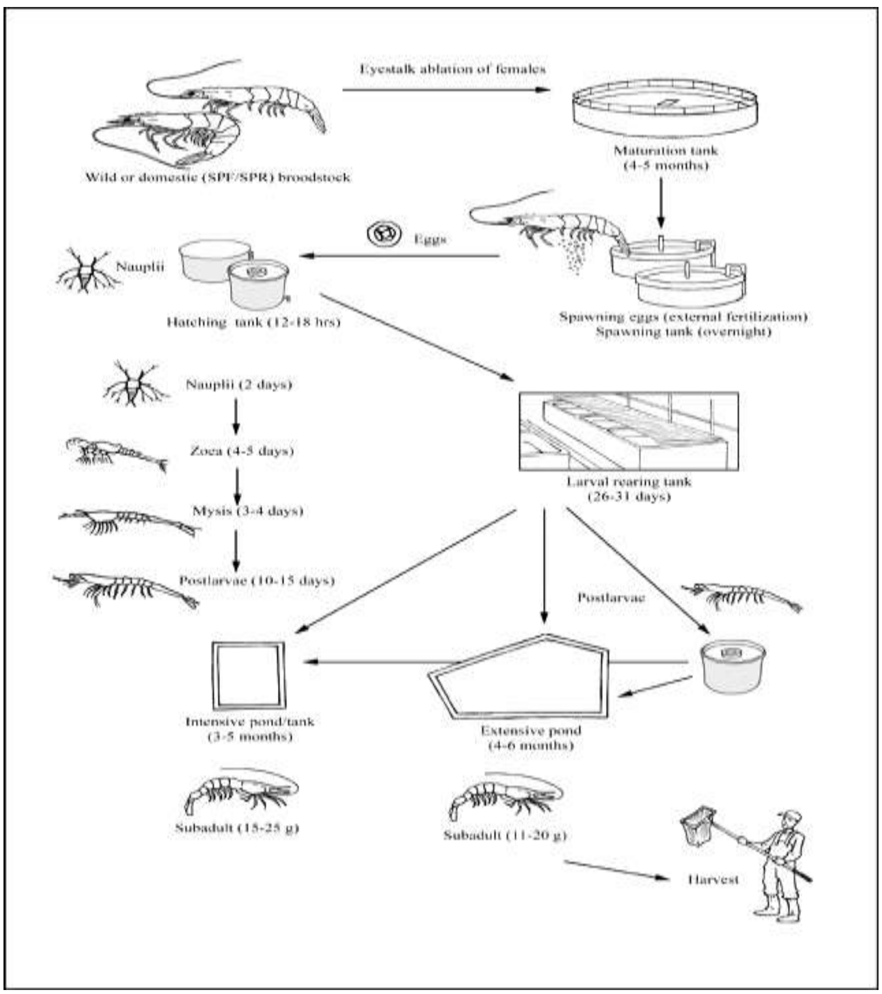

Figure 3. Generalized production cycle of penaeid shrimp.

In *L. vannamei*, the eggs hatch and the larvae develop as zooplankton. The first stage (nauplii) have five substages that last approximately 2-3 days, during which they change from a totally planktonic larvae subsisting on their own egg yolk to having rudimentary feeding appendages. The protozoal stage follows and consists of 3 substages lasting 3-5 days. At this stage, the larvae feed on phytoplankton and occasionally on zooplankton, in addition to their egg yolk. During this development stage the body becomes more elongate and a carapace, compound eyes and uropods are present.

After the protozoal stage, the mysis stage follows and also lasts for 3-5 days, with three substages. At this stage, development is characterized by elongation of the body, telson and pleopods, with the larvae able to swim and seek food. The diet changes from phytoplankton to zooplankton.

Usually it takes 12-15 days from egg to PL stage, depending on temperature and food availability. After 5-6 days, the PL change from a pelagic to a benthic organism, migrating from the open ocean into nearshore muddy bottoms and estuaries where water temperature ranges from 25-32°C, salinity from 28-34 ppt and depth is usually lower than 70 cm. These areas serve as nurseries. Adults prefer higher salinity (34-35 ppt) and deeper water (30-50 m). Typically prawns feed on a wide range of food items and their diet changes as they increase in size. Small crustaceans such as amphipods and copepods are important components of the juvenile diet, with subadults and adults feeding on polychaetes and molluscs.

Significance to aquaculture and fisheries

Beginning in early 1970s, various penaeid species including *L. vannamei* were introduced to a number of countries (including the northwestern Pacific coast of the Americas and to the eastern Atlantic coast from Carolina and Texas in the north through Mexico, Belize, Nicaragua, Columbia, Venezuela and to Brazil when French researchers in Tahiti developed techniques for their intensive breeding and rearing. In the USA, the first spawning of *L. vannamei* was achieved in Florida in 1973 from nauplii spawned and shipped from a wild-caught mated female from Panama. Following good pond results and the discovery of unilateral ablation to promote maturation in Panama in 1976, commercial culture began in South and Central America. Introductions of *L. vannamei* to Asia began in 1978/79 to the Philippines and in 1988 to mainland China. Of these trials, only mainland China maintained production and initiated a culture industry. Introductions on a commercial scale began in 1990 in mainland China and Taiwan POC and quickly spread to the Philippines, Indonesia, Viet Nam, Thailand, Malaysia and India. Since this time, *L. vannamei* has become the primary cultured species in many countries in Latin America and in the USA, Brazil, China, Thailand, Viet Nam, Indonesia, Taiwan POC, the Philippines, Malaysia, India and elsewhere.

Total production of *L. vannamei* was approximately 316,000 tonnes 2002 in Asia, and this increased to nearly 500,000 tonnes in 2003, worth around US$ 4 billion on the export market. In 2008, 67% (1,823,531) of the world production of cultured penaeid shrimp consisted of *L. vannamei*, representing an 18-flold increase in production in Asia. The commercial success of introducing *L. vannamei* into Asia can be attributed to several factors that include: increased availability of genetically selected, viral-pathogen-free domesticated broodstock; high larval survival; faster growth rate; better tolerance of high stocking density; lower dietary protein requirements; more efficient utilization of plant proteins in formulated diets; stronger adaptability to low salinity; better tolerance to ammonia and nitrite levels; and lower susceptibility to serious viral pathogens that infect *P. monodon*. China is currently the biggest producer of *L. vannamei*, increasing production from 33% in 2001 to 47% in 2008, mainly from inland freshwater ponds. The culture of *L. vannamei* in freshwater is expected to continue to increase in China, Thailand and other countries in Asia due to higher profits compared with comparable freshwater aquaculture species and also due to higher land availability in inland rather than coastal areas.

Production systems

Seed supply

Captured wild seed were used in Latin America for extensive pond culture until the late 1990s. Domestication and genetic selection programs in the 1980s provided more consistent supplies of high-quality, disease-free and/or resistant PLs, that are produced in hatcheries. Some were shipped to Hawaii in 1989, resulting in production of specific pathogen free (SPF) and specific pathogen resistant (SPR) lines that were used later in industry in the USA and Asia.

Broodstock maturation, spawning and hatching

There are three sources for broodstock:

1. Where they occur naturally, broodstock are sea-caught (usually at 1 year of age and weighing >40 g) and spawned.
2. Cultured shrimp harvested from ponds (after 4-5 months at 15-25 g), are on-grown for 2-3 months and then transferred to maturation facilities at >7 months of age when they weigh 30-35g.
3. Purchased from tank-reared SPF/SPR broodstock from the USA and elsewhere, (at 7-8 months of age and weighing 30-40 g).

Broodstock are stocked in maturation tanks in dark rooms supplied with clean, filtered seawater. Feeds consist of a mixture of fresh and formulated broodstock feeds. One eyestalk from each female is ablated, leading to repeated maturation and spawning. Females of 8-10 months of age reproduce effectively, while males peak at >10 months. Spawning rates of 5-15% per night are achieved, depending upon broodstock source. Females are spawned in either communal or individual tanks (to avoid disease transmission). The following afternoon, healthy nauplii are attracted by light, collected and rinsed with seawater. They are then disinfected with iodine and/or formalin, rinsed again, counted and transferred to holding tanks or directly to larval rearing tanks.

Hatchery production

Hatchery systems range from specialized, small, unsophisticated, often inland, backyard hatcheries to large, sophisticated and environmentally controlled installations, together with maturation units. Nauplii are stocked into flat, or preferably "V" or "U" shaped tanks with a volume of 4-100 m^3, made from concrete, fibreglass or other plastic-lined material. The larvae are either cultured to PL10-12 in a single larval rearing tank, or harvested at PL4-5 and transferred to flat-bottomed raceways/tanks and reared to PL10-12. Survival rates to PL10-12 should average >60%. Water is exchanged regularly (at 10-100% daily) to maintain good environmental conditions. Feeding normally consists of live food (microalgae and *Artemia*), supplemented by micro-encapsulated, liquid or dry formulated diets. From hatching, it takes about 21 days to reach harvest at PL12. Care is taken to reduce bacterial/pathogen contamination of the larval facilities using a combination of periodic dry-outs and disinfections, inlet water settlement, filtration and/or chlorination, disinfection of nauplii, water exchange and the use of antibiotics or (preferably) probiotics.

Nursery

Most farming operations do not use nurseries, but transport PL10-12 at reduced temperature either in plastic bags or oxygenated transportation tanks to the pond and introduce them directly. In some instances, nursery systems are used and comprise of separate concrete nursery tanks or earthen ponds, or even net pens or cages located within production ponds. Such nursery systems may be used for 1-5 weeks. Nurseries are useful in colder areas with limited growing seasons, where PLs are nursed to a larger size (0.2-0.5 g) in heated tanks/ponds, before stocking into ponds. The use of super-intensive, temperature-controlled, greenhouse-enclosed, concrete or lined raceways have also provided good results in the USA.

Grow-out techniques

Grow-out techniques can be subdivided into four main categories: extensive, semi-intensive, intensive and super-intensive, which represent low, medium, high and extremely high stocking densities, respectively.

Extensive: Commonly found in Latin American countries, extensive grow-out is conducted in tidal areas where minimal or no water pumping or aeration is provided. Ponds are of irregular shape, usually 5-10 ha (up to 30 ha) and 0.7-1.2 m deep. Originally, wild seed entering the pond tidally through the gate or purchased from collectors were used; since the 1980s, hatchery-reared PL are stocked at 4-10/m². Shrimp feed mainly on natural foods enhanced by fertilization, and once-daily feeding with low-protein formulated diets. Despite low stocking densities, small shrimp of 11-12 g are harvested in 4-5 months. The yield in these extensive systems, is 150-500kg/ha/crop, with 1-2 crops per year.

Semi-intensive: Semi-intensive ponds (1-5 ha) are stocked with hatchery-produced seed at 10-30 PL/m²; such systems are common in Latin America. Regular water exchange is by pumping, pond depth is 1.0-1.2 m and aeration is at best minimal. The shrimp feed on natural foods enhanced by pond fertilization, supplemented by formulated diets 2-3 times daily. Production yield in semi-intensive ponds range from 500-2,000 kg/ha/crop, with 2 crops per year.

Intensive: Intensive farms are commonly located in non-tidal areas where ponds can be completely drained, dried and prepared before each stocking, and are increasingly being located far from the sea in cheaper, low-salinity areas. This culture system is common in Asia and in some Latin American farms that are trying to increase productivity. Ponds are often earthen, but liners are also used to reduce erosion and enhance water quality. Ponds are generally small (0.1-1.0 ha) and square or round. Water depth is usually >1.5 m. Stocking densities range from 60-300 PL/m². Heavy aeration at 1 HP/400-600 kg of harvested shrimp is necessary for water circulation and oxygenation. Feeding with artificial diets is carried out 4-5 times per day. FCRs are 1.4-1.8:1.

Since the outbreak of viral diseases, the use of domesticated SPF and SPR stocks, the implementation of biosecurity measures, and reduced water exchange systems have become commonplace. However, feed, water exchange/quality, aeration and phytoplankton blooms require carefully monitoring and management. Production yields of 7,000-20,000 kg/ha/crop, with 2-3 crops per year can be achieved, and up to a maximum of 30,000-35,000 kg/ha/crop.

In the "bacterial floc" system, the ponds (0.07-1.6 ha) are managed as highly aerated, recirculating, heterotrophic bacterial systems. Low-protein feeds are fed 2-5 times per day, in an effort to increase the C:N ratio to >10:1 and divert added nutrients though bacterial rather than algal pathways. Stocking at 80-160 PL/m², the ponds become heterotrophic and flocs of bacteria are formed, which are consumed by the shrimp, reducing dependence on high-protein feeds, and increasing FCR and cost efficiency. Such systems have realized productions of 8,000-50,000 kg/ha/crop in Belize and Indonesia.

Super-intensive: Recent research conducted in the USA has focused on growing *L. vannamei* in super-intensive raceway systems enclosed in greenhouses, using no water exchange (only the replacement of evaporation losses) or discharge, stocked with SPF PL. They are thus biosecure, eco-friendly and can produce cost-efficient, high-quality shrimp. Stocking 282 m² raceways with 300-450 0.5-2 g juveniles/m² and on-growing for 3-5 months has realized production of 28,000-68,000 kg/ha/crop at growth rates of 1.5 g/week, survival of 55-91%, mean weight of 16-26 g and FCRs of 1.5-2.6:1.

Feed supply

Litopenaeus vannamei is very efficient at utilizing the natural productivity in shrimp ponds, even under intensive culture conditions. Additionally, feed costs are generally less than for the more carnivorous *P. monodon*, due to the lower requirement for protein (18-35% compared with 36-42%), especially where bacterial floc systems are used. Feed prices for *L. vannamei* range from US$ 0.6/kg in Latin America and Thailand to US$ 0.7-1.1/kg elsewhere in Asia; FCRs of 1.2-1.8:1 are generally obtained.

Harvesting techniques

Extensive and semi-intensive ponds are harvested by draining the pond at low tide through a bag net installed in the outlet sluice gate. If the tide does not allow harvesting, the water can be pumped out. In some larger farms, harvesting machines pump shrimp and water up to the pond bank where they are dewatered. Intensive ponds may be harvested similarly, and small 2-6 man seine nets are dragged around the pond to corral shrimp to the side of the pond from where they are removed by cast or dip net or perforated buckets.

Partial harvesting is common in Asian intensive culture after the first 3 months. In Thailand, artificial sluice gates are temporarily installed inside one corner of the pond to harvest closed-system ponds. Shrimp are then trapped in nets attached to this temporary gate when the pond is pumped out.

In super-intensive systems, the shrimp are simply harvested with large scoop nets when required for processing.

Handling and processing

If shrimp are sold directly to processing plants, specialized teams for harvesting and handling are commonly used to maintain quality. After sorting, shrimp are washed, weighed and immediately killed in iced water at 0-4 °C. Often sodium metabisulphate is added to the chilled water to prevent melanosis and red-head. Shrimp are then kept in ice in insulated containers and transported by truck either to processing plants or domestic shrimp markets. In processing plants, shrimp are placed in iced bins and cleaned and sorted according to standard export sizes. Shrimp are processed, quickly frozen at -10 °C and stored at -20 °C for export by ship or air cargo.

Diseases and control measures

Major disease problems affecting *L. vannamei* include white spot disease (WSD) Taura syndrome virus (TSV), infectious hypodermal & haematopoietic necrosis (IHHN) causing runt deformity syndrome (RDS), baculoviral midgut gland necrosis (BMN) and vibriosis.[2] The availability of SPF and SPR broodstock provide a means of avoiding these and many other diseases, although biosecurity procedures are also important, including:

- thorough drying/scraping of pond bottoms between cycles
- reducing water exchange and fine screening of any inlet water
- use of bird netting or scarers
- putting barriers around ponds
- sanitary procedures

Once viruses do enter the ponds, there are no chemicals or drugs available to treat the infections, but good management of pond, water, feed and the health status of stocks can reduce their impacts.

Market and trade

FAO statistics show that the total farmed production of *L. vannamei* increased steadily from 8,000 tonnes in 1980 to 194,000 tonnes in 1998 (Figure 4). After a small decline in 1999 and a more significant decline in 2000 due to the arrival of WSSV in Latin America, FAO data show a rapid increase in production to over 1,386,000 tonnes in 2004, due to the rapid spread of this species to Asia. Main producer countries in 2004 were: China (700,000 tonnes), Thailand (400,000 tonnes), Indonesia (300,000 tonnes) and Viet Nam (50,000 tonnes).

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[2] See the pathogen risk analysis by Drs Richard Arthur and Victoria Alday-Sanz (Annex 6) for current information of the diseases of penaeid shrimp.

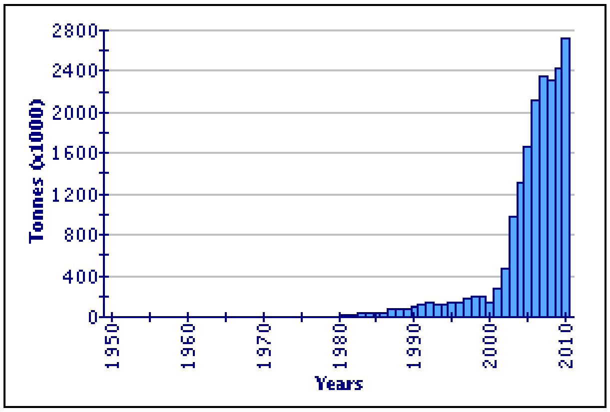

Figure 4. Global aquaculture production of *Litopenaeus vannamei* (FAO Fishery Statistic).

Products*:* Frozen head-on, head-off, and peeled shrimp were formerly the major products for export to the main global markets of the USA, the European Union and Japan. The trend now is for the processing of value-added products. This is due to the lack of antidumping tariffs for processed products to the US market, fewer people eating out and the desire for ready-to-cook or ready-to-eat products for home dining.

Prices and market statistics*:* The major market for shrimp is the USA, which was expected to import approximately 477,000 tonnes worth US$ 3.1 billion in 2005. The US market was traditionally supplied with small frozen or processed headless shrimp from Latin America. More recently, the USA has looked to Asia to meet increasing demand (1.9kg/capita in 2004). Major suppliers to the USA in 2005 were Thailand, Ecuador, India, China and Viet Nam. However, rapidly increasing production of *L. vannamei* has led to serious price depression in the international markets. Similarly, farm gate value for 15-20 g size *L. vannamei* steadily decreased from US$ 5/kg in 2000 to about US$ 3.0-3.5/kg in 2005.

The next most important market is the European Union (importing 183,000 tonnes in the first half of 2005), which favours small (31/40 count), whole, frozen shrimp. In Japan, the market mainly requires large headless (16/20 count) shrimp, and is typically supplied by *P. monodon* from large extensive Asian farms.

Market regulations*:* Standards for sanitation and the use of drugs and chemicals, and common food safety regulations for seafood (particularly shrimp) are already high in all major importing countries. However, the European Union market has more strict regulations (zero tolerance) on residues of chemicals and antibiotics, as well as the Generalized System of Preference (GSP) on import tax. The US market enforces more strictly on a sanitary standard such as HACCP or Sensory Assessment, but has also instigated strict controls over banned antibiotics in shrimp. From June 2005, the final antidumping tariffs on cultured shrimp imported into the USA from six main shrimp producing countries were finalized and set (for the general rate) at approximately 113% for China, 26% for Viet Nam, 10% for India, 7% for Brazil, 6% for Thailand and 4% for Ecuador. Mexico and Indonesia escaped these tariffs.

Responsible aquaculture practices

Due to rapid expansion and increasing awareness of the negative impacts of shrimp farming practices on the environment and its own production, many shrimp-producing countries are making efforts to comply with the concept of responsible aquaculture as detailed in Article 9 of the FAO *Code of Conduct for Responsible Fisheries* (CCRF). The formulation and adoption of better management practices (BMPs) (or Good Aquaculture Practices – GAPs) is gaining popularity to enhance biosecurity, increase cost efficiency, reduce chemical residues and increase traceability. Organic certification for shrimp farming is being seriously considered. HACCP and ISO standards, already used in processing/feed plants, are being adopted in farms and hatcheries. FAO and other organizations have developed a system of guidelines and BMPs to help shrimp producing countries comply with the various aspects of the CCRF.

Introductions

The introduction of *L. vannamei* to non-native areas of the Americas, Asia and the Pacific have had a significant positive effect on the production capacities of the countries involved. For example, *L. vannamei* was introduced to Brazil in 1983 and soon after commercial production began. It was not until 1995 however, when this species became the major species produced there due mainly to importation of a highly productive Panamanian stock in 1991 that was followed by mastering of its captive maturation, fast growth, high survival rates obtained in ponds and its good market potential in Europe and USA.

While six species of penaeid shrimp (*L. vannamei*, *P. monodon*, *L. stylirostris*, *P. japonicus*, *F. chinensis* and *F. indicus*) had been introduced to Hawaii for culture and research purposes, only *L. vannamei* is currently under commercial pond culture there.

The first commercial shipment of SPF *L. vannamei* broodstock from the Americas to Asia was from Hawaii to Taiwan POC in 1996, and following successes in captive maturation, larval rearing and culture in ponds of this species, led to huge demand for broodstock and to introductions of wild broodstock from many sources in Latin America in 1997. This was followed by introduction of *L. vannamei*, both SPF and SPF/SPR (for TSV) from USA, and non-SPF from Latin America and Taiwan POC to Philippines (1997), Thailand (1998), Indonesia and Viet Nam (2000), Malaysia and India (2001), and Myanmar and Bangladesh, and many other Asian countries, that in some cases were introduced without official government approval.

Litopenaeus vannamei gained prominence across Asia and production increased significantly due mainly to problems with the growth rate of *P. monodon* (the preferred species prior to introduction of *L. vannamei*). In addition, *L. stylirostris*, which was the major species cultured in Mexico has been replaced or out-competed by *L. vannamei* in every other country in the Americas. While SPF *L. stylirostris* had been promoted to many Asian countries around 2000-2003, this species has only had a significant impact in Brunei, and trials in Taiwan POC, Myanmar, Indonesia, Thailand and China have not led to commercial culture or have yet to make an impact on the shrimp production in these countries.

There have been at least two introductions of SPF/SPR *L. vannamei* from Hawaii into Iran. It is not clear whether or not a feral population has been established in the Persian Gulf. *Litopenaeus vannamei* has escaped from farms in Thailand and established a population that may be self sustaining in the Bangpakong estuary (no mature individuals were found as of 2009) in the Gulf of Thailand. *Litopenaeus vannamei* is not native to these areas and there is no reported evidence of any direct or indirect impacts on local shrimp species.

Preliminary List of Suppliers of Specific Pathogen Free *Litopenaeus vannamei* Broodstock

State of Hawaii

The following suppliers of *Litopenaeus vannamei* SPF broodstock are listed by the State of Hawaii, Department of Agriculture (updated 13 September 2012) as having been in operation for over 24 months with continuous negative PCR surveillance testing (http://hawaii.gov/hdoa/adp/shrimpstock):

Keawa Nui Farms LLC. (Molokai)
Contact: Mr. John Austin
HC 1-479
Kaunakakai, HI 96748
Telephone: 808-558-8931
FAX: 808-558-8934
E-mail: kiwi1961@mac.com
Website: http://keawanui.nexcess.net/
Relevant Company Information:

- *L. vannamei* SPF and SPR certified broodstock are free of WSSV, TSV, YHV, IHHNV, HPV, MBV, IMNV, microsporidians, haplosporidians, gregarines, BP/MBV, NHP.
- Facilities have been disease-free for over 12 years.
- Each shipment is accompanied by A Health Status Report, Certificate of Origin, US Fish & Wildlife permit, Invoice, Packing Information, Export declaration, Airway bill

Kona Bay Marine Resources – Waimea Aquatic Laboratory (Kauai)
Contact: Mr. Jim Sweeney
7550 Kaumualii Hwy.
Kekaha, HI 96752
Phone: 808-338-0331
FAX: 808-338-0332
E-mail: info@konabaymarine.com
Website: http://www.konabaymarine.com
Relevant Company Information:

- World's leading provider of SPF and SPR *L. vannamei*. Supplies broodstock to customers around the world and specializes in shipping throughout Asia. Kona Bay GSR-Taura™ is the world's leading line of Taura virus resistant shrimp ; developed by Kona Bay to be highly resistant to TSV and to be very high growth.
- Kona Stock of *L. vannamei* is from the SPF population at the Oceanic Institute in Hawaii. Weight of males ranges between 40 to 45 g and 45 to 50 g for females. Age of shrimp broodstock is from 9 to 12 months from postlarval stage.

- Commercial hatcheries in Asia, Latin America and the US that use Kona Bay GSR-Taura™ are assured of having the finest white shrimp available. Kona Bay GSR-Taura™ broodstock are highly efficient producers of both nauplii and postlarvae.
- Certified SPF by the State of Hawaii, Kona Bay GSR-Taura™ is raised in an advanced bio-secure facility. All shipments include a Health Status Report prepared by the State of Hawaii certifying its disease-free status.

Molokai Sea Farms International (Molokai)
Contact: Mr. Steve Chaikin
P. O. Box 560
Kaunakakai, HI 96748
Phone: 808-553-3547
FAX: 808-553-5216
E-mail: shrimp@broodstock.com
Website: http://www.broodstock.com
Relevant Company Information:

- Specializes in the production of SPF and SPR broodstock. Domesticated families of broodstock have a stock origin from the SPF/SPR populations of the USDA Marine Shrimp Farming Program at the Oceanic Institute in Hawaii.
- SPR broodstock are resistant to TSV. The Oceanic Institute refined these families from extensive research and development.
- The State of Hawaii Aquaculture Disease Prevention Program's Veterinarian Medical Officer lll Allen C. Riggs DVM, MS routinely samples our population for WSSV, IHHNV, HPV, TSV, YHV, INMV MVB and other serious pathogens of marine shrimp. Shrimp are tested using state of the art technology by D.V. Lightner at the Aquaculture Pathology Laboratory, University of Arizona.
- Facilities have remained free of serious shrimp pathogens since the inception of shrimp operations in 1984. Molokai Sea Farms International is the longest running aquaculture operation in the State of Hawaii.
- A Health Status Report, Certificate of Origin, US Fish & Wildlife permit, Invoice, Packing Information, export declaration and airwaybill accompany each shipment. We can request special documents such as Disease History Reports or Sanitary Certificates from the Disease Prevention Program if required.

The Oceanic Institute – Makapu'u Facility (Oahu)
Contact Mr. Steve Arce
41-202 Kalanianaole Highway
Waimanalo, HI 96795
Phone: 808-259-7951
FAX: 808-259-9762
E-mail: sarce@oceanicinstitute.org
Website: www.oceanicinstitute.org
Relevant information:

- Oceanic Institute (OI) is primarily a "not-for-profit" research and development organization that makes limited quantities of SPF *L. vannamei* broodstock available to private industry when there are excess stocks from research/breeding activities.
- OI typically only makes stocks available 3 times per year in 1-2 month windows (when the stocks are between 8-9 months of age), based on their research/production schedule. The next window of availability will begin in January 2013. After January, OI anticipates having stocks available in April/May.
- OI offers genetically improved *L. vannamei* broodstock from its selective breeding program for sale to select, successful companies which have sound management and biosecurity practices.
- The broodstock are SPF and will be accompanied by a State of Hawaii health certificate and certificate of origin.
- Disease screenings are conducted by the University of Arizona's Aquaculture Pathology Laboratory (an OIE Reference Laboratory) in conjunction with the State of Hawaii's Shrimp Surveillance and Certification Program. All OI shrimp stocks are screened for TSV, WSSV, YHV/GAV/LOV, IHHNV, MBV, BP, BMN, IMNV, HPV, PvNV, MoV, NHP, RLB-MHD, microsporidians, haplosporidians and gergarines.
- The broodstock are from a select group of OI's top-performing families selected for rapid growth and high growout survival. In research trials conducted at OI, the families were stocked at a mean size of 2.0 g, evaluated for growth at a stocking density of 209 shrimp/m^2 and were harvested at 20.4 g. At these high stocking densities, mean growth of the selected families was 0.29 g/day (2.0 g/wk) and mean survival was 88%.
- There is a minimum order of 600 broodstock and the price per broodstock is USD$25 excluding materials and freight charges. Broodstock are offered on a first come, first served basis, and interested buyers can secure their order by forwarding a 50% deposit to OI. The remainder of the balance will be due at least 7 days prior to shipment. (source, S. Arcie, Oceanographic Institute, pers. comm.)

Shrimp Improvement Systems - Hawaii LLC (Big Island)
Contact: Mr. Kenneth Tay
73-4460 Kaahumanu Highway Suite #108
Kailua-Kona, HI 96740
Phone: +65 6397 0555
E-mail: kenneth@shrimpimprovement.com
Relevant Company Information: see below (note: recently expanding in Hawaii via purchase of High Health Shrimp; also see: http://hawaiitribune-herald.com/sections/news/local-news/shrimp-farm-expanding-big-island.html)

State of Florida

Shrimp Improvement Systems (LLC SIS)
88081 Old Overseas Hwy
Islamorada FL 33036
Phone: (305) 852-0872

Fax: (305) 852-0874
Website: http://www.shrimpimprovement.com/
Relevant Company Information:

- Since the start of operations at Plantation Key, representative samples of all SIS stocks have been routinely monitored and have been found to be free of WSSV, TSV, IHHNV, YHV and NHP.
- On an ongoing basis, representative tissue and/or hemolymph samples from all stocks are submitted to an independent outside shrimp disease specialist to confirm the disease-free status of the NBC.
- All shrimp stocks in the NBC are tested twice a year by PCR for WSSV, TSV, IHHNV, YHV, IMNV and NHP using OIE approved methodologies and primers. Samples are collected from larval tanks, hatching tanks, PL tanks, maturation tanks and broodstock raceways using sample size statistical guidelines that assure a 95% confidence interval.
- In addition to routinely monitoring the disease status of the shrimp stocks within the NBC, wild-caught indigenous shrimp and crab species from the Plantation Key area are also monitored twice yearly by PCR for WSV, TSV, and IHHNV as a means of assessing thy potential for possible contamination of the facilities by local crustacean species.

Asia-Pacific

Aquaculture Promotion Co., Ltd.
22nd Floor, CP Tower, 313 Silom Road
Bangrak, Bangkok, Thailand 10500
Mr. Suravut Bavornvipat
E-mail : cpbroodstock@gmail.com
Tel. 668-4465-3022, 662-625-8300-7 Fax. 662-638-2737
Website: http://www.cpbroodstock.com
Relevant Company Information:

- CP SPF SPR broodstock has been developed over the past 10 years for Asian culture systems. The broodstock has a proven track record in producing superior results in maturation units, hatcheries, and farms. No other broodstock is comparable in providing both hatcheries and farms with the animal performance to deliver superior profits.
- CP introduced SPF *P. vannamei* into Asia in 2002, with the importation of certified disease free SPF shrimp from Hawaii. Subsequently, over 10 independent populations of SPF shrimp have been added to the CPF breeding program. Only shrimp that were cleared of a strenuous quarantine process were put into the nuclear breeding center. The breeding center is where all selection takes place and new founding shrimp have not been added since 2005. Only select juveniles are passed out of the center to closed system, biosecure broodstock farms where the final broodstock for hatcheries are produced.
- Both nuclear breeding centers and broodstock farms are monitored for disease, and are certified by the government of Thailand as SPF for TSV, WSSV, HPV, IHHNV, MBV, YHV, GAV, IMNV, BP and NHPB.

Saipan Aquaculture
As Falipe Saipan
Commonwealth of Northern Mariana Islands 96950
USA
Tel. No. (670) 233-4770
E-mail: inquiry@saipanaquaculture.com
Website: http://www.saipanaquaculture.com/
Relevant Company Information:

- Our breeding programs are derived from large number of families, with a broad genetic base and incorporate intense selection on each generation using combination of family selection, mass selection (WFS) and marker assisted selection. The programs maintain population inbreeding at less than 1% per year to enable sustainable long term genetic improvement. The broodstock are offspring from a select group of top performing families selected for rapid growth, TSV resistance, and high pond survival.
- TSV resistance is determined by bioassay laboratory challenge tests. Families are exposed to TSV isolates from US (USTX95), Belize (BH01), Thailand (TH04) and Venezuela (VE05). Between and within family selection Genetic selection is undertaken from data collated and analysed from raceway, pond and bioassay trial.
- Product Guarantees include: Produced in Premium Health SPF facilities, Produced using no antibiotics, Produced using non-GMO feeds and non-GMO technology. Certified negative to class I viruses (WSSV, TSV, IHHNV, YHV)
- Our production system also complies with Best Aquaculture Practices (BAP) and all existing government and environmental laws on protection and conservation.
- To maintain our High Health Status, samples from on going cultures are periodically sent to the world renowned Shrimp Pathology Laboratory of University of Arizona. The facility has already established more than 3 years of SPF status. In addition, all incoming shrimp are certified SPF and quarantined until established as high health animals.
- Our production system allows for full traceability of the genealogy of the animal. Full production parameters are also recorded for each tank and culture batches, for reference if the need arises.
- The farm is certified SPF facility and has been under the disease surveillance of the University of Arizona Pathology Laboratory for more than 3 years now. The farm is located 220 feet above sea level, 2 miles from the nearest ocean and water sourced from a deep well 260 feet down. We have no neighboring shrimp farm. The closest shrimp farms to us are the small farms in Guam more than 200 kilometers away.

Other potential suppliers

The following additional suppliers were recently approved by the Government of India (list dated 12.04.2011) to supply SPF *L. vannamei* to Indian shrimp growers (http://aquaculture.tn.nic.in/pdf/attn_importspfbroodstock-020109.pdf):

M/s. SyAqua
159, Serm – Mit Tower 11th FlK

Sukhumvit 21 (Asoke) Road
North Hlongtoey, Wattana
Bangkok – 10110, Thailand
Phone No: 66- 2- 661-7607- 9
Email: syaqua@syaqua.com

M/s. Vannamei 101 Co. Ltd., with joint venture
Partner Sibsaen Aqua Marine
178/5 Moo I, Paklok, Thalang
Phuket – 83110, Thailand
Phone No: (66)76- 529724
Email: mattbriggs101@gmail.com; david@ Vannamei 101.com
sibsaenshrimp@hotmail.com

M/s. Charoen Pokphand Foods Public Co. Ltd.
Shrimp Genetic Improvement Center
313, CP Tower, Silom Road, Bangrak
Bangkok – 10500, Thailand.
Phone No: (638) - 2000
Email: kungrankij@yahoo.com; cpudomask@yahoo.com

Shrimp Improvement Systems Pte. Ltd.
No.90, Lim Chu Kang Lane,
6F SINGAPORE 718873.
Tel. No.: (65) 6397 0555.
Fax No.: (65) 6397 0880.
E-Mail: sis_shrimp@singnet.com.sg
Website: www.shrimpimprovement.com
Relevant Company Information: see above.

Annex 3(A)

ICES Quarantine Procedures and Standards for Introductions and Transfers of Live Aquatic Animals[7]

QUARANTINE

Quarantine is the separate holding, rearing, or both, of taxa in a facility or site, under conditions which prevent the escape or other movement of these taxa and associated organisms (i.e. disease agents, pathogens, epibionts) out of the location. Different periods of quarantine and security level may be required depending on the risk of introducing reportable disease agents or previously undetected disease agents of concern.

During the quarantine period, the taxon is held in a quarantine unit. To accomplish this, general principles which apply to all quarantine units for aquatic species are given below. The individual construction and approval of the unit and the length of the quarantine period. Further is there a need to build quarantine systems according to the species considered as some might have peculiarities remains with the operator and the jurisdiction into which the introduction or transfer takes place.

For the operation of an effective quarantine unit, the operators will need to take the topics below into account when constructing and maintaining the quarantine unit

Effluent and Waste Disposal

All effluent and wastes generated within a quarantine facility**7** should be treated in a manner that effectively destroys all disease agents, parasites, fouling organisms on oysters etc.

A quarantine facility is defined as land based facility or portion of a facility approved by the ITC where shellfish can be held in a manner which prevents the movement of shellfish or shellfish disease agents from the facility.

To ensure continuous operation and complete containment, quarantine effluent treatment systems should be equipped with fail-safe backup mechanisms to ensure continuous operation and complete containment.

Treated effluent and waste may contain substances deleterious to the environment (e.g. active disinfectants). The discharge should therefore be disposed of in a manner that minimizes environmental impact.

Further information on disinfecting effluent and solid wastes are presented below (under heading of Disinfection).

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[7] Reproduced from: ICES (2012), Annex 6. Appendices to the ICES Code of Practice (CoP) on the Introductions and Transfers of Marine Organisms (2005), APPENDIX C: QUARANTINE, pp. 263-265.

Discharge of treated effluent and waste must comply with all other restrictions and regulations applicable to the facility (e.g., federal, provincial, municipal or other environmental legislation with respect to quarantine effluent discharge and waste disposal).

A detailed log of effluent and solid waste treatment should be prepared, listing the operational personnel responsible for treatments, timing and monitoring of the system is useful to monitor effective operation and act as a early warning system for possible failures. Details of the information that should be monitored are provided below (under heading of Record Keeping).

Physical Separation
Aquatic organisms held in quarantine must be separated from other organisms in a system to ensure containment of animals and disease agents, to prevent entry by birds and other animals, to prevent entry by unauthorised personnel and to prevent spills from contaminating surrounding areas. Water lines must be constructed such that there is no possibility of backflow from the quarantine areas to other animals or the environment.

Personnel
Access to a quarantine facility must be restricted to authorised personnel. Personnel working in the quarantine facility must ensure that footwear, hands, and any material used within the facility are disinfected before exit from the facility.

Equipment
Upon receipt all life-stages, tanks, water, shipping containers and equipment in contact with the taxon— including the transport vehicles — should be handled to ensure that there is no escape of the taxon or associated disease agents from the facility. All shipping and packing material must be disinfected or burned.

All equipment and supplies used within a quarantine facility must be disinfected in a manner that will effectively destroy disease agents before removal from quarantine. Protocols for effective management and disinfection must be approved by the WGITMO.

Mortalities and Disposal
Daily records of mortalities must be maintained and be available for inspection, where required.
All mortalities must be kept on site. No mortalities, body parts or shells, bones etc. can be discarded without approved treatment to ensure complete disinfection so that no body parts etc. may re-enter the aquatic environment. Where autoclave access is outside the quarantine facility, the organisms and associated solid waste can be chemically sterilised, or frozen prior to transport to the autoclave. Alternately, materials can be formalin fixed and then discarded in e.g. a landfill site.

The cause of mortalities must be determined in a timely manner. Mortalities should be reported immediately to the WGITMO in order to expedite protocols for examination of the affected animals. This may require that samples be collected or preserved for transportation to a laboratory in an approved manner.

Inspection and Testing

Regular inspections for reportable disease agents must be carried out as specified under the conditions of the licence for introductions or transfers.

If a reportable disease agent, or previously undetected disease agent, is identified in any life-history stage of animals in a quarantine facility, actions necessary to control the disease will be required. These actions may include destruction of all animals in the facility and disinfection of the facility.

If no reportable disease agent is detected in the animals during quarantine, a pathogen status report will be provided to terminate the quarantine requirement.
Following removal of all life stages of the taxon from the quarantine facility, further monitoring and testing of the taxon for reportable disease agents and imposition of additional restrictions may be required.

Duration
The required duration of quarantine will vary according to the aquatic taxon, seasonality of pathogens of concern, rearing conditions and reason for quarantine containment. The quarantine period will be specified on the licence for introductions or transfers that specifies quarantine as a condition.

RECORD KEEPING

All quarantine and isolation facilities and sites must maintain accurate records of the following:

- entry /exit times of personnel, all of whom should have authorization for access
- numbers of mortalities and method of storage or disposal
- effluent and/or influent treatments and monitoring of residuals
- any abnormal conditions affecting quarantine / isolation operations (power outages, building damage, serious weather conditions, etc.)

DISINFECTION

The general principles pertaining to disinfection of aquaculture facilities (hatcheries, holding facilities, land-based ponds, etc.) involve the application of treatments in sufficient concentrations, and for sufficient periods of time, to kill all harmful organisms which otherwise would gain access to surrounding aquatic ecosystems. Since the inherent toxicity of disinfectants prohibits safe use in open-water, or in flow-through systems, disinfection can only be applied with reasonable control within hatcheries, tank or isolated pond-holding facilities. All disinfectants should be neutralised before release into the surrounding environment and facilities based on seawater should deal with the residual oxidants produced during chemical disinfection.

A log of neutralisation of disinfection procedures and monitoring results is highly recommended for ensuring that neutralisation is adequate to prevent negative environmental impacts.

There is a wealth of detailed information on disinfection available from official and commercial organisations. New and improved products and protocols are being continuously developed and so it is not appropriate to provide detailed guidance here.

For example, The World Animal Health Organization (Office International des Epizooties) has provided information on the disinfection of aquaculture establishments (see: International Aquatic Animal Health Code, 2002, Office International des Epizooties 2002 - http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/2010/1.1.3_DISINFECTION.pdf). A list of some commercially available disinfectants approved for use in Australia by AQIS can be found on their website. (http://www.daff.gov.au/aqis/import/general-info/qap/aqis_approved_disinfectants_for_all_classes).

FAO Recommended Standards of Construction, Security and Operation for Quarantine Facilities for "High-risk" Movements (Introductions and Transfers)[8]

GENERAL

Chapter 4.0 outlines the recommended minimum standards for the construction, security and operation of quarantine facilities used to hold aquatic animals considered to pose a "high risk" of introducing serious aquatic animal disease. These include imported live aquatic animals that are destined for use in aquaculture development, fisheries enhancement or other activities that involve intended release or probable escape into natural waters (i.e. introduced or transferred species) and whose individual health status and/or the health status of the population from which they originate is partially or completely unknown and which are considered to be potential carriers of serious aquatic animal diseases of concern to the importing country. The following procedures are adapted primarily from the protocols developed by AQIS (undated(a)), MAF (2001) and ICES (2005).

The protocols outlined in ICES *Code of Practice on the Introductions and Transfers of Marine Organisms* (ICES, 2005) recommend that such "high-risk" aquatic animals should normally be held in strict quarantine throughout their lives, during which repeated observation, sampling and testing for pathogens should be conducted. Progeny reared from the imported parent stock (F1 generation), following additional observation and testing, may be approved for limited release under controlled conditions, during which further monitoring of their health status should be conducted. If no pathogens are detected during this initial period (which can be expected to last several years), subsequent generations may be approved for wider use in aquaculture or for release into the wild. In all cases, the original parent stock should not be released from quarantine and should be destroyed, preferably by concurrent lethal sampling and testing for pathogens.

The standards recommended in this Chapter should be applied only to the quarantine of those aquatic species that have received written approval by the regulating agency or CA (typically the national Quarantine Service, Ministry of Fisheries, Department of Agriculture, Veterinary Service, etc.) to be introduced or transferred into the national territory. Such approval should be based on a risk analysis (Arthur *et al.*, 2004; ICES, 2005; OIE, 2006a; FAO, 2007) and an application for a Permit to Introduce or Transfer Live Aquatic Animals, as specified in the appropriate national regulations.

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8 Reproduced from Section 4 of Arthur, J.R., Bondad-Reantaso, M.G. & Subasinghe, R.P. 2007. Procedures for the quarantine of live aquatic animals: a manual. *FAO Fisheries Technical Paper.* No. 502. Rome, FAO. 60 p.

The approval of an application to introduce or transfer an aquatic animal should, among others, be contingent upon the use (construction, establishment or lease) of an approved Quarantine Facility meeting the minimum standards outlined in this Section. Each application should be considered on its individual merits with consideration being given to the quarantine risk and serviceability associated with each establishment's location and construction and on the capability of the applicant to successfully operate such a facility. Examples of the approval processes and application forms used for quarantine facilities for ornamental aquatic animals and their operators are given in MAF (2001). The *Import Health Standard for the Importation into New Zealand of Ornamental Fish and Marine Invertebrates from All Countries* (Anon., 2002) provides a useful example of a set of procedures and legal requirements (i.e. an aquatic animal import health standard) to import aquatic animals. Similar requirements for Australia are given in AQIS (undated (b), undated(c)). Approval of a Quarantine Facility should be contingent upon the operator's formal agreement to undertake any training or other courses or briefings as mandated by the CA.

It is the responsibility of the operator of an approved Quarantine Facility to ensure that the premises and all operations comply with all local, state and federal regulations. Documented evidence of compliance with these requirements must be produced to the supervising Quarantine Officer on request.

The operator should ensure that all staff entering the Quarantine Facility are adequately trained in the husbandry of the species being held in quarantine and are familiar with the applicable standards of performance as outlined in the aquatic animal import standard and any SOPs.

The CA should require that notification in writing be given at least 30 days prior to any intended change in ownership, senior management, quarantine operating procedures/arrangements or contemplated modifications to the Quarantine Facility.

Non-compliance with the criteria outlined in these recommendations, once they have been modified to fit specific national needs and circumstances and formally adopted into national legislation, may be justification for withdrawal or suspension of approval of the Quarantine Facility, the possible destruction of stock and the instigation of legal action.

The importation of "high risk" aquatic animals destined for introduction or transfer into aquaculture facilities or natural waters often entails a significant risk that serious pathogens accompanying them may escape and become established in natural or cultured populations. Thus, an extremely high level of biosecurity must be maintained at all times. The Quarantine Facility should be constructed and operated in a manner that will assure a high level of security, guaranteeing the isolation of the imported aquatic animals, such that the animals, any pathogens they may carry and any pests or other living organisms contained in their transport waters will not be released from the facility. The possible entry of pathogens of domestic origin that might infect the stock held under quarantine, thorough contaminated influent water, or their entry along with personnel, feeds and fomites must also be prevented.

During the quarantine period, the operator should ensure that no living aquatic animals, equipment or materials are removed from the Quarantine Facility.

PERIOD OF QUARANTINE

No set period of quarantine should be established. The period of holding in the Quarantine Facility will depend on the results of observation and testing of the imported stock and the resulting F1 generation. In all cases, once the CA is satisfied that the F1 or a subsequent generation is safe for limited release, the parent stock should be destroyed and the Quarantine Facility thoroughly disinfected.

An application to introduce or transfer an aquatic animal entails a commitment to maintain the animals under conditions of strict quarantine for a number of years. The quarantine period will need to take into account the life history of the aquatic animal being introduced or transferred.

If a pathogen or infectious disease is detected at any point while the imported aquatic animals and their progeny are under quarantine, the supervising Quarantine Officer may require treatment and further testing. If the disease is of a serious and/or untreatable nature, destruction of all aquatic animals held in the facility should be ordered and complete disinfection of the building, water and all equipment should be required before permission to restock is granted.

STANDARDS OF CONSTRUCTION

Location of quarantine facilities

The location of a Quarantine Facility should be determined on a case-by-case basis. Premises should not be approved in the vicinity of private or government fish hatcheries, aquaculture facilities, watercourses or areas subject to frequent flooding.

General requirements

Access to the Quarantine Facility should be through property owned or leased on a long-term basis by the operator and should be available to Quarantine Officers during normal business hours and at such time that aquatic animals are entering or leaving the facility. The operator should notify the supervising Quarantine Officer of the times when the premises will be attended and any alterations to the regular hours.

The Quarantine Facility should be located within a single operational entity that is structurally separated from all other operations and is dedicated solely to the holding of the shipment. It should not share a building having areas that are used for different purposes and should not serve as an access way to other buildings or activities. The Quarantine Facility should not to be used for any purpose, what-so-ever, other than as a place for the performance of quarantine.

The Quarantine Facility should be weatherproof and maintained in a state of good repair.

The Quarantine Facility should be a secure, lockable building that is surrounded by a lockable person-proof security fence.

The holding capacity of the Quarantine Facility should be commensurate with the proposed quantities of the species of aquatic animal for which a permit is granted. Provision must be made for the growth and maturation of the original parent stock and the holding of all F1 and subsequent generations.

The Quarantine Facility should be equipped for the sterilization of all equipment that comes in contact with aquatic animals or tank water during the quarantine period.

The Quarantine Facility should be equipped with back-up systems for essential components (e.g. electricity, water circulation, aeration, temperature control, filtration, etc.) to maintain biosecurity and the health of stocks in the case of electrical or mechanical failures.

Specific construction and equipment requirements

The Quarantine Facility should comply with the following specific construction and equipment requirements:

(a) Windows should be screened to prevent the entry of insects.
(b) Floor and walls should be constructed of concrete, tiles or other impervious material to enable hose down and disinfection with retention of all wastewater. The floor should be sufficiently smooth and with sufficient grade to drain to an enclosed holding tank.
(c) Floor to wall junctions and all gaps and cracks in the walls, floor and ceiling should be effectively sealed such that the quarantine area is capable of containing all leaks and floods that might occur.
(d) Lighting should be of sufficient intensity to allow proper inspection of all aquatic animals.
(e) Floor drainage with an insertable plug or other mechanism to prevent the accidental escape of aquatic animals or uncontrolled release of water should be installed. Drainage should be to an approved holding tank. The holding tank should be of suitable size to contain the total volume of all tanks used for the holding of aquatic animals.
(f) Doors should be equipped with self-closing mechanisms to ensure that they remain shut after entry, or there should be a self-closing insect-proof screen door installed.
(g) Access to the Quarantine Facility should only be through a personnel entrance leading to a separate outer change room provided with facilities for staff and Quarantine Officers to wash their hands and change outer clothing prior to entering or leaving the quarantine area.
(h) A footbath containing disinfectant should be placed at the entrance door to the quarantine facility.
(i) All holding tanks for aquatic animals should:
   - be identified with permanent numbers so that individual tank records can be correlated with them;
   - be fitted with lids or other approved coverings so as to prevent transmission of pathogens between adjacent tanks due to splash from the aeration/filter system, and to prevent the escape of aquatic animals;
   - have water intake lines equipped with automatic shut-off valves;
   - be arranged in a manner that permits ready access for inspection purposes, including a minimum width of 75 cm for corridors between rows of tanks or tanks and walls;

- other than the aquatic animals, contain only sterilizable materials (e.g. plastic) that do not interfere with inspection;
- have at least the front transparent to provide good visibility of their contents, and be stacked for adequate viewing; and
- have its own set of nets, buckets, beakers and other items associated with the tank use, to ensure that none are shared between tanks (also Chapter 4.6.1).

(j) As all aquatic animals within the facility should be considered to have the same quarantine status, the use of a shared water recirculation system is permissible but not advisable, as it may facilitate the spread of pathogens between tanks.

(k) All entry and exit points to the Quarantine Facility should prominently display a permanently affixed, professionally made, quarantine sign that states "Quarantine Area–Authorized Persons Only". Such signs should be highly visible (e.g. black lettering of about 5 cm in height on a yellow background).

(l) A suitable wash-up trough should be located in the quarantine area for the cleaning and disinfecting of equipment. An approved disinfectant should be available at the wash-up trough. A suitable draining rack should be provided for air drying of equipment.

(m) A designated refrigerator or freezer should be provided solely for the storage and preservation of dead aquatic animals. The refrigerator or freezer should be clearly identified as being for quarantine use only, be lockable, and located within the quarantine area.

(n) Equipment necessary to carry out the disinfection all wastewater (both the overseas transport water and all domestic waters used in the Quarantine Facility) should be supplied.

(o) Secure storage facilities for food used for aquatic animals should be provided such that contamination or infestation by pests is prevented.

(p) A fully stocked first aid cabinet should be provided and maintained.

(q) Amenities that should be provided for use by Quarantine Officers include access to a desk and chair, a telephone with a direct outside line, toilet facilities, hand washing facilities (within the quarantine area) and a hygienic means of drying hands, and suitable arrangements for daily cleaning of amenities.

STANDARDS OF OPERATION

Influent water

All influent water entering the Quarantine Facility should be from an approved groundwater source certified to be free from biological material, including any possible infective agents. Alternatively, water from other sources may be used, however, it should be filtered to remove suspended matter and then sterilized using a method approved by the CA before being used in the Quarantine Facility.

Wastewater sterilization and disposal

All wastewater to be discharged from the Quarantine Facility should be appropriately sterilized. Sterilized wastewater should not be discharged directly into natural waterways. Disposal of wastewater should also conform to any state and local government requirements.

Wastewater should be sterilized in accordance with one of the following methods:

(a) Chlorination[9]

(i) All water should pass through an approved filter capable of removing suspended organic material prior to hypochlorite treatment.
(ii) All water should pass to a retention vessel where sufficient hypochlorite is be added to achieve a minimum concentration of 200 parts per million (ppm) (200 mg/liter) at 1 hr post-treatment. Sodium hypochlorite (bleach) should be used at 1.6 ml of hypochlorite solution (12.5 percent available chlorine) per liter of water, while calcium hypochlorite powder (e.g. Pool Chlor®, 65–70 percent available chlorine) should be used at 0.3 g of powder per liter of water.
(iii) Before the treatment period commences, the chlorinated effluent should be brought to a pH between 5.0 and 7.0.
(iv) Following addition of hypochlorite, wastewater should be agitated for at least 10 min to ensure thorough mixing of hypochlorite.
(v) After a retention period of not less than 1 h, the chlorine concentration is measured using an approved method (e.g. commercially available chlorine test kit). Tanks not achieving a minimum chlorine concentration of 200 ppm (200 mg/liter) at the allotted time should be re-treated until the requirement is met.
(vi) The chlorine in the wastewater should be neutralized by adding sodium thiosulphate at a rate of 1.25 g (2.5 ml of 50 percent sodium thiosulphate solution) per l of treated wastewater, then agitated for not less than 10 min before discharge.[10]
(vii) Chlorination records should be maintained noting: the amount of compound added, the volume of effluent, the time that treatment period commenced, the pH at commencement of the treatment period, the 1 hr post-treatment concentration, the amount of sodium thiosulphate added to achieve neutralization and the of residual chlorine concentration at discharge.
(viii) Chlorinated water should not be discharged directly into adjacent waterways.

(b) Heat treatment
Prior to discharge, wastewater shall be heated to at least 85 °C for a minimum of 30 minutes. Water heating units should be approved by the CA and be fitted with temperature and flow recorders.

(c) Ultraviolet (UV) light radiation
As particles in the water may shade pathogens from the effects of UV light, all water to be treated should pass through an approved filter capable of removing suspended organic material prior to irradiation.

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[9] Chlorine compounds are corrosive, relatively unstable and are inactivated by the presence of organic matter. They can be highly toxic to fish and humans and thus must be handled and applied with due care. For detailed reviews of their use as disinfectants in aquaculture, DAFF (2006) and Danner and Merrill (2006).
[10] Theoretically, 1 mg thiosulphate is required to reduce 1 mg chlorine dioxide (Siemens, 2006). Thus the specified treatment would potentially reduce chlorine at levels of 1 250 mg/liter and below. To reduce the quantity of chemical required, residual chlorine level at the end of treatment may be measured and the amount of thiosulphate required for neutralization calculated.

Commercial UV water treatment units operating in the spectral range of 190-280 nm (254 nm recommended) delivering doses of at least 130 mWs/cm^2 are required. As UV bulbs will burn long after their effectiveness has waned, the burning time of the UV lamp should be monitored, and the lamp replaced according to manufacturer's specifications.

DISINFECTION OF EQUIPMENT

Before removal from the quarantine area, and before any restocking, all tanks and tank equipment must be thoroughly cleaned and disinfected with (i) hypochlorite solution at 200 ppm concentration for 5 min or with (ii) an approved iodophore solution containing iodine at 0.5 percent available iodine for 5 min or (iii) by another disinfection procedure approved by the supervising Quarantine Officer.

Filter material should be disposed of by autoclaving followed by incineration or deep burial.

DISPOSAL OF DEAD AQUATIC ANIMALS

Dead aquatic animals should only be disposed of as directed by the CA. Aquatic animals that have died while under quarantine should held in an approved freezer, an approved refrigerator, or preserved using another method as specified by the CA until removed for laboratory examination or released for disposal by the supervising Quarantine Officer. Upon approval, dead aquatic animals should be disposed of by sterilization using of an approved autoclave, followed by incineration or deep burial.

DISPOSAL OF PACKING MATERIALS

All containers (bags, boxes and cartons) used to hold aquatic animals during transit should be disinfected using the methods of disinfection specified under "Disinfection of Equipment" (Chapter 4.3) and then disposed of by incineration, deep burial or another method approved by the supervising Quarantine Officer.

WORK PRACTICES

4.6.1 Cleanliness and sanitation

The Quarantine Facility and holding tanks should be kept clean at all times. Adequate cleaning facilities (e.g., pressurized water supplies, brooms, shovels, etc.) should be provided to enable maintenance of appropriate standards of hygiene.

No animals other than aquatic animals and live food for aquatic animals should be permitted in the quarantine area. All feeds used within the Quarantine Facility should have prior approval of the supervising Quarantine Officer and be of assured sanitary condition. Live food should not be used unless no other alternative food is acceptable to the animals under quarantine. Live food should be certified to the specifications set by the Competent Authority to ensure their freedom from potential disease agents.

Equipment used in the handling of aquatic animals and tank cleaning and maintenance should not be shared between tanks. A separate set of equipment (nets, cleaning equipment, etc.) should be kept for each tank or series of tanks operated on an individual water filtration system. In the case where several tanks are linked by a shared water recirculation system, a single set of equipment can be used for all tanks within the shared system.

All nets and other equipment should be regularly disinfected by an approved method of disinfection. Equipment or other material should not be removed from the quarantine area during the period that the shipment is under quarantine conditions. In exceptional circumstances, and with the written approval of the supervising Quarantine Officer and his verification that proper disinfection has been accomplished, a request to remove specific items of equipment may be granted.

All footwear and protective clothing used in the quarantine area should be restricted to this site.

The operator should provide protective clothing (jumpsuits, waterproof apron or outer-wear and rubberized footwear) to staff and visitors to use in the facility. Protective clothing should be kept inside the quarantine area (street footwear should left outside the quarantine area and within the changing area). Cloth protective clothing that should be routinely washed may be removed from the quarantine area after washing for the purpose of drying. During the period in which aquatic animals are under quarantine, protective clothing (with the exception of washed clothes removed for drying) should be removed only for destruction. Should removal of unusable protective clothing become necessary, it should first be sterilized by autoclaving or use of an approved disinfectant such as Betadine® (5 percent solution) and then removed and destroyed by incineration under the supervision of the Quarantine Officer.

A footbath containing hypochlorite, Betadine® or another approved disinfectant should be maintained at the entrance of the quarantine area proper. The bath should be routinely replenished for adequate disinfection and a record of bath maintenance maintained.[11] A sign stating "Footwear must be Immersed in Footbath on Exit/Entry from Quarantine Area" should be appropriately displayed.

All wastewater disposals should meet any state and local government requirements, be by an approved method (Chapter 4.4.2), and should not flow directly into natural waterways.

[11] Once iodophor solutions have lost their brown color to become colorless, they are no longer active and should be replaced (DAFF, 2006).

All filter material should be disinfected by autoclaving on another method approved by the supervising Quarantine Officer prior to removal from the Quarantine Facility and then disposed of by incineration or deep burial.

Staff and visitors who have had contact with water or aquatic animals should wash their hands and forearms with soap and water prior to exiting the Quarantine Facility.

Staff and visitors should plan their daily activities such that when they leave the Quarantine Facility they do not visit any other aquatic animal facility that day.

Handling of aquatic animals

Upon arrival of a shipment of aquatic animals at the approved port of entry, and following verification of the accuracy of details of the shipment and its preliminary inspection and clearance by customs officers, the shipment should be resealed by the supervising Quarantine Officer with an approved tamperproof seal (e.g. Tyden seal, lead seal or padlock) and then transferred to the custody of the operator, who should guarantee the secure transport of the aquatic animals, under quarantine conditions, to the Quarantine Facility.

Upon their arrival at the Quarantine Facility, the integrity of the seal should be verified by the supervising Quarantine Officer, the seal removed and the animals transferred to new water. The overseas water should be subjected to an approved disinfection treatment (Chapter 4.4.2).

In the event that a shipment of imported aquatic animals is incorrectly represented in any manner, the shipment may be destroyed under supervision of the Quarantine Officer.

The progeny of aquatic animals that breed during the quarantine period may be removed to another tank or room in the facility but are subject to all quarantine conditions.

A standard Tank Record Sheet should be maintained for each tank (Chapter 4.8.2).

Periodically throughout the day, the operator should observe all aquatic animals for signs of illness and abnormal behavior.

All dead aquatic animals should be held for inspection by a Quarantine Officer. All animals found dead on arrival or that die during the quarantine period should be placed in a labeled plastic bag as soon as possible and kept under refrigeration or preserved as specified by the Quarantine Officer until diagnostic examination can be completed. Information on labels should identify the shipment, species, tank number, number of mortalities, date of death and name of collector.

Any equipment that has been in contact with dead aquatic animals should be disinfected before re-use.

Any unusual levels of mortality, changes in behavior or unusual signs of disease, parasites or pests should be immediately reported to the supervising Quarantine Officer.

The use of any drug or chemical to treat aquatic animals should have the prior approval of the CA and be recorded on tank record sheets.

The operator should ensure that no aquatic animals leave the quarantine area under any circumstances without the approval of the supervising Quarantine Officer (i.e. the granting of biosecurity clearance).

On approval by the CA F1 or subsequent generation aquatic animals may be released from the Quarantine Facility for limited trials in aquaculture facilities or for stocking in enclosed water bodies. The CA may specify the precise conditions, period and any further risk management measures under which the aquatic animals are to be maintained. Prior to removal from the Quarantine Facility, aquatic animals should be transferred into clean water..

All original stock and any F1 or subsequent generation aquatic animals not approved for release from quarantine should remain under quarantine conditions. When determined by the CA or at the request of the operator, the operation of the Quarantine Facility may be terminated under the direct supervision of the supervising Quarantine Officer. In which case, all remaining aquatic animals, including all original parent stock, should be humanely killed by a method approved by the supervising Quarantine Officer, tested for pathogens if required, appropriately sterilized (.e.g. heat sterilization through autoclave, etc.) and then disposed of by incineration or deep burial. The facility and all tanks and equipment should be thoroughly cleaned and disinfected using approved disinfectants, and all filters, clothing and other similar materials autoclaved or disinfected and then destroyed by incineration or deep burial. Upon written sanitary certification by the supervising Quarantine Officer, the premises may then be disposed of as seen fit by the operator, or may be used as the basis for a new application for an approved Quarantine Facility.

OCCURRENCE OF AN OUTBREAK OF A SERIOUS EXOTIC DISEASE

If a serious exotic disease is diagnosed, the operator should be immediately notified. In such cases, the supervising Quarantine Officer or other representative of the CA may direct the management of disease control. Disease control measures may include the extension of quarantine, treatment and/or the destruction of stock.

Measures to be taken are likely to include:

- treatment and/or destruction of stock from infected tanks or of all aquatic animals present in the facility at the time of the outbreak, and their sanitary treatment, removal and incineration;
- decontamination of the interior of the facility, all tanks and equipment, and all waters present in the facility at the time of the outbreak; and
- approval of the CA prior to the reuse of the facility.

RECORD KEEPING REQUIREMENTS

Summary records

A complete history of the stock of aquatic animals being contained in the Quarantine Facility should be maintained. The operator should, for auditing purposes, maintain all documentation (shipping bills, health certificates, biosecurity clearance, etc.) and records for a minimum period of 36 months after closure of the Quarantine Facility, during which time they will, upon request, be readily made available to a Quarantine Officer. The following summary information concerning the quarantined stock(s) should be recorded:

- overseas supplier, country of origin and waybill;
- date of arrival of parent stock;
- date(s) of release of F1 or subsequent generation from quarantine;
- total number of animals in original shipment(s) and total mortalities in each shipment upon arrival;
- original number of animals stocked in each tank;
- details of any clinical signs of disease and number of affected individuals, by tank;
- details of any mortalities, by tank;
- details of any health certificates;
- details of any diagnostic tests and examinations;
- details of any F1 progeny produced (date and number) and their corresponding transfer tank number;
- for parent stock, and for any F1 or subsequent generation aquatic animals that for any reason have not been approved for release from quarantine upon termination of the quarantine license: number and size of aquatic animals destroyed, date and method of destruction and disposal and signature of the supervising Quarantine Office; and
- for F1 or subsequent generation aquatic animals, if approved for limited release from quarantine: number and size of aquatic animals released, date of release, destination, summary of any risk management measures or restrictions to be employed and signature of the supervising Quarantine Officer.

Tank record sheets

A corresponding approved Tank Record Sheet should be maintained for each holding tank and must be kept up to date at all times. Tank Record Sheets should be retained for a minimum of 36 months following release from quarantine of the portion of the shipment held in the specific tank, or their destruction. This sheet should display the following information:

- tank number;
- number of aquatic animals in tank;
- exporter identification details, including country of export;
- importer’s name;
- date of arrival;
- shipment or airway bill number;
- number of aquatic animals dead on arrival;
- details of any observed disease conditions and number of sick aquatic animals;
- daily record of number of aquatic animal deaths in tank;
- details of any prophylactic or therapeutic treatments given;
- disposal details;
- disinfection details; and

- details of any F1 progeny produced (date and number) and their corresponding transfer tank number.

Operations and entry logbooks

Details of wastewater treatment (including chlorination records, if applicable),; filter cleaning, replacement or disposal; internal audit; and general maintenance should be recorded in an operations logbook.

A separate entry logbook should be used to record details of the entry and exit of authorized personnel into the Quarantine Facility.

AUDITING

The operator should undertake systematic periodic internal audits at least on a quarterly basis, to ensure that the standards for the operation of the Quarantine Facility as in the relevant legislation are maintained and to identify and correct any deficiencies. The operator should record in the logbook, any variations from the prescribed criteria encountered and the corrective measures taken.

Periodic external audits of the Quarantine Facility should be conducted by the supervising Quarantine Officer or other approved personnel to verify the security and proper functioning of the facility.

SECURITY

Control and security of the Quarantine Facility is of the utmost importance and is the responsibility of the operator. The Quarantine Facility and its perimeter fencing should be securely locked when the facility is not in active use or when unattended. Increased after working hour's security should be considered to prevent unauthorized entry and theft, particularly where valuable broodstock or foodfish are being held.

Procedures should be adopted to ensure that access to the premises is limited to authorized persons only. Signs should be prominently displayed on all sides of the external perimeter fencing and on all entrances to the facility to show that it is a Quarantine Facility and that unauthorized entry is prohibited.

The entry of staff into the Quarantine Facility should be restricted to the minimum required to perform necessary maintenance and observation of the quarantined animals. A list of authorized staff should be provided to the supervising Quarantine Officer by the operator. Except in an emergency situation, no other persons should enter the Quarantine Facility unless written prior approval has been granted from the supervising Quarantine Officer.

A logbook of all entry and exit into and out of the Quarantine Facility should be maintained. All personnel entering the facility should be required to enter the following information:

- name of authorized person;
- date of entry/exit;
- time of entry;
- reason for entry;
- time of exit;
- signature at exit; and
- notation of any irregularities.

Signature at exit indicates that the exiting staff has confirmed that the Quarantine Facility was in proper order at the time of his/her exit and that the premises have been left in a secure manner. The operator should ensure that all staff conform to these requirements and should verify the accuracy of record keeping on a weekly basis. The logbook should be made available for examination by the supervising Quarantine Officer upon request.

CONTINGENCY PLANS

The operator should develop a contingency plan addressing actions to be taken in the event of a vehicle breakdown during transport of aquatic animals from customs arrival to the Quarantine Facility, and due to on-site emergencies that may arise, such as fire, flood, electrical failure or breakdown of essential equipment (aerators pumps, etc.). In the case of emergency, the supervising Quarantine Officer should be notified as soon as possible.

Annex 4

Report of the Genetic Risk Assessment

Annex 5

Report of the Ecologic/Environmental Risk Assessment

Annex 6

Report of the Pathogen Risk Analysis

Genetic Risk Assessment

Introduction of *Litopenaeus vannamei*

to the Kingdom of Saudi Arabia

for Aquaculture Development

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Executive summary

In summary, the risk of any direct genetic impact on native populations of L. vannamei, *or on populations of other species in the same genus, is extremely low.*

The genetic risk assessment follows the structure and content guidelines recommended by the ICES Working Group on Introduction and Transfers of Marine Organisms as well as FAO documents that deal with genetic risks associated with marine introductions.

The risks assessed include both direct and indirect genetic risks to wild organisms in the Red Sea, as well as adjacent areas that might exchange genetic material with the Red Sea (Persian Gulf, Arabian Sea and western Indian Ocean).

Farmed *Litopenaeus vannamei* has escaped and established local populations on both coasts of the Americas, and possibly also in the Middle East and Asia. Escaped *L. vannamei* have had no reported genetic impact of any sort on natural *L vannamei* populations anywhere in the world.

There are no wild *L. vannamei* populations in the Red Sea or anywhere within dispersal range. There is therefore very low risk of direct genetic impact on natural conspecific populations.

Non-native (feral populations) of *L. vannamei* may already exist in the Persian Gulf and oceanic regions within dispersal range of the Red Sea. By definition, non-native species have no conservation value and are not relevant to the genetic risk assessment (2012 Report of the ICES Working Group on Introductions and Transfers of Marine Organisms).

Experimental interspecific hybridization within the genus *Penaeus* (sensu lato) has had little success, even with species that appear morphologically compatible (now usually grouped in the genus *Litopenaeus*). There are no species in this group in or near the Red Sea. The risk of genetic impact on congeneric species is therefore very low.

Shrimp species in the Red Sea and areas within dispersal range have a gonadal structure that renders natural hybridization with open-thelycum species in the genus *Litopenaeus* virtually impossible. The risk of direct genetic impact through hybridization or introgression with other shrimp species is therefore very low.

Possible indirect impacts might stem from any evolutionary increase in the invasiveness of *L. vannamei;* by competing with other shrimps, the latter populations may decline, losing adaptive genetic variability or by stimulation of the emergence of recombinant pathogens in feral *L. vannamei* (which would be a novel host for RNA viruses in the Red

Sea). The risk of high-impact indirect genetic effects are non-calculable, but expected to be low.

Mitigation of indirect genetic effects will involve (1) introduction of breeders which will produce highly inbred feral populations and (2) monitoring, so that any unexpected evolutionary change can be recognized early and its genetic basis understood in a timely way.

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Assumptions and terms of reference

This genetic risk assessment follows the structure and content guidelines recommended by the ICES Working Group on Introductions and Transfers of Marine Organisms (ICES 2012). The issues addressed here also include, but are not entirely limited to, those in two FAO technical documents that discuss genetic risks associated with introductions of aquatic organisms (Hallerman 2008; Arthur *et al.* 2009).

The risks assessed include both direct and indirect genetic risks to wild organisms in the Red Sea, as well as in adjacent areas that might be colonized by, or exchange genetic material with, feral *L. vannamei*. These neighboring oceanic regions are considered to consist of the Persian Gulf, Arabian Sea and western Indian Ocean.

The risk assessment assumes that the source population is known, that escapes from the introduced, land-based culture system will take place, and that some part of the gene pool of the introduced population will become a self-sustaining feral population.

Taxonomic note
The genus now generally called *Litopenaeus* includes species which were formerly included in the genus *Penaeus*. The designation *Litopenaeus vannamei* (*L. vannamei*) and *Penaeus vannamei* (*P. vannamei*) refer, in this document, to the same taxonomic entity.

Review of the genetic impact of previous *L. vannamei* introductions

Hazard identification: genetic impact of feral *L. vannamei*

"A harm is defined as gene pool perturbation resulting in negative impacts to a species, a hazard is an agent or process that has the potential to produce harm." (Hallerman 2008, Arthur *et al.* 2009).

The *hazardous agent* in this case is a new, feral stock of *L. vannamei* that is presumed to become established in the Red Sea.

The *direct genetic harm* of most concern is movement of genes from the feral stock into wild populations of the same species, or into other species with which *L. vannamei* may hybridize or introgress.

Hybridization or introgression, if it should occur, can create potentially harmful changes in the genetic adaptation of local populations of the same or related species. There are a number of mechanisms (genetic swamping leading to reduced genetic variance and loss of adaptability, outbreeding depression, etc.) by which this loss of local adaptation might occur.

The possibility of *indirect genetic harm* originating as genetic phenomena in feral *L. vannamei* but manifesting itself ecologically or epidemiologically may also exist. This possibility is also assessed in this report.

Natural distribution of *L. vannamei* in the wild

Litopenaeus vannamei is found abundantly in coastal waters of the eastern Pacific Ocean from the Mexican state of Sonora as far south as northern Peru. The native range and the history of the worldwide translocation of *L. vannamei* have been described in detail (Briggs *et al.* 2004). No *Litopenaeus* species are native to the eastern Mediterranean Sea, Red Sea, Arabian Sea, Persian Gulf or northwest Indian Ocean.

Known *L. vannamei* introductions and farm escapes

Populations that could exchange genetic material with the Red Sea

There have been at least two introductions of specific pathogen free (SPF) or specific pathogen resistant (SPR) *L. vannamei* from Hawaii into Iran (Matinfar 2010, Matinfar *et al.* 2010). It is not clear from the cited publications whether or not a feral population has been established in the Persian Gulf. *Litopenaeus vannamei* is not native to this area, and there is no reported evidence of any direct or indirect genetic impact on local shrimp species.

Litopenaeus vannamei has escaped from farms in Thailand and established a population that *may be* self sustaining in the Bangpakong estuary (no mature individuals were found as of 2009) in the Gulf of Thailand (Senanan *et al.* 2007, 2009). *Litopenaeus vannamei* is not native to this area, and there is no reported evidence of any direct or indirect genetic impact on local shrimp species.

Litopenaeus vannamei has been introduced to India (Briggs *et al*. 2004). To be consistent with the precautionary principle underlying the present risk assessment, we shall assume that feral populations are established in Indian coastal waters, even if they have not yet been reported.

Populations unlikely to exchange genetic material with the Red Sea

Litopenaeus vannamei aquaculture in mainland China, Taiwan POC, the Philippines, Indonesia, Viet Nam and Malaysia (Briggs *et al.* 2004) may have generated feral populations, but any such populations are highly unlikely to exchange genetic material with feral populations in eastern Mediterranean Sea, Red Sea, Arabian Sea, Persian Gulf or northwest Indian Ocean. It appears that as of 2004 no feral populations were definitely known to exist in these waters or anywhere else in the Eastern Hemisphere; if this is still the case, it is interesting to hypothesize whether there is a reason other than lack of sampling.

Feral populations do exist elsewhere, however:

- A population of *L. vannamei* has been found in the southern Gulf of Mexico, presumably escaped from coastal shrimp farms (Wakida-Kusunoki *et al.* 2011). *Litopenaeus vannamei* is not native to this area, and there is no reported evidence of any direct or indirect genetic impact on local shrimp species.

- *Litopenaeus vannamei* has been reported in coastal regions of northeastern Brazil (Loebmann *et al.* 2010). *Litopenaeus vannamei* is not native to this area, and there is no reported evidence of any direct or indirect genetic impact on local shrimp species.

Assessment of genetic risks and impacts from *L. vannamei* introductions

As defined by Hallerman (2008), Risk is *the product of the probability of exposure, P(E), and the conditional probability of harm given that exposure has occurred, P(H|E). That is,* $R = P(E) \times P(H|E)$.

In the case of *L. vannamei* importation into The Kingdom Saudi Arabia, the TOR specifies that a feral population will undoubtedly become established in the adjacent Red Sea.

Although the form of the exposure probability function $P(E)$ is unknown in general, it must equal zero when there is no susceptible population present even if the presence of *L. vannamei* has a probability of 1.0. Under the terms of reference for this Risk Assessment it is assumed that the introduced *L. vannamei* will escape into the wild, so the probability of exposure will, in fact, equal unity. This assumption is conservative, in the sense of being precautionary, and it also reflects the real likelihood that some introduced animals will escape into the wild. The conclusion that $P(E) = 0$ even if escape is inevitable because there is no susceptible population present does not in any way reduce the importance of minimizing the rate of escape. The mathematical conclusion applies only to genetic risks to natural populations of *L. vannamei* in the geographic area under consideration, of which there are none.

For species other than *L. vannamei*, that are present and might suffer indirect genetic harms, $P(E)$ will not necessarily equal zero. The form of the $P(E)$ function is highly dependent upon risk management measures adopted, that is, upon the effectiveness of confinement measures incorporated into the production system(s) utilized. These are discussed elsewhere in the Risk Assessment.

Different sorts of confinement measures may be implemented, including physical confinement, reproductive confinement, and appropriate operations management practices (Hallerman 2008). Physical confinement measures might include passage of pond effluent through one or more filters with openings small enough to retain even the smallest life-stages of shrimp. Effective reproductive confinement measures for shrimps have not been established and demonstrated. Operations management measures include regular inspections and maintenance of physical confinement infrastructure, safeguard of farm sites from animal or human intrusion, and passage of aquaculture effluents through effective filters. Measures for minimizing $P(E)$ have been well elaborated for genetically modified aquatic organisms, and notable discussions may be found in ABRAC (1995) and Kapuscinski *et al.* (2007).

The form of the conditional harm probability function $P(H|E)$ is unknown, but will approach zero even when a susceptible population is present, if that population is defined as having negligible or no conservation value (that is, harm, H, is zero). A recent ICES report of the Working Group on Introductions and Transfers of Marine Organisms (ICES 2012, p. 258), emphasizes that the harm concern is the preservation of the genetic integrity of *species that are native to the area*. Specifically, "genetic impacts which can affect the capacity of native species to maintain and transfer to successive generations their current identity and diversity". Thus, when the susceptible population is another feral population of *L. vannamei*, there is no harm, and the value of $P(H|E)$ will be zero.

Direct genetic risks and impacts

Potential for conspecific hybridization in L. vannamei

Natural populations of *Litopenaeus vannamei* appear to have a lot of genetic variation. Significant biogeographic and population genetic structure has been found within the natural range of the species, as measured by selectively neutral genetic marker allele frequencies. This differentiation is thought to be related to water mass and differences in spawning times (Benzie 2009).

There is also significant quantitative genetic differences in Taura syndrome virus (TSV) resistance in founder stocks collected from various locations that extend from Mexico to Ecuador (Argue *et al.* 2002 and unpublished personal observations).

Several commercial SPF/SPR stocks could be candidates for the proposed importation. For the most part, the details of their origin and current genetic composition are not publicly available (Benzie 2009), but it is unlikely that they incorporated a large fraction of the natural genetic variation in the species.

The origins of some commercial stocks are known, however, notably the SPF/SPR stocks of the Oceanic Institute in Hawaii. The commercial SPF/SPR stocks are known not to be entirely independent (*i.e.* they share some founder individuals), but are likely to have diverged considerably from their founders and from each other through random genetic drift and intense selection over 20 or more generations.

It appears from the known history of commercial *L. vannamei* production that conspecific hybridization among the existing SPF/SPR strains is unlikely to cause outbreeding depression. Conspecific hybridization may help remove some inbreeding depression, at least temporarily (Moss *et al.* 2007).

The most important point regarding genetic risk of hybridization with other *L. vannamei* populations in the Red Sea and adjacent waters is that all such populations within dispersal range are themselves feral. By definition, they are not native species and have no or very little conservation value (ICES 2012, p. 258). Furthermore, they are believed to have originated from the same, genetically depleted SPF/SPR broodstocks as the proposed importation, which would minimize genetic perturbations resulting from any hybridization events.

***Potential for hybridization within the genus* Litopenaeus**

There are five species in the genus *Litopenaeus*:

- *L. occidentalis*, native to western Central and South America;
- *L. schmitti*, native to the western Atlantic from the Carolinas to Brazil;
- *L. setiferus*, native to the Carolinas and Gulf of Mexico;
- *L. stylirostris*, western Mexico and Central America; and
- *L. vannamei*, native to the western Pacific from Mexico to Peru.

Misamore and Browdy (1997) report that "Based on the low levels of behavioral interaction during interspecific crosses, the lack of natural interspecific matings, and absence of fertilization in interspecific crosses for both artificial inseminations and *in vitro* fertilizations, the potential for hybridization between *P. setiferus* and *P. vannamei* appears to be negligible."

Benzie (2009) noted that "Crosses between shrimp species have been experimental, largely unsuccessful, and have not resulted in the development of useful hybrids..... This approach has been largely abandoned and is not considered further [cited review paper]."

In summary, we can conclude that hybridization among species within the genus *Litopenaeus* is unlikely to occur naturally even where the species come in contact, which they do not in the Red Sea and adjacent waters.

Potential for hybridization with other genera

Shrimp species in the area of concern for the proposed introduction (Red Sea – Indo–West Pacific) have a closed-thelycum gonadal structure that renders natural hybridization with the open-thelycum species in the genus *Penaeus* (sensu lato) virtually impossible (Asakura 2009).

Risk from genetically modified organisms (GMOs)and chromosome manipulation

The proposed introduction does not include genetic material affected by recombinant DNA technology or by chromosome set manipulation (forced polyploidy, gynogenesis).

SUMMARY TABLE: Total direct genetic risk assessment

This table summarizes the genetic risk assessment, as risk is defined in (ICES 2012, p. 258), namely, *genetic impacts which can affect the capacity of native species to maintain and transfer to successive generations their current identity and diversity*. Risk is defined more precisely by (Hallerman 2008), *the product of the probability of exposure, P(E), and the conditional probability of harm given that exposure has occurred, P(H|E).* Risk by Hallerman's definition is evaluated in column 5.

Col. 1:	Type of potential genetic impact, as defined above.	**Col. 2:**	Type of genetic harm to native gene pools.
Col. 3:	Severity of genetic harm if populations are impacted	**Col. 4:**	Biological and other factors used in assessment
Col. 5:	Evaluation of risk equation, *R = P(E) x P(H|E)*.	**Col. 6 & 7:**	Confidence in the risk equation evaluation.

	1	**2**	**3**	**4**	**5**	**6**	**7**
	Genetic hazard (process)	**Genetic harm category**	**Potential harm level if impact occurs**	**Major factors in harm probability assessment**	**Genetic risk/impact**	**Confidence in risk assessment**	**Basis of confidence in assessment**
1	Hybridization with natural *L. vannamei* populations	Disruption of natural gene pools	Very high	No native conspecifics within dispersal range	Very low $P(E)\approx 0$	Very certain	Cited peer-reviewed research
2	Hybridization/ introgression with other *Litopenaeus* species	Disruption of natural gene pools	Very high	No species in range. Low hybridization success	Very low $P(E)\approx 0$, $P(H\|E)\approx 0$	Very certain	Cited peer-reviewed research
3	Hybridization/ introgression outside genus *Litopenaeus*	Disruption of natural gene pools	Very high	Incompatible mating morphology and behavior	Very low $P(H\|E)\approx 0$	Very certain	Cited peer-reviewed research
4	Hybridization with other feral *L. vannamei* populations	Disruption of other feral gene pools	None	Impacted populations have no conservation value	None $P(H\|E)=0$	Very certain	Follows from TOR

Indirect genetic risks and impacts

An indirect genetic effect is one that is manifested through ecological processes including, presumably, both ordinary ecology and host-pathogen dynamics. Direct exchange of genetic material with the impacted species is not involved.

As defined by Hallerman (2008, p. 53), indirect (ecological) harm results because, "Through competition or predation, by reducing the abundance of affected populations, the cultured stocks may reduce their effective population size, causing loss of genetic variability and ability to adapt in face of changing selective pressure, and also increase the likelihood of subsequent inbreeding and extinction".

Arthur *et al.* (2009) define indirect genetic risk in a related way: "genetic improvement that increases fitness of a highly invasive species for introduction into a vulnerable community raises a high level of concern".

By these definitions, the indirect consequence of a genetic increase in *L. vannamei* fitness or invasiveness is primarily manifested as local extinction of native species. This could occur through competition, predation and interference with reproduction. Extinction could happen relatively quickly as a purely ecological effect, or slowly if the population size of an affected species is reduced to a level where inbreeding and random genetic drift drive it into a negative fitness loop (an "extinction vortex").

Given the evidence above, that *L. vannamei* populations have not become established in Asia or the Middle East, the likelihood of competition-, predation-, or reproductive interference-related processes becoming important are remote.

It is also conceivable that genetic recombination among pathogens in a novel host like feral *L. vannamei* could cause the emergence of new pathogens in the Red Sea, e.g. (Dennehy *et al.* 2010). This is mentioned as an indirect effect that would become manifest as a disease problem.

Evolutionary potential of feral L. vannamei to become invasive

SPF/SPR broodstock imported from the USA are likely to have been deliberately inbred and/or to consist of only a few sets of siblings. This is the standard practice of commercial broodstock suppliers as a way of protecting their intellectual property. The scope for evolution in a population of escapees from gene pools that have been artificially restricted in this way will be reduced because of reduced genetic variation.

Nevertheless, despite considerable (suspected) diversity loss under cultivation, well-managed *L. vannamei* populations continue to give sustained response to selection for growth rate and TSV resistance (reviewed by Benzie (2009); personal observation).

Behavior also is changing. It appears that in some broodstocks, the response to selection is actually accelerating, presumably owing to the increasing influence of initially rare alleles and the breakdown of genetic correlations and other epistatic interactions during selection (unpublished personal observations).

Even though introduced *L. vannamei* will probably have gone through one or more severe genetic bottlenecks, some of them deliberate, this need not necessarily put an end to further evolution in a feral population. It is known that genetic mechanisms exist to *increase* the additive genetic variance in populations that have a small number of founders (Barton and Turelli 2004, Sobel *et al.* 2009), sometimes (in theory) to a startling extent. Individually, such genetic events may be rare and sample-dependent, but a lot of second- and third-order interactions are possible even in severely depleted gene pools.

Furthermore, the proposed cultured and feral population in the Red Sea may coexist in a "source/sink" relationship (Pulliam 1988, Runge *et al.* 2006), in which escapes occur over a prolonged period, not just in a single episode. If this is the case, a feral population might persist long enough for habitat-specific evolution to occur even if the population is – initially – unable to sustain itself.

In summary, the combination of different kinds of genetic events renders the likelihood of feral *L. vannamei* evolving to become invasive literally incalculable. "Incalculable" means we cannot calculate whether the probability is small or large by evaluating any plausible probability distribution. There is no numerical basis for estimating – or even guessing – the probability of invasiveness from, say, the power-law or other "thick tailed" distribution which has a non-negligible frequency of extreme events. It is even less reasonable to use the Gaussian distribution, a.k.a., the bell curve, in which high-impact events are vanishingly rare. (The Gaussian distribution underlies almost all quantitative genetic theory and practice.)

Any imported stock of *L. vannamei* is likely to have non-negligible genetic variation both among and between commercial sources. Even small feral populations can be expected to continue to evolve and adapt, either as independent entities or as components of a source/sink metapopulation. Consequently, both the likelihood and the impact of an indirect, deleterious genetic impact may increase as time passes, but we do not know what that probability is.

We cannot say that likelihood of genetically-driven increase of invasiveness is “high”, “medium”, “low” or “near-zero”. The most we can confidently say about *L. vannamei* evolving into an invasive species in the future is that *it hasn't happened yet*.

Incalculable risks with potentially high impacts

Extreme risks are events that have serious consequences, but non-calculable probabilities. Examples in ordinary life would be terrorist attacks, major earthquakes, and stock market crashes. Examples from population translocation history are massive occupation of new habitat (rabbits in Australia), and generation of new recombinant viruses from human activity (SARS, influenza). By definition, the entities, populations or processes affected by an extreme risk event cannot be specified with any confidence before the event has actually happened.

A risk which is not recognized as such until after it has happened has come to be called a "black swan event" (Taleb 2010). (In recent military parlance, black swans are called "unknown unknowns"). In risk analysis theory, a black swan is a harmful event that comes as a complete surprise, but after it happens can be rationalized and explained with the benefit of hindsight. The clarity of 20-20 hindsight usually places blame on the source of the problem, which in this instance would be a decision to import *L. vannamei*.

Black swan events thus constitute (indirect) genetic risks that fall within the TOR for this assessment (Nuñez and Logares 2012) even though, by definition, their nature cannot be specified *a priori*. They are potentially very consequential, but rare and hard-to-predict events that are beyond the realm of normal expectation on the basis of what is known about the biology of the situation.

The nature of an indirect genetic impact (extreme, or black swan event) cannot be identified *a priori*. As pointed out by Hallerman (2008), "Exact probabilities of risk are difficult or impossible to determine for all types of possible harm. Indeed, it is unlikely that all possible harms would be known a priori, particularly with respect to any indirect effects". Also, "...it will be necessary to update the risk analysis as knowledge accumulates using an adaptive management approach".

Genetic monitoring of the imported and feral *L. vannamei* populations will be needed so that any genetic changes can be recognized and their consequences understood and mitigated. This point is discussed further in the section on mitigation.

Monitoring and mitigating potential genetic impacts

Minimizing impact before introduction takes place

Given the TOR assumption that a viable, feral *L. vannamei* population is inevitable, the best available risk management option *before introduction takes plac*e is to choose a source stock that is least likely to be well adapted to the Red Sea environment, or to evolve so as to become adapted. Commercial SPF/SPR stocks from the USA are the safest candidates in this regard because they: (a) have been released from natural selection pressures for many generations, and (b) have low genetic diversity and high inbreeding potential.

Genetic measures can be taken to reduce the likelihood that animals introduced as aquacultural breeders can succeed in establishing a feral population even if containment fails. The simplest of these measures is to ensure that F2 and subsequent generations will be highly inbred (Doyle *et al.* 2006). These measures will be highly situation-specific, so the proponents of the introduction should design a procedure for accomplishing the inbreeding objective in the context of their commercial situation, then subject it to expert review.

Finally, from a genetic risk management perspective it is not a good idea to set up a *L. vannamei* selection program for tolerance to the Red Sea environment or pathogens.

Mitigating and monitoring impact after introduction

Direct genetic impacts

No direct genetic impacts are assessed to have significant hazard probabilities, so mitigation should not be needed for hybridization or introgression with the genus *Penaeus* or related genera.

Indirect genetic impacts

Indirect genetic impacts such as an evolutionary increase in invasiveness are possible and potentially very harmful, but their probabilities are non-calculable. Minimization of the likelihood of indirect genetic or ecological impacts could be achieved by adoption of a set of effective confinement measures, including physical confinement and well-chosen operations management procedures. Mitigation of indirect genetic "unknown unknowns" will involve environmental monitoring so that unexpected ecological or evolutionary changes can be recognized early and their ecological or genetic basis understood quickly. Then, corrective measures can be designed and implemented, e.g., targeted collection of shrimp from a feral population.

If done properly, this regime will involve: 1. collecting and examining the genetic properties of population samples and searching the resulting data for unexpected patterns, 2. environmental sampling to search for feral *L. vannamei*, and 3. revising and as appropriate redesigning confinement practices.

Researchers in ecological genomics will soon be sequencing *everything*. It is likely that within a few years, the whole genome of animals in a population sample, plus their attendant and incorporated viral genomes, can be resequenced quickly and relatively cheaply. This does not mean that a molecular genetics laboratory needs to be set up. It is possible to store samples safely and cheaply until they need to be analysed – on a contingency basis – at some indefinite time in the future.

The cost and technological issues involved in an ecological or genetic monitoring program are outside the TOR for this assessment but one suggestion will be made: it might be desirable to set up a monitoring program in collaboration with King Abdullah University of Science and Technology (KAUST). If a feral population of *L. vannamei* becomes an object of long-term study from an ecological, genetic, or genomics point of view, it could well fall within the mandate of KAUST. For instance, it might be possible to pinpoint genes or gene combinations involved in adaptation to the high temperatures and salinities of the Red Sea environment.

The details of a monitoring program should be left to the proponents of the introduction because the monitoring will be extremely situation-specific. The proponents should be required to design a monitoring program (including genetic monitoring) and subject it to expert review.

CONCLUSIONS: total genetic risk assessment

Genetic impact of previous *L. vannamei* introductions

Litopenaeus vannamei has undoubtedly escaped from farms and become established in the wild in tropical coastal waters on both coasts of the Americas. Feral populations in Asia and the Middle East that could exchange genetic material with the Red Sea *may* exist, although this is not certain. There is no indication of hybridization, introgression or genetic swamping or native populations associated with these feral populations. No genetic or ecological impact of any sort on natural populations has been reported.

Direct genetic risks and impacts

1) Genetic exchange among native, wild populations of *L. vannamei* is certain to occur if they come into contact. However, there are no wild *L. vannamei* in the area of concern or anywhere within conceivable dispersal range. Thus, the value of the exposure probability function *P(E)* in the risk equation equals zero for genetic impact on other *Penaeus* (sensu lato) populations. The whole risk equation evaluates at zero impact on natural conspecifics. **(See row 1 in the Summary Table).**

2) Genetic exchange within the genus *Litopenaeus* is highly unlikely for two reasons. There are no congenerics within the plausible dispersal range. Thus, the value of the exposure probability function *P(E)* in the risk equation equals zero for genetic impact on other *Litopenaeus* species. Furthermore, inter-species hybridization has had a low success rate in laboratory studies, i.e., the value of the conditional harm probability function *P(H|E*) is low. The whole risk equation, therefore, evaluates at zero impact on congenerics. **(See row 2 in the Summary Table).**

3) Wide hybridization with other genera is judged to be nearly impossible owing to behavioral and anatomical incompatibility. Here, too, the value of the conditional harm probability function *P(H|E*) is low, and the risk equation evaluates at zero impact for wide crosses. **(See row 3 in the Summary Table).**

4) Genetic exchanges among other feral populations of *L. vannamei* is possible. However, these are not natural populations and are of little or no genetic conservation value within the terms of reference of the assessment (ICES 2012, p. 258). Thus, the value of the conditional harm probability function *P(H|E)* in the risk equation equals zero for genetic impact, by definition. The whole risk equation, therefore, evaluates at zero impact on conspecifics. **(See row 4 in the Summary Table).**

Indirect genetic risks and impacts

We cannot say that likelihood of genetically-driven increase of invasiveness is “high”, “medium”, “low” or “near-zero”. The probabilities are non-calculable. It is noteworthy, however, that they have never occurred elsewhere in Asia or the Americas.

The same analysis applies to other high-impact indirect genetic effects such as a stimulation of the emergence of recombinant pathogens in feral *L. vannamei*, which would represent a novel host in the Red Sea.

Mitigation of indirect genetic effects will involve monitoring so that unexpected ecological or evolutionary changes can be recognized early and their genetic basis understood in a timely way. Should feral populations be detected, targeted harvest may remove or reduce such populations to the point that ecological or disease risks are minimized.

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Professional qualifications of the author

Dr. Roger W. Doyle is currently president of Genetic Computation Ltd., a consulting company in aquaculture and conservation genetics. He is a retired professor of Biology and founding director of the Marine Gene Probe Laboratory at Dalhousie University, in Canada.

Dr. Doyle is former president of the International Association for Aquaculture Genetics and former Coordinator of the Aquaculture Genetics Network in Asia (IDRC).

He received his MSc and PhD in biology from Yale University, and his MSc in oceanography and Hons. B.Sc. in biology and chemistry from Dalhousie University.

Dr Doyle has been awarded research and study fellowships from NATO (Denmark), the FAO Cooperative Program (Rome), the International Atomic Energy Agency (Vienna) and the International Development Research Centre (Singapore).

He is the author of approximately 100 publications in ecology, aquaculture, theoretical and applied genetics, and has received numerous editorial appointments, invited keynote lectures, consultancies and visiting professorships in aquaculture, fisheries, fisheries genetics and conservation genetics. His awards include the Elvira E.O. Tan Research Award (South East Asian Fisheries Development Center) and the APICS research gold medal.

Dr Doyle has extensive research, development and commercial experience in tilapia, salmon, carp and shrimp aquaculture genetics in the Americas, the Middle East, Africa and Asia. His ongoing research programs focus on the effect of long-term domestication on commercially important fitness traits in aquaculture, and on the maintenance of founder genome diversity in aquacultural broodstocks.

Annex 5(A)

Independent Review of Dr. Roger W. Doyle's

Genetic Risk Assessment

Introduction of *Litopenaeus vannamei* to the Kingdom of Saudi Arabia

for Aquaculture Development

by

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Department of Fish and Wildlife Conservation
Virginia Polytechnic Institute and State University
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The Saudi Aquaculture Society (SAS) is considering the introduction of Pacific white shrimp, *Litopenaeus vannamei*, into the Kingdom as the basis for shrimp production there. It is entirely fitting that a genetic risk assessment be conducted before such an action. Internationally noted aquaculture geneticist Roger Doyle was contracted to draft such a risk assessment, and has produced a creditable document. It draws well upon the existing literatures on risk assessment, shrimp ecology, and shrimp production. The analysis is straightforward, clear, and defensible. I have typed a number of suggested editorial revisions directly onto the document in order to sharpen the presentation, especially for non-population geneticists or risk assessors. While generally defensible and complete, the discussion would be strengthened by fuller treatment of possible ecological impacts – I have added brief passages to the text, and could add more if the SAS or others would value that. More importantly, the discussion does not adequately treat the topic of risk management and how adoption of suitable risk management measures would minimize probability of exposure and hence risk itself. I have added passages to the text, and I am willing to elaborate these if desired. Finally, the topic of monitoring could be addressed in a more straightforward and complete fashion – monitoring, in addition to that for genetic changes, should also address possible establishment of feral *L. vannamei*, which if found, could be targeted for reduction. Overall, this is a credible risk assessment, which with minor revision, can serve as the basis for informed decision-making for the possible production of *L.vannamei* in the Kingdom of Saudi Arabia.

Annex 5(B)

Response to the Independent Review of Dr. Eric Hallerman

Prepared by Roger W. Doyle
December 2012

First of all, I would like to thank Dr. Hallerman for the thoughtful, thorough and useful suggestions for improving my genetic risk assessment manuscript. I have incorporated without alteration all the changes he made directly on the draft. These changes include his additional text and references concerning containment as a risk management measure.

The responses to his more general comments are detailed below:

1. Dr. Hallerman suggests that the consideration of risk management should be expanded, writing, "More importantly, the [draft] discussion does not adequately treat the topic of risk management and how adoption of suitable risk management measures would minimize probability of exposure and hence risk itself." I have not yet done this in the revised manuscript on the grounds that risk management/ containment is involved in all three areas of the assessment: ecology, disease and genetics.

   Since the TOR for the genetic risk assessment assumes that escape will inevitably occur, the containment issue is almost, but not entirely, moot for genetic effects. It is moot for direct genetic effects because there are no natural *P. vannamei* to be impacted. The rate of escape could conceivably have indirect genetic effects, e.g. on possible recombination of viral pathogens in *P. vannamei* as a novel host, so management procedures that minimize exposure are far from being irrelevant for genetic impact. However, the greater direct concern must be in the areas of disease and ecology.

   I assume that the disease and ecology risk assessments cover the issue raised by Dr. Hallerman. If they do not, then I will gladly collaborate with the authors of those assessments to write a joint section on risk management and how adoption of suitable risk management measures would minimize probability of exposure and hence risk itself.

2. I added the following short section on risk mitigation that I simply hadn't thought of when writing the draft. It may be quite important and is mentioned in the revised Executive Summary as well:

   "Genetic measures can be taken to reduce the likelihood that animals introduced as aquacultural breeders can succeed in establishing a feral population even if containment fails. The simplest of these measures is to ensure that F2 and subsequent generations will be highly inbred (Doyle *et al.* 2006)."

3. Dr. Hallerman also writes, "Finally, the topic of monitoring could be addressed in a more straightforward and complete fashion – monitoring, in addition to that for genetic changes, should also address possible establishment of feral *P. vannamei*...."

   Part of my response to this is, again, that ecological monitoring properly belongs to the Ecological Risk Assessment since the TOR for genetics assumes that containment measures fail. If it is not in other sections of the total risk assessment then I will be glad to collaborate in writing it up.

4. The draft and revised manuscripts indicate that genetic sampling should be part of the overall monitoring program and that DNA samples should be stored so an adaptive response can be made to identifying and reacting to future genetic problems. I also suggest that a collaboration with KAUST should be considered for design and implementation of genetic monitoring.

   Dr. Hallerman wants more detail about this, but in the revised manuscript I now indicate that the details of a genetic monitoring program are extremely situation-specific (including the current state-of-the-art in molecular genetics) and therefore difficult to include in the Assessment. As a solution to this problem, in the revision I suggested that the proponents of the introduction should themselves be required to design the monitoring program (including genetic monitoring) and subject it to expert review. I made the same suggestion regarding the measures that should be taken to ensure that the feral populations are highly inbred from F2 onwards. The proponents should propose a procedure for accomplishing this in the context of their specific commercial situation, then subject it to expert review.

5. I also added the following section in light of Dr. Hallerman's comment on the exposure probability function. In the case of *P. vannamei* importation into Saudi Arabia, the TOR specifies that a feral population will undoubtedly become established in the adjacent Red Sea.

   "Although the form of the exposure probability function *P(E)* is unknown in general, it must equal zero when there is no susceptible population present even if the presence of *vannamei* has a probability of 1.0. Under the terms of reference for this Risk Assessment it is assumed that the introduced *P. vannamei* will escape into the wild, so the probability of exposure will, in fact, equal unity. This assumption is conservative, in the sense of being precautionary, and it also reflects the real likelihood that some introduced animals will escape into the wild. The conclusion that $P(E) = 0$ even if escape is inevitable because there is no susceptible population present does not in any way reduce the importance of minimizing the rate of escape. The mathematical conclusion applies only to genetic risks to natural populations of *P. vannamei* in the geographic area under consideration, of which there are none."

"For species other than *P. vannamei*, that are present and might suffer indirect genetic harms, *P(E)* will not necessarily equal zero. The form of the *P(E)* function is highly dependent upon risk management measures adopted, that is, upon the effectiveness of confinement measures incorporated into the production system(s) utilized. These are discussed elsewhere in the Risk Assessment...."

[If not, I'll be glad to help write them.]

Ecological and Environmental Risks Analysis for the Introduction of Whiteleg Shrimp, *Litopenaeus vannamei*, to Kingdom of Saudi Arabia

Prepared by

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A report prepared for

Saudi Aquaculture Society

December 2012

Executive Summary

Saudi Aquaculture Society (SAS) has been requested by the Ministry of Agriculture of the Kingdom of Saudi Arabia to support a consultancy for an ecological/environmental risk analysis to determine the potential impacts resulting from a proposed introduction of whiteleg shrimp (*Litopenaeus vannamei*) for aquaculture. This approach is a country initiative by the Saudi Aquaculture Society.

The report covers the results of the ecological analysis for the proposed introduction of *L. vannamei* to the Kingdom of Saudi Arabia (KSA). The ecological risk analysis focuses on the invasiveness and “pest potential” of the species to be translocated and considers the likelihood of its escape and/or release into the natural environment of the KSA and the nature and extent of any potential ecological impacts such escape or release may entail. To assist in assessing the ecological risks, a thorough literature search was carried out.

The ecological risk analysis is characterized by a high level of certainty, and the estimated risk potential is low based on information available, for example, on impacts of *L. vannamei* escapees into the wild that include follow-up studies from previous introductions of this species and its subsequent escape into the wild, as seen in Thailand.

Mitigation measures are not identified since the overall estimated risk potential is low; however, additional recommendations have been made to be aware of the meteorological conditions of the farm site and the climatic conditions of the region to reduce the likelihood of escapes from farms and hatcheries. In addition, establishment of a monitoring program for the presence of *L. vannamei* in the wild to allow detection of the geographical spread of escapees (should this occur) and to assess their impacts on wild species is also recommended.

The ecological risk analysis suggests, despite a lack of some specific literature on species currently farmed at the Shrimp Cultivation Projects in KSA, that the benefits of introduction outweigh any potential negative effects. However, it is emphasized that the results should not be taken as a sole basis for a decision by the Kingdom of Saudi Arabia and the Saudi Aquaculture Society to approve or not approve a request for the proposed species translocation. Such a decision may require additional consideration by the Kingdom of policy, legislation, etc. and should include extensive stakeholder consultation.

Acknowledgements

The consultants acknowledge the kind assistance of the following people who provided information and/or logistical support essential to the completion of this risk analysis: Dr Richard Arthur and Dr Aralar of SEAFDEC.

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1.0 INTRODUCTION

1.1. Purpose

Under contract from the Saudi Aquaculture Society (SAS), the consultants were engaged to undertake a risk analysis involving the proposed introduction of whiteleg shrimp, *Litopenaeus vannamei* to KSA. The report covers the results of the ecological analysis for the proposed introduction of the shrimp to the Kingdom of Saudi Arabia (KSA). The ecological risk analysis focuses on the invasiveness and "pest potential" of the species to be translocated and considers the likelihood of its escape and/or release into the natural marine environment of KSA and the nature and extent of any potential ecological impacts such escape or release may entail. This report is was commissioned as part of a larger proposal that includes other aspects such as pathogen and genetic risk analyses.

1.2. Terms of Reference (TOR)

The objective of this component of the consultancy is to undertake a risk analysis of ecological and environmental risk associated with a proposed introduction of specific pathogen free (SPF)whiteleg shrimp (*Litopenaeus vannamei* to the Kingdom of Saudi Arabia for aquaculture development.

The consultancy will entail the following:

1. As background to the study, conduct a detailed review and assessment of any reported ecological/environmental impacts associated with past introductions of *L. vannamei* on a global basis (note that risks due to pathogens should not be considered, while risks due to "fellow travellers" should be included, but will, to a large part, be addressed by the use of broodstock derived from SPF sources and held under high security quarantine).

2. Conduct a comprehensive analysis of the ecological/environmental risks associated with the proposed introduction of *L. vannamei* to Saudi Arabia. The risk analysis should consider the likelihood (probability) that introduced *L. vannamei* will escape from shrimp farms located along the Red Sea coast and will become established in the local marine ecosystems of Saudi Arabia.

3. The risk analysis will follow the general methods outlined in FAO Fisheries and Aquaculture Technical Paper No. 519 (in particular, the papers by Leong and Dudgeon, 2008; and Phillips and Subasinghe 1988) and in No. 519/1 (in particular, section 4.3: Overview of the ecological (pests and invasives) risk analysis process and section 4.5 Overview of the Environmental Risk Analysis process) and will conform as far as possible, with the guidelines given in the Code of Practice for the Introductions and Transfers of Marine Organisms (ICES, 2005) and the procedures outlined in Annex B: Risk Review of the ICES Code (as given in ICES 2012, Appendix 6, Annex 6 (Appendix B: Risk Review, pp. 356-262).

4. The risks to be assessed will include both direct and indirect ecological and environmental risks to the potential receiving environment (the Red Sea; flora, fauna and physical environment). Analysis of potential direct genetic impacts on native fauna should not be considered, as these will be addressed by a separately commissioned genetic risk analysis.

5. The consultants will deliver a draft version in Microsoft Word (electronic format) of the risk analysis to NPC by 1 December 2012, and will provide a final version in similar format addressing any comments or corrections resulting from independent review.

1.3. Commodity Description

Table 1 defines the precise nature of the commodity to be imported.

Table 1. Commodity description for the proposed introduction of whiteleg shrimp (*Litopenaeus vannamei*) to Kingdom of Saudi Arabia.

Species to be introduced: *Litopenaeus vannamei* (whiteleg shrimp)
Proposed date of importation: beginning of January 2013, for a period of 3 years
Life cycle stage to be imported: broodstock only
Importers: Participating members of Saudi Aquaculture Society, Jeddah, Saudi Arabia (list of Approved Importers)
Exporter: Approved SPF facilities (list of Approved Suppliers to be developed)
Source: High security SPF culture facilities (list of Approved Suppliers)
Proposed number of shipments: as required
Volume: as required
Proposed destination: participating shrimp farms along the Red Sea coast, KSA

1.4. International Framework/Context of the Risk Analysis

There have been major changes in the patterns of world trade in the past 20 years due primarily to the liberalization of international trade through the General Agreement on Tariffs and Trade (GATT) and establishment of the World Trade Organization (WTO) in 1995. With the adoption of the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), WTO member countries are now required to use the risk analysis process as a means to justify any restrictions on international trade based on risks to human, animal or plant health that exceed those measures allowed by international agreement (e.g., those given in the *Aquatic Animal Health Code,* see OIE, 2012) (WTO 1994, Rodgers 2004, Arthur *et al*. 2004). Risk analysis has thus become an internationally accepted standard method for deciding whether trade in a particular commodity (e.g., a live aquatic animal or its product) poses a significant risk to human, animal or plant health, and if so, what measures could be adopted to reduce that risk to an acceptable level.

Aquaculture issues are covered specifically in the SPS Agreement; the World Organisation for Animal Health (OIE, formerly the Office International des Epizooties) with its 178 member countries is recognized as the international organization responsible for the development and promotion of international animal health standards, guidelines and recommendations affecting trade in live terrestrial and aquatic animals and their products. Publications relevant to this risk analysis include the OIE's *Aquatic Animal Health Code* 2012 (OIE 2012), FAO Technical Papers No. 519 and 519/1 (Bondad-Reantaso *et al*. 2008, Arthur *et al.* 2009) and ICES (2005 2012).

Following from the above, in its recent correspondence SAS is contemplating importing whiteleg shrimp, *Litopenaeus vannamei,* for aquaculture development and has commissioned this risk analysis to evaluate the ecological and environmental risks associated with the introduction of this species.

2.0 METHODS

2.1 Project Team

The project team for this component of the study is comprised of three scientists based at Queensland University of Technology (QUT) having expertise in aquaculture, aquatic ecology and crustacean biology. The team members are:

- Professor Peter Mather (Project Leader, Aquatic Ecologist, Population Genetics Specialist)
- Dr Satya Nandlal (Aquaculture Specialist)
- Dr David Hurwood (Aquatic Ecologist, Population Genetics Specialist)

The project began with a series of email exchanges with Dr Richard Arthur (Coordinating Consultant) and the SAS to ensure the objectives of the project were understood, and all the relevant background information was collected and collated. The email exchanges also agreed upon the major tasks and the timeframe for the risk analysis.

2.2 Literature Review

This task was to review and document reports and information resources available to the project. The review collated and evaluated a range of resource materials identified via literature searches undertaken using journals and the Internet, on the species concerned and the habitats in KSA. The information was reviewed and a list of potential issues identified, such as the relative invasiveness and the ecological risks associated with introduction of *L. vannamei* to Shrimp Cultivation Projects in KSA for aquaculture in ponds.

3.0 APPROACHES FOR THE RISK ANALYSIS

3.1 General Approach for the Ecological Risk Analysis

The approach taken for assessing the ecological risks of introducing *L. vannamei* into KSA was to review the applicable scientific literature and technical reports covering the ecology of the species as well as those dealing with local species that could potentially be negatively impacted. In broad terms, the assessment examined:

- the risk of escape,
- the potential for *L. vannamei* to establish sustaining local populations,
- the potential for widespread dispersal, and
- the possible effects on native species should a population of *L. vannamei* become established in the wild.

Results from the literature review were summarized and tabulated using a modification of the method promoted by FAO (Technical Paper Nos. 519 and 519/1) the International

Council for the Exploration of the Sea's *Code of Practice on the Introductions and Transfers of Marine Organisms* (ICES 2005, 2012).

3.2 Terminology

The terms used to describe the risk analysis process follow those definitions given by the World Organization for Animal Health (OIE 2012).

3.3 Review Process

The commodity-specific data presented in Table 1, and other information essential to completion of the risk analysis were obtained and verified, as far as possible and were reviewed for accuracy by members of this Project team (Dr. Richard Arthur's Team), the proponents and stakeholders in KSA.

Following completion of the risk analysis, the draft document was circulated for critical comment. While the comments and suggestions of the reviewers have, where possible been addressed, the conclusions and recommendations presented herein, and any errors, remain solely those of the consultants.

3.4 Limitation of the Risk Analysis

The consultants and SAS recognize that the purpose of this document is to provide technical guidance and assessment on the likely risks involved in the proposed translocation and thus the consultants recommend that this risk analysis should not be taken as a basis for a decision by KSA to approve or not approve a request for a proposed species translocation. Such a decision would require additional consideration by the government of policy, legislation, etc. and should include extensive stakeholder consultation. In common with other countries, information on KSA's coastal flora and fauna is limited.

4.0 BACKGROUND ON THE SPECIES PROPOSED FOR INTRODUCTION

4.1 Taxonomy, Distribution and Life Cycle

4.1.1 Taxonomy and distribution

The following information on *L. vannamei* is obtained from http://www.fao.org/fishery/culturedspecies/Litopenaeus_vannamei/en and other relevant sources are cited accordingly.

Litopenaeus vannamei is a decapod crustacean that belongs to the family Penaeidae. Important distinctive features (Fig. 1) include; the presence of teeth on both the upper and lower margins of the rostrum, and a lack of setae on the body (Wyban and Sweeney 1991). The rostrum is moderately long with 7-10 dorsal and 2-4 ventral teeth. In mature males the petasma is symmetrical and semi-open. Spermatophores are complex, consisting of sperm mass encapsulated by a sheath. Mature females have an open thelycum. Colouration is normally white and can change depending on substratum, feed and water turbidity. Females commonly grow faster and to larger size than males. The taxonomical position of

Litopenaeus vannamei Boone, 1931 is taken from the Integrated Taxonomic Information System (ITIS 2004).

Kingdom: Animalia
Phylum: Anthropoda
Subphylum: Crustacea
Class: Malacostraca
Subclass: Eumalacostraca
Superorder: Eucarida
Order: Decapoda
Suborder: Dendrobranchiata
Superfamily: Penaeoidea
Family: Penaeidae
Genus: *Litopenaeus vannamei* (Boone, 1931)

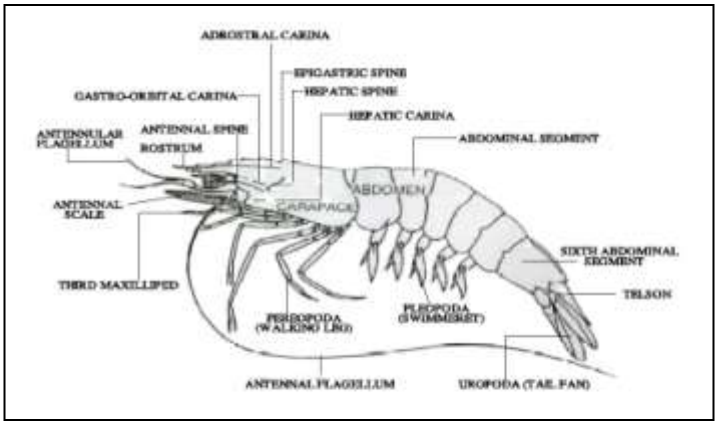

Figure 1. External anatomy of an adult *Litopenaeus vannamei* (from Rosenberry 2005).

Litopenaeus vannamei is native to the western Pacific coast of Latin America, from southern Mexico in the north to northern Peru in the south, between latitudes 32°N and 23°S, (Fig. 2) in areas where water temperatures are usually above 20°C across the year (Wyban and Sweeney 1991). This species is highly abundant along the coast of Ecuador to Esmeraldas (the border province of Columbia) and is fished commercially in the Gulf of California and Gulf of Tehuantepec (ICES/FAO 2005).

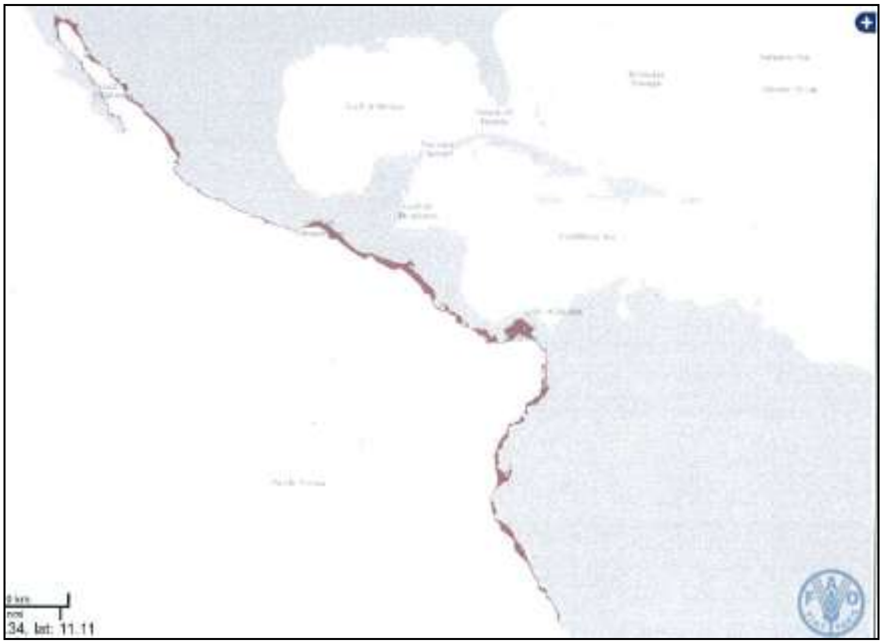

Figure 2. Map showing the natural range of *Litopenaeus vannamei*

4.1.2 Life cycle

In general, prawns in the genus *Litopenaeus* mate and spawn in deeper near-shore waters at a temperature of 26-28°C and a salinity of approximately 35‰. Males become mature from 20g and females from 28g onwards at the age of 6-7 months. *Litopenaeus vannamei* weighing 30-45g will spawn 100,000-250,000 eggs of approximately 0.22mm in diameter. Hatching occurs about 16 hours after spawning and fertilization. The life cycle of *L. vannamei* with various development stages is shown in Figure 3. In general, the first stage larvae, nauplii, swim intermittently and are positively phototactic. The next larval stages (protozoea, mysis and early postlarvae, respectively) remain planktonic for some time and are carried towards the shore by tidal currents. The postlarvae (PL) change their planktonic habit approximately 5 days after moulting into PL and move inshore and settle to the bottom where they begin feeding on benthic detritus, worms, bivalves and crustaceans. After several months in an estuary, juvenile shrimp return offshore where sexual maturation, mating and spawning occur (Rosenberry 2005).

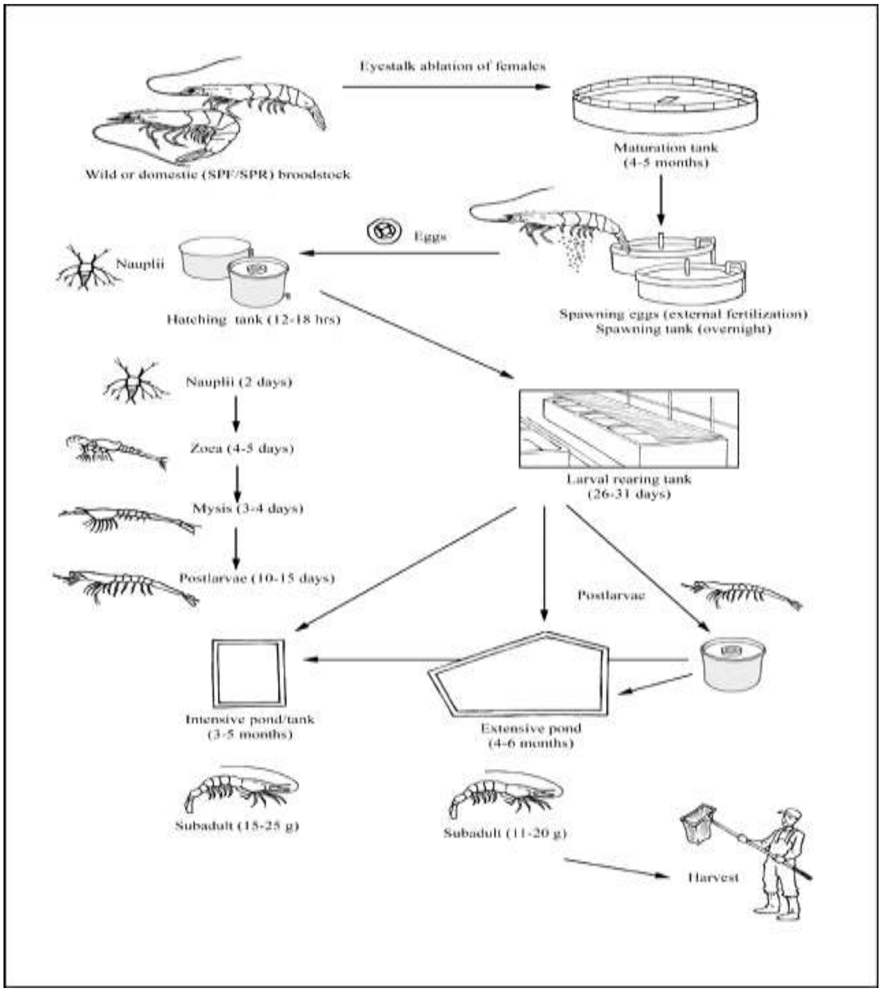

Figure 3. Generalized production cycle of penaeid shrimp (from Rosenberry 2005).

In *L. vannamei*, the eggs hatch and the larvae develop as zooplankton. The first stage (nauplii) have five sub-stages that last approximately 2-3 days where they change from a totally planktonic larvae subsisting on their own egg yolk to having rudimentary feeding appendages. The protozoeal stage follows and consists of 3 sub-stages lasting 3-5 days. At this stage, the larvae feed on phytoplankton and occasionally on zooplankton in addition to their egg yolk. During this development stage the body becomes more elongate and a carapace, compound eyes and uropods are present.

After the protozoeal stage, the mysis stage follows and also lasts for 3-5 days with three sub-stages. At this stage, development is characterized by elongation of the body, telson and pleopods, with the larvae able to swim and seek food. Their diet changes from phytoplankton to zooplankton.

Usually it takes 12-15 days from egg to PL stage, and this depends on temperature and food availability. After 5-6 days, the PL change from a pelagic to a benthic organism migrating from the open ocean into nearshore muddy bottoms/areas and estuaries where according to Liao and Chien (2011) water temperature ranges from 25-32°C, salinity from 28-34ppt and water depth is usually lower than 70cm. These areas serve as nurseries. Adults prefer higher salinity (34-35ppt) and deeper water (30-50m). Typically prawns feed on a wide range of food items and their diet changes as they increase in size. Small crustaceans such as amphipods and copepods are important components of the juvenile diet, with subadults and adults feeding on polychaete worms and molluscs.

4.2 Significance to Aquaculture and Fisheries

Beginning in early 1970s, various penaeid species including *L. vannamei* have been experimentally introduced to a number of countries. including the northwestern Pacific coast of the Americas and to the eastern Atlantic coast from Carolinas and Texas in the north through Mexico, Belize, Nicaragua, Columbia, Venezuela and to Brazil when French researchers in Tahiti developed techniques for their intensive breeding and rearing (Briggs *et al*. 2004).

In the USA, the first spawning of this species was achieved in Florida in 1973 from nauplii spawned and shipped from a wild-caught mated female from Panama. Following good pond results and the discovery of unilateral ablation to promote maturation in Panama in 1976, commercial culture began in South and Central America. Introductions of *L. vannamei* to Asia began in 1978/79 to the Philippines and in 1988 to mainland China (Briggs *et al*. 2004). Of these trials, only mainland China maintained production and initiated a culture industry. Introductions on a commercial scale began in 1990 in mainland China and Taiwan and quickly spread to the Philippines, Indonesia, Viet Nam, Thailand, Malaysia and India. Since this time, *L. vannamei* has become the primary cultured species in Latin America from USA to Brazil, and China has a large flourishing industry based on this species, having increased its production from 270,000 tonnes in 2002 to 300,000 tonnes in 2003, which was higher than the total production from Latin America at that time. Thailand, Viet Nam, Indonesia, Taiwan, the Philippines, Malaysia and India have also established industries based on this species, each country producing thousands of tonnes annually.

Total production of *L. vannamei* was approximately 316,000 tonnes in 2002 in Asia, and this increased to nearly 500,000 tonnes in 2003, worth around USD 4 billion on the export market. *Litopenaeus vannamei* is now farmed and established in several countries in east, southeast and southern Asia and is playing a more significant role in shrimp aquaculture production. In 2008, 67% (1,823,531 tonnes) of the world production of cultured penaeid shrimp consisted of *L. vannamei,* representing an 18-flold increase in production in Asia (Liao and Chien 2011).

The commercial success of introducing *L. vannamei* into Asia can be attributed to several factors that include: higher availability of genetically selected viral-pathogen-free domesticated broodstock, high larval survival, faster growth rate, better tolerance to high stocking density, lower dietary protein requirements, more efficient utilization of plant proteins in formulated diets, stronger adaptability to low salinity, better tolerance to ammonia and nitrite levels, and lower susceptibility to serious viral pathogens that infect *Penaeus monodon* (Liao and Chien 2011). China is currently the biggest producer of *L. vannamei*, increasing production from 33% in 2001 to 47% in 2008, mainly from inland freshwater ponds (Liao and Chien 2011). The culture of *L. vannamei* in freshwater is expected to continue increasing in China, Thailand and other countries in Asia due to higher profits compared with comparable freshwater aquaculture species and also due to higher land availability in inland rather than coastal areas.

4.3 Production Systems

4.3.1 Seed supply

Captured wild seed were used in Latin America for extensive pond culture until the late 1990s. Domestication and genetic selection programs in the 1980s have provided more consistent supplies of high-quality, disease-free and/or resistant PLs that are produced in hatcheries. Some were shipped to Hawaii in 1989, resulting in production of SPF and SPR lines that were used later in industry in the USA and Asia.

4.3.2 Broodstock maturation, spawning and hatching

There are three sources for broodstock

1. Where they occur naturally, broodstock are sea-caught (usually at 1 year of age and weighing >40g) and spawned.
2. Cultured shrimp harvested from ponds (after 4-5 months at 15-25g) are on-grown for 2-3 months and then transferred to maturation facilities at >7 months of age when they weigh 30-35g.
3. Purchased from tank-reared SPF/SPR broodstock from the USA, (at 7-8 months of age and weighing 30-40g).

Broodstock are stocked in maturation tanks in dark rooms supplied with clean, filtered seawater. Feeds consist of a mixture of fresh and formulated broodstock feeds. One eyestalk from each female is ablated, leading to repeated maturation and spawning. Females of 8-10 months of age reproduce effectively, while males peak at >10 months. Spawning rates of 5-15%/night are achieved, depending upon broodstock source. Females are either spawned in communal or individual tanks (to avoid disease transmission). The following afternoon, healthy nauplii are attracted by light, collected and rinsed with seawater. They are then disinfected with iodine and/or formalin, rinsed again, counted and transferred to holding tanks or directly to larval rearing tanks.

4.3.3 Hatchery production

Hatchery systems range from specialized, small, unsophisticated, often inland, backyard hatcheries to large, sophisticated and environmentally controlled installations, together with maturation units. Nauplii are stocked into flat, or preferably 'V' or 'U' shaped tanks with a volume of 4-100m^3, made from concrete, fibreglass or other plastic-lined material. The larvae are either cultured to PL10-12 in a single larval rearing tank, or harvested at PL4-5 and transferred to flat-bottomed raceways/tanks and reared to PL10-12. Survival rates to PL10-12 should average >60%. Water is exchanged regularly (at 10-100% daily) to maintain good environmental conditions. Feeding normally consists of live food (microalgae and *Artemia*), supplemented by micro-encapsulated, liquid or dry formulated diets. From hatching, it takes about 21 days to reach harvest at PL12. Care is taken to reduce bacterial/pathogen contamination of the larval facilities using a combination of periodic dry-outs and disinfections, inlet water settlement, filtration and/or chlorination, disinfection of nauplii, water exchange and the use of antibiotics or (preferably) probiotics.

4.3.4 Nursery

Most farming operations do not use nurseries, but transport PL10-12 at reduced temperature either in plastic bags or oxygenated transportation tanks to the pond and introduce them directly. In some instances, nursery systems are used and comprise of separate concrete nursery tanks or earthen ponds, or even net pens or cages located within production ponds. Such nursery systems may be used for 1-5 weeks. Nurseries are useful in colder areas with limited growing seasons, where PLs are nursed to a larger size (0.2-0.5g) in heated tanks/ponds, before stocking into ponds. The use of super-intensive, temperature-controlled, greenhouse-enclosed, concrete or lined raceways have also provided good results in the USA.

4.3.5 Grow-out techniques

Grow-out techniques can be subdivided into four main categories: extensive, semi-intensive, intensive and super-intensive, which represent low, medium, high and extremely high stocking densities, respectively.

Extensive**:** Commonly found in Latin American countries, extensive grow-out is conducted in tidal areas where minimal or no water pumping or aeration is provided. Ponds are of irregular shape, usually 5-10ha (up to 30 ha) and 0.7-1.2m deep. Originally, wild seeds entering the pond tidally through the gate or purchased from collectors were used; since the 1980s hatchery-reared PLs are stocked at 4-10/m^2. Shrimp feed mainly on natural foods enhanced by fertilization, and once-daily feeding with low protein formulated diets. Despite low stocking densities, small shrimp of 11-12g are harvested in 4-5 months. The yield in these extensive systems is 150-500kg/ha/crop, with 1-2 crops per year.

Semi-intensive: Semi-intensive ponds (1-5 ha) are stocked with hatchery-produced seed at 10-30 PL/m^2; such systems are common in Latin America. Regular water exchange is by pumping, pond depth is 1.0-1.2m and aeration is at best minimal. The shrimp feed on natural foods enhanced by pond fertilization, supplemented by formulated diets 2-3 times daily. Production yield in semi-intensive ponds range from 500-2,000kg/ha/crop, with 2 crops per year.

Intensive: Intensive farms are commonly located in non-tidal areas where ponds can be completely drained, dried and prepared before each stocking, and are increasingly being located far from the sea in cheaper, low salinity areas. This culture system is common in Asia and in some Latin American farms that are trying to increase productivity. Ponds are often earthen, but liners are also used to reduce erosion and enhance water quality. Ponds are generally small (0.1-1.0ha) and square or round. Water depth is usually >1.5m. Stocking densities range from 60-300 PL/m^2. Heavy aeration at 1 HP/400-600kg of harvested shrimp is necessary for water circulation and oxygenation. Feeding with artificial diets is carried out 4-5 times per day. Food conversion ratios (FCRs) are 1.4-1.8:1.

Since the outbreak of viral diseases, the use of domesticated specific pathogen free (SPF) and specific pathogen resistant (SPR) stocks, implementation of biosecurity measures and reduced water exchange systems have become commonplace. However, feed, water exchange/quality, aeration and phytoplankton blooms require carefully monitoring and management. Production yields of 7,000-20,000kg/ha/crop, with 2-3 crops per year can be achieved, up to a maximum of 30,000-35,000kg/ha/crop.

In the "bacterial floc" system, the ponds (0.07-1.6ha) are managed as highly aerated, recirculating, heterotrophic bacterial systems. Low protein feeds are fed 2-5 times per day, in an effort to increase the C:N ratio to >10:1 and divert added nutrients though bacterial rather than algal pathways. Stocking at 80-160 PL/m^2, the ponds become heterotrophic and flocs of bacteria are formed, which are consumed by the shrimp, reducing dependence on high-protein feeds and FCR and increasing cost efficiency. Such systems have realized productions of 8,000-50,000kg/ha/crop in Belize and Indonesia.

Super-intensive*:* Recent research conducted in the USA has focused on growing *L. vannamei* in super-intensive raceway systems enclosed in greenhouses, using no water exchange (only the replacement of evaporation losses) or discharge, stocked with SPF PL. They are thus biosecure, eco-friendly, have a small ecological footprint and can produce cost-efficient, high-quality shrimp. Stocking 282m^2 raceways with 300-450 0.5-2g juveniles/m^2 and on-growing for 3-5 months has realized production of 28,000-68,000kg/ha/crop at growth rates of 1.5g/week, survivals of 55-91%, mean weight of 16-26g and FCRs of 1.5-2.6:1.

4.3.6 Feed supply

Litopenaeus vannamei are very efficient at utilizing the natural productivity in shrimp ponds, even under intensive culture conditions. Additionally, feed costs are generally less for *L. vannamei* than for the more carnivorous *P. monodon*, due to their lower requirement for protein (18-35% compared with 36-42%), especially where bacterial floc systems are used. Feed prices for *L. vannamei* range from USD 0.6/kg in Latin America and Thailand to USD 0.7-1.1/kg elsewhere around Asia; FCRs of 1.2-1.8:1 are generally obtained.

4.3.7 Harvesting techniques

Extensive and semi-intensive ponds are harvested by draining the pond at low tide through a bag net installed in the outlet sluice gate. If the tide does not allow harvesting, the water can be pumped out. In some larger farms, harvesting machines pump shrimp and water up to the pond bank where they are dewatered. Intensive ponds may be harvested similarly and small 2-6 man seine nets are dragged around the pond to corral shrimp to the side of the pond from where they are removed by cast or dip net or perforated buckets.

Partial harvesting is common in Asian intensive culture after the first 3 months. In Thailand, artificial sluice gates are temporarily installed inside one corner of the pond to harvest closed system ponds. Shrimp are then trapped in nets attached to this temporary gate when the pond is pumped out.

In super-intensive systems, the shrimp are simply harvested with large scoop nets when required for processing.

4.3.8 Handling and processing

If shrimp are sold directly to processing plants, specialized teams for harvesting and handling are commonly used to maintain shrimp quality. After sorting, shrimp are washed, weighed and immediately killed in iced water at 0-4°C. Often sodium metabisulphate is added to the chilled water to prevent melanosis and red-head. Shrimp are then kept in ice in insulated containers and transported by truck either to processing plants or domestic shrimp markets. In processing plants, shrimp are placed in iced bins and cleaned and sorted according to standard export sizes. Shrimp are processed, quickly frozen at -10 °C and stored at -20 °C for export by ship or air cargo.

4.4 Diseases and Control Measures

The major disease problems suffered by *L. vannamei* include; white spot disease (WSD) Taura syndrome (TS), infectious hypodermal and haematopoietic necrosis IHHN) causing runt deformity syndrome (RDS), baculovial midgut gland necrosis (BMN) (also known as midgut gland cloudy disease, white turbid liver disease and white turbidity disease), and vibriosis. The availability of SPF and SPR broodstock provides a means of avoiding these diseases, although biosecurity procedures are also important, including:

- Thorough drying/scraping of pond bottoms between cycles.
- Reducing water exchange and fine screening of any inlet water.
- Use of bird netting or scarers.
- Putting barriers around ponds.
- Sanitary procedures.

Once viruses do enter the ponds, there are no chemicals or drugs available to treat the infections, but good management of pond, water, feed and the health status of stocks can reduce their impacts.

4.5 Market and Trade

FAO statistics show that the total farmed production of *L. vannamei* increased steadily from 8,000 tonnes in 1980 to 194,000 tonnes in 1998 (Fig. 4). After a small decline in 1999 and a more significant decline in 2000 due to the arrival of WSSV in Latin America, FAO data show a rapid increase in production to over 1,386,000 tonnes in 2004, due to the rapid spread of this species to Asia. Main producer countries in 2004 were: China (700,000 tonnes), Thailand (400,000 tonnes), Indonesia (300,000 tonnes) and Viet Nam (50,000 tonnes).

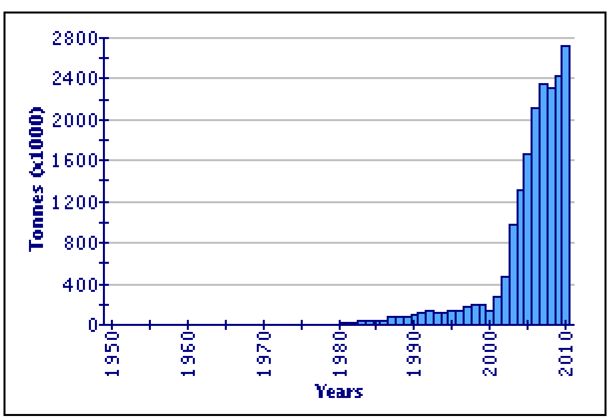

Figure 4. Global aquaculture production of *L. vannamei* (FAO Fishery Statistic)

***Products**:* Frozen head-on, head-off, and peeled shrimp were formerly the major products for export to the main global markets of the USA, the European Union and Japan. The trend now is for the processing of value-added products. This is due to the lack of antidumping tariffs for processed products to the US market, fewer people eating out and the desire for ready-to-cook or ready-to-eat products for home dining.

Prices and market statistics*:* The major market for shrimp is the USA, which was expected to import approximately 477,000 tonnes worth USD 3.1 billion in 2005. The US market was traditionally supplied with small frozen or processed headless shrimp from Latin America. More recently, the USA has looked to Asia to meet increasing demand (1.9kg/capita in 2004). Major suppliers to the USA in 2005 were Thailand, Ecuador, India, China and Viet Nam. However, rapidly increasing production of *L. vannamei* has led to serious price depression in the international markets. Similarly, farm gate value for 15-20g size *L. vannamei* has steadily decreased from USD 5/kg in 2000 to about USD 3.0-3.5/kg in 2005.

The next most important market is the European Union (importing 183,000 tonnes in the first half of 2005), which favours small (31/40 count), whole, frozen shrimp. In Japan, the market mainly requires large headless (16/20 count) shrimp and is typically supplied by *P. monodon* from large extensive Asian farms.

Market regulations*:* Standards for sanitation and the use of drugs and chemicals, and common food safety regulations for seafood (particularly shrimp) are already high in all major importing countries. However, the European Union market has more strict regulations (zero tolerance) on residues of chemicals and antibiotics, as well as the Generalized System of Preference (GSP) on import tax. The US market enforces more strictly on a sanitary standard such as Hazard Analysis and Critical Control Point (HACCP) or Sensory Assessment, but has also instigated strict controls over banned antibiotics in shrimp. From June 2005, the final antidumping tariffs on cultured shrimp imported into the USA from 6 main shrimp producing countries were finalized and set (for the general rate) at approximately 113% for China, 26% for Viet Nam, 10% for India, 7% for Brazil, 6% for Thailand, and 4% for Ecuador. Mexico and Indonesia escaped these tariffs.

4.6 Responsible Aquaculture Practices

Due to rapid expansion and increasing awareness of the negative impacts of shrimp farming practices on the environment and its own production, many shrimp-producing countries are making efforts to comply with the concept of responsible aquaculture as detailed in Article 9 of the FAO *Code of Conduct for Responsible Fisheries* (CCRF). The formulation and adoption of Better Management Practices (BMPs) (or Good Aquaculture Practices – GAP) is gaining popularity to enhance biosecurity, increase cost efficiency, reduce chemical residues and increase traceability. Organic certification for shrimp farming is being seriously considered. HACCP and ISO standards, already used in processing/feed plants, are being adopted in farms and hatcheries. FAO and other organizations have developed a system of guidelines and BMPs to help shrimp-producing countries comply with the various aspects of the CCRF (FAO 1995).

4.7 Status of Knowledge of Pathogens and Parasites of *L. vannamei*

There are now concerns, as evidence of viruses that were previously confined to Latin America, such as Taura syndrome virus (TSV) are affecting *L. vannamei* aquaculture in many countries in Asia, including reports of "runt deformity syndrome" (RDS) caused by IHHNV, which is endemic in *P. monodon* in Asia (Briggs *et al*. 2004). According to Liao and

Chien (2011), while TSV is the most economically significant viral pathogen of *L. vannamei*, it is not reported to be detrimental to aquaculture production and has also not affected indigenous cultured or wild shrimp populations in Asia; however, precautionary measures have been advocated or enforced by government authorities and executed by some private sectors. Following from the above, concerns about the potential risk of introducing serious viral disease along with other exotic species have led several Asian countries to allow controlled importations of *L. vannamei* only from certified sources or by certified agencies. The primary concern is that *Litopenaeus* spp. may carry TSV, which has been detected in a number of countries that culture *L. vannamei* (de la Pena 2004, Sunarto *et al*. 2004, Van 2004).

4.8 Penaeid Shrimp in Kingdom of Saudi Arabia

4.8.1 Native species

The native white shrimp, *Fenneropenaeus indicus* constitutes all of the aquaculture production in KSA, comprising approximately 78% of the total aquaculture production in 2004 (Fisheries Statistics, 2008). This species is present in the waters around Saudi Arabia, with its natural distribution extending from the Indo-West Pacific and southeast Africa to New Guinea and across northern Australia. *Fenneropenaeus indicus*, commonly known as the Indian white prawn, is tolerant of relatively high water salinity. Because of its capability to breed and grow well in highly saline waters, this species was found to be the best shrimp species suitable for aquaculture in KSA. Early attempts to culture exotic *P. monodon* and *P. semisulcatus* were initially very successful but have not been so successful lately, due to water salinity issues.

4.8.2 History of previous introductions

The first experimental introductions began in the early 1970s and later, in the 1970s and the 1980s, *L. vannamei* were translocated from their natural range on the Pacific coast of Latin America from Mexico to Peru. From here, they were introduced to the northwestern Pacific coast of the Americas in the USA and Hawaii, and to the eastern Atlantic coast from Carolina and Texas in the north through Mexico, Belize, Nicaragua, Colombia, and Venezuela and on to Brazil (Briggs *et al*. 2004). Most of these countries now have culture industries farming *L. vannamei*. Experimental introductions into Asian countries began between 1978 and 1979 and commercially into Taiwan and China since 1996 and 1998, respectively. Subsequently they have been introduced into the Philippines, Indonesia, Viet Nam, Thailand, Malaysia and India (SEAFDEC 2005). There are no records or reports indicating that *L. vannamei* had been introduced to KSA in the past. A summary of the introductions including reasons for introduction is presented in Table 2.

Table 2. Importation of *L. vannamei* in Latin American and Asian countries and the Pacific.[1]

Country	First introduction of *L. vannamei*	Original source	Original cultured species	Reason for importing	Reference
Tahiti	1970/1980	Pacific coast-Mexico to Peru	-	Experiments on larval rearing	Briggs *et al*. 2004
USA	1970/1980/1985	"	-		"
Mexico	1980	"			"
Belize	1980	"			"
Nicaragua	1980	"			"
Colombia	1980	"			"
Venezuela	1980	"			"
Brazil	1983	"	Local species	Problem with local species	"
Pacific islands	1972	Mexico, Panama	M, Me, J, Fi,Ma	Experiments, cold tolerance	"
China	1988	Texas, USA	C, M, J, P, Me	Diversification	"
Taiwan	1995/1996	Hawaii, USA	M, J, Ma	Problems with *P. monodon*	" ; Wyban 2002
Philippines	1997	Taiwan	M, I, Me	Problems with *P. monodon*	"
Thailand	1998	Taiwan	M,J, Ma	Problems with *P. monodon*	"
Vietnam	2000	China	M	Problems with *P. monodon*, cold tolerance	"
Indonesia	2001	Hawaii, USA	M, Me	Problems with *P. monodon*	"
Malaysia	2001	Taiwan	M, S	Problems with *P. monodon*	"
India	2001	Taiwan	M, I, Ma	Problems with *P. monodon*	"
Cuba	2003	USA	*P. schmitti*	Experiments	Tizol *et al*. 2004

[1]Cultured species: M = *P. monodon*, J= *M. japonicus*, Me = *F. merguinensis*, C = *F. chinensis*, S = *P. stylirostris*, P = *F. penicillatus,* Fi = *F. indicus,* Ma = *Macrobrachium rosenbergii*

Introductions of *L. vannamei* to non-native areas of the Americas, Asia and the Pacific have had a significant positive effect on the production capacities of the countries involved. For example, *L. vannamei* was introduced to Brazil in 1983 and soon after, commercial production began. It was not until 1995 however, when this species became the major species produced there due mainly to importation of a highly productive Panamanian stock in 1991 that was followed by mastering of its captive maturation, fast growth, high survival rates obtained in ponds and its good market potential in Europe and the USA.

While six species of penaeid shrimp (*L. vannamei*, *P. monodon*, *L. stylirostris*, *Marsupenaeus .japonicus*, *F. chinensis* and *F. indicus*) had been introduced to Hawaii for culture and research purposes, only *L. vannamei* is currently under commercial pond culture there (Eldridge 1995, Henning *et al*. 2003, Briggs *et al*. 2004).

The first commercial shipment of SPF *L. vannamei* broodstock from the Americas to Asia was from Hawaii to Taiwan in 1996 (Wyban 2002) and following successes in captive maturation, larval rearing and culture in ponds of this species, led to huge demand for broodstock and to introductions of wild broodstock from many sources in Latin America in 1997 (Briggs *et al*. 2004). This was followed by introduction of *L. vannamei*, both SPF and SPF/SPR (for TSV) from the USA, and non-SPF from Latin America and Taiwan to the Philippines (1997), Thailand (1998), Indonesia and Viet Nam (2000), Malaysia and India (2001), and Myanmar and Bangladesh, and many other Asian countries, that in some cases were introduced without official government approval (Fegan 2002, Taw *et al*. 2002, Wyban 2002).

According to Briggs *et al*. (2004) and other reports, *L. vannamei* gained prominence across Asia and production increased significantly due mainly to problems with the growth rate of *P. monodon* (that had been the preferred species prior to introduction of *L. vannamei*). In addition, *L. stylirostris* that had been the major species cultured in Mexico has been replaced or out-competed by *L. vannamei* in every other country in the Americas. While SPF *L. stylirostris* had been promoted to many Asian countries around 2000-2003, this species has only had a significant impact in Brunei, and trials in Taiwan, Myanmar, Indonesia, Thailand and China have been less successful and have not yet led to commercial culture or have yet to make an impact on the shrimp production in these countries (Briggs *et al*. 2004).

5.0 JUSTIFICATION FOR INTRODUCTION AND ALTERNATIVE STRATEGIES

5.1 Justification for Introduction

The proposed introduction of *L. vannamei* will be used by Shrimp Cultivation Projects in KSA to produce shrimp and associated shrimp products to meet local demand and also for exports. The KSA "national policy encourages the export of farmed fish to neighbouring Arab countries and Europe. We want the private sector to be competitive with other aquaculture producers and exporters in South East Asia." (www.fao.org/english/newsroom/news/2002/9346-en). Six Saudi Shrimp Cultivation Projects produced over 26,000 tonnes of *F. indicus* in 2010 (source FAO), the only indigenous species that is cultured commercially in KSA.

Markets have been developed in KSA itself and the Middle East, and a major share of the production is being exported to Asian, European and the US markets. The companies' infrastructure includes broodstock facilities, hatcheries, feed mills, grow-out farms, processing plants, cold stores, water treatment plants, etc. The Saudi Shrimp Cultivation Projects have the support of the Saudi Aquaculture Society (SAS) and the Ministry of Agriculture of the KSA, who will assist in the importation of *L. vannamei*. Despite the potential for problems with disease transfer, *L. vannamei* is believed to offer numerous advantages over the presently cultured indigenous species, *F. indicus* and other species (*P.*

monodon). The advantages of farming *L. vannamei* (in addition to information provided in section 4.0) include:

- ***Ease of breeding***: *L. vannamei* has an open thelycum, meaning that they can be induced to mate and spawn easily in captivity, i.e., it is easier to operate hatcheries. This allows much more control and enhancement of the stocks, with higher survival rates of 50-60% compared to other species. This allows for rapid development of SPF and SPR stocks. In fact, a reliable supply of SPF and SPR lines are commercially available. *Litopenaeus vannamei* also shows high survival during larval rearing, and this allows for a greater marketing advantage compared with other species.

- ***Ease of domestication***: Along with ease of hatchery operations, *L. vannamei* broodstock are easily produced in culture ponds. This relieves the necessity for returning to wild broodstock, PLs or juveniles and permits domestication and genetic selection for favourable traits such as growth, disease resistance and rapid maturation. Additionally, this eliminates the problems associated with wild broodstock, as cheap broodstock are readily available from ponds. Compared with the domestication of *P. monodon* that has been going on for some time in the USA, Australia and Thailand, *L. vannamei* offers many advantages (Briggs *et al*.,2004). The minimum spawning size for *P. monodon* females is 100g, which will take at least 10-12 months under commercial pond conditions, while in *L. vannamei* this can be achieved in only 7 months.

- ***Resistance to certain diseases (related to SPR stocks)***: *L. vannamei* is generally considered to be more disease resistant than other white shrimp (Wyban and Sweeney 1991). While this species is susceptible to WSSV and TSV and is a carrier of IHHNV and lymphoid organ vacuolization virus (LOVV) (Briggs *et al*. 2004), farms in Asian countries are not experiencing problems from these viruses. Survival rates of *L. vannamei* are currently higher than for *P. monodon* in Asia, and Thailand, Malaysia and Indonesia have not suffered major WSSV or YHV- related epidemic, with survival rates of 80-90% being achieved on some farms (Briggs *et al.* 2004).

- ***Rapid growth rate:*** Growth of *L. vannamei* is usually around 1-1.15g/wk, with 80-90% survival common in high-density pond systems (60-150/m^2) in Thailand and Indonesia compared with *P. monodon* (1g/wk) under commercial culture conditions (Briggs *et al.* 2004). In addition, *L. vannamei* are amenable to culture at very high stocking densities, as high as 400/m^2 in controlled recirculated tank culture, resulting in better productivity per unit area than that currently achievable with other species in Asia. Size at harvest is generally 20g at a stocking density to up to 150/m^2.

- ***Salinity tolerance***: *L. vannamei* is extremely euryhaline (Menz and Blake 1980), capable of tolerating a wide range of salinities from 0.5-45ppt), particularly at low salinities of around 10-15ppt, and thus is more amenable to a range of sites, including inland (not suitable for *P. monodon* or *L. stylirostris*) and coastal sites.

- ***Temperature tolerance:*** *L. vannamei* can tolerate temperatures ranging from 15-33°C (Wyban and Sweeny 1991) and grows best between 23-30°C, with optimum growth at 30°C for small (1g) and 27°C for larger (12-18g) shrimp (Briggs *et al*. 2004). This wide temperature tolerance range allows *L. vannamei* to be cultured in the cool season in Asia, resulting in increased yearly harvests compared with other species except for *L. stylirostris,* which can tolerate even colder temperatures than *L. vannamei*, *P. monodon* or *F. indicus*.

- ***Dietary protein requirements:*** *L. vannamei* require lower protein feed (20-35%) during culture than *P. monodon* or *L. stylirostris* (36-42%) and are more amenable to utilizing the natural productivity of shrimp ponds, even under intensive culture conditions (Wyban and Sweeny 1991). In addition, feeding efficiency is better with *L. vannamei* , with a lower FCR of 1.2 as compared with approximately 1.6 for other species (for example *P. monodon*). This results in lower operational costs and therefore overall lower production costs. Following from above, results using recycled systems in Belize, China, Indonesia and elsewhere have shown that protein levels as low as 20% can be successfully used provided the natural bacterial productivity of the ponds is correctly stimulated (McIntosh *et al*. 1999).

- ***Post-harvest characteristics:*** *L. vannamei* are resistant to melanosis provided they are treated with plenty of ice (Briggs *et al*. 2004).

- ***Marketing:*** *L. vannamei* and *L. stylirostris* are the preferred species for consumption in the USA – the world's largest shrimp market (Briggs *et al*. 2004). In addition, it offers a higher meat yield at 66-68% than *P. monodon* (62%). According to Rosenberry (2002), *L. vannamei* can be mixed together and sold as western white shrimp, with consumers preferring its taste over *P. monodon* in USA. There is also a strong demand for *L. vannamei* in local markets in mainland China, Taiwan and Thailand (Peterson 2002).

5.2 Alternative Strategies

The only alternative strategy available apart from introduction of *L. vannamei* for culture in KSA is to continue culture of *F. indicus*, with the inherent constraints on production, or to introduce another exotic species, such as *L. stylirostris*, which is not as tolerant of a wide range of salinities.

An accreditation scheme, similar to that being set up in Thailand and other Asian countries for importation of *L. vannamei* could be developed by the SAS that would simplify importation of PLs originating from pre-approved hatchery facilities.

6.0 DESCRIPTION OF THE CULTURE SYSTEMS AND CURRENT PRACTICES

6.1 Culture Systems at Saudi Shrimp Cultivation Project Sites

Saudi shrimp aquaculture basically evolved out of two initiatives, an initiative of Saudi Fisheries Company which led to the commercial operation of a Shrimp Cultivation Project with a pilot phase as early as 1995, and an initiative of the Al-Ballaa family which started in 1982 and led to the establishment of the National Prawn Company.

Six Shrimp Cultivation Projects are in operation KSA. All Saudi Shrimp Cultivation projects grow *Fenneropenaeus indicus*. All projects are located on the Red Sea coast.

The Saudi Shrimp Cultivation Projects are:

- *Arabian Shrimp Company* is an integrated shrimp cultivation operation located North of Jizan. The total project area is about 6,200 ha with about 3,000 ha allocated to production ponds.
- *Island Prawn* is based about 150km south of Jeddah and has 35 ponds of 2.2 ha; 77 ha of pond surface area.
- *Jazadco Development Company* is located in the Jizan area and has been in shrimp production operation since 2003; it was fully developed with 440 ha of water area in 2007-2008.
- *National Prawn Company (NPC),* located about 180km south of Jeddah began with a research project in 1982. Its current set up, as a vertically integrated operation, from a breeding center to cold stores and other buildings, has been developed since 1999. The technical production capacity of the project once fully developed, with a pond surface area of about 4,200 ha, will be between 25,000 and 28,000 tonnes annually.
- *Red Sea Aquaculture* was started in 2008 with an overall focus to develop a totally integrated marine aquaculture project in Saudi Arabia. As a startup, the company has developed a water area of 200 ha or an overall land area of 350 ha for shrimp aquaculture at the Red Sea coast 15 km south of Al-lith.
- *Saudi Fisheries Company (SFC)* entered commercial production in 1995. It is located about 500km south of Jeddah. SFC is currently operating its Shrimp Cultivation Project on an area of 750 ha with 108 ponds and an annual production of 1,500 tonnes.

Saudi Shrimp Cultivation Projects maintain biosecurity programs at different levels and have obtained business-relevant certifications such as ISO9001/14001/22000, HACCP and British Retail Consortium (www.robian.com.sa/home.html) enabling them to sell on both local and international markets to overcome the problem of restrictions placed on detection of banned antibiotic residues in shrimp in the European Union and the USA, which has introduced much stricter controls over testing for banned antibiotics (chloramphenicol and nitrofurans).

7.0 DESCRIPTION OF RECEIVING ENVIRONMENT AND CONTIGUOUS AREAS

KSA has a total area of 1,960,582 km^2, occupies 80% of the area of the Arabian Peninsula and is surrounded on three sides by water - with the Arabian Sea to the southwest, the Red Sea to the west and the Persian Gulf to the east (Fig. 5) that lies between Iran and the Arabian Peninsula. The Kingdom has a shelf area approximately 95,040km^2 and lengthy continental coastline (length of 2,640km), of which approximately 580km is to the Gulf and remainder to the Red Sea. It is a large country, most of which is desert.

The following information about the Red Sea is taken from UNEP (1994) and several other sources, including http://wwf.panda.org/about_our_earth/ecoregions/red_sea.cfm.

7.1 The Red Sea

The Red Sea is a flooded valley that can be described as a young ocean (about 70 million years), created by the pulling apart of Africa and Arabia and extends SE-NW between 12°N, 43'E and 30°N, 32'E and has a surface area of 44000 km^2 (UNEP 1994). It is connected at its northern end with the Mediterranean Sea through the man-made Suez Canal and at its southern end with the Indian Ocean through the Strait of Bab el Mandab. Near the latitude 28°N, the Red Sea branches into the Gulf of Suez and Aqaba.

The Gulf of Suez extends for about 255km with widths of 17-45km and a maximum depth of 83m. Throughout its geological history, this Gulf has always been a site of immense sediment accumulation, resulting in its bottom morphology being smooth and simple and having gentle submarine coastal slopes. This excessive sedimentation has also restrained the development of coral reefs. In contrast, the Gulf of Aqaba is shorter, narrower, and much deeper. It extends for 180km with widths varying from 25km in its southern part to 16km at the north. The Gulf proper is divided into three elongated deep basins striking north east. The northern deep is the shallowest (900m deep) and has flat bottom, while the other two deeps have irregular bottom topography and much greater depths. The maximum water depth reaches up to 1850m in the central basin, and fringing coral reefs grow luxuriously along the entire coastline with the exception of the northernmost part of the Gulf.

The Red Sea extends for about 2000km. The distance between the eastern and western Red Sea coasts is 180km at its narrowest part and double this value at the widest part. The continental shelf is 15-30km wide in the north and about 120km in the south, with the most southern part of the sea considered as part of a shallow shelf extending to the center of the sea. The inner part of the shelf contains reefs, rocky shoals, banks and islands, resulting in a rough bottom topography. The islands typically range in height from less than one meter to hilly ones rising up to 300m above the sea level. The outer shelf slopes gently with a distinct break at 500-600m depth marking the edge of the main trough. According to UNEP (1994) the most important feature of the Red Sea is the deep, narrow axial trough cutting the medial axis of the large main trough that extends from north to south. The axial trough is 10-30km wide and has steep-sided walls, generally filled with hot brine and important metal deposits. The water depth in the deepest pool is 2850m, and this Red Sea rift system is characterized by extensive volcanic activity throughout the whole area.

The coast of the Gulf of Suez is bordered by relatively wide and moderately high to low sandy plains, while the coasts of the Gulf of Aqaba are steep throughout the gulf with coastal plains very narrow or absent. The coastal plains of the Red Sea proper are bordered by high mountains rising about 1000m in the north and more than 3500m in the south. These plains are generally narrow and high in the north and more wide and low in the south.

The Red Sea coastlines frequently protrude out in the form of rocky headlines. In addition, the coastlines and the outlying fringing reefs are cut into at irregular intervals by creeks that are typically drowned stream valleys. The southern Red Sea coastal zones have well-developed mangroves and marshes. The best example of these mangroves and marshes is found at Jizan in Saudi Arabia. It extends for about 1km inland. In addition, sublittoral and supralittoral sand dunes, muddy embayments and sand spits have formed due to intensive accumulation of sediments in the south.

The Red Sea contains representatives of all major tropical marine communities except estuaries because there are no permanent rivers or streams that flow into the Red Sea, and is partially isolated from the open ocean. Together, these features contribute to a unique flora and fauna and some specific features include:

- *Coral reef s* - The northern and central Red Sea have the best developed reefs. In the central sections, reef complexes are found along the coast at about 3-10km offshore

- developed on a series of narrow underwater banks of tectonic origin. In the southern third of the Red Sea, these banks are much wider and give rise to several archipelagos which may resemble atolls. A high coral diversity (about 129 species of hermatypic and 120 species of soft corals) has been recorded from the reefs.

- *Mangroves and wetlands*- The mangrove forest or mangal of the Red Sea include the hard-bottom mangal that are more prevalent in the northern Red Sea. These are found on a substrate of relatively thin sediment over sub-fossil or raised coral/rock and typically occur in regions with high salinity (up to 47ppt). Four species of mangroves are reported: *Avicennia marina,* which is found throughout the region; *Rhizophora mucronata,* found in few restricted locations including *Bruguiera gymnorhiza* and *Ceriops tagal.*

  According to Khalil (2004), based on the distribution and density of the *Avicennia* mangroves along the eastern coast near Al-Lith (the proposed site for introduction of *L. vannamei*), the Red Sea coast may be broadly divided into two areas:

  1. the area north of Al-Lith where mangrove distribution is sparse; and
  2. the area south of Al-Lith where the mangroves are relatively dense, fringing most of the shoreline. The distribution of mangroves increases towards the south, coinciding with the gradual disappearance of stony corals and increased availability of muddier substrate and rainwater.

  According to UNEP (1994) the mangals of the Red Sea is a mosaic habitat, inhabited by species typical of muddy, sandy or rocky shore devoid of mangrove vegetation. Compared to Indian Ocean mangals, the number of mangrove and associated species in the Red Sea is low; however, they play similarly important ecological roles as nurseries for fishes and protect coral reefs by trapping sediment loads from seasonal rainwater runoff.

- *Seagrasse s*- In the Red Sea, 12 species of seagrasses (Lipkin and Zakai 2003, El Shaffai 2011) are found from midtidal level on shores receiving regular tides to about 70m depth. The commonest species are *Halophila stipulacea*, *H. ovalis*, *Halodule uninervis, Thalassomadendrum ciliatum* and *Syringodium isoetifolium*. *Halophila stipulacea* and *T. ciliatum* have the greatest distribution, the former extending from the lower shore to at least 70m depth, and the latter from extensive low-water level to at least 40m depth. The remaining species are restricted to seabed under less than 10m of water.

  Seagrasses are food for sea turtles, fishes and dugongs, and also support complex food webs because of their physical structure and primary productivity (El Shaffai 2011), as well as animals in the detrital food chain and annelids, molluscs, crustaceans, echinoderms and fishes (McRoy and Helfferich 1980). Dead seagrass decomposes through the action of physical breakup and bacteria. The bacteria are cropped by protozoan grazers, which in turn are eaten by carnivorous microzoa that become the prey of yet other larger fauna (Roy and Helfferich 1980). In addition, seagrasses are also extremely important for nursery and shelter and provide 20 times more surface area for small sessile flora and fauna as compared to unvegetated areas.

- *Seaweeds* - In the Red Sea, areas with hard substrate are dominated by algae. These occur in shallow coral reef areas where the algae tend to be filamentous greens and small browns which grow as "algal lawns". Algal communities in most of the areas show a strong seasonality and many appear to be annual.

  There are about 500 species of algae recorded from the Red Sea, with about 9% of species endemic to the region. Most species comprising the algal turf in northern and central areas of the Red Sea are macroscopic, non- calcarious forms of green, brown and red algae, and commonest is brown *Sphacelaria tribuloides* , which serves as a substrate for other epiphytic and turf algae. All these groups contribute to algal lawns and have trophic importance to marine herbivores as well as in the detrital food chain.

Following from above, the Red Sea coast contains numerous wetland habitats of considerable importance to fisheries and wildlife. These include the extensive coastal plain areas with mangroves and other terrestrial vegetation, intertidal sand flats, intertidal mudflats and many other littoral and shallow water enclosed soft-bottom habitats that contribute to fisheries of Red Sea.

Traditional or artisanal fisheries as well as industrial fisheries operate in both the Red Sea and the Arabian Gulf areas. In the Arabian Gulf, the industrial sector is solely concerned with shrimp production while the artisanal sector uses fish traps, gillnets, handlines, trolling, and small shrimp trawl nets.

In the Red Sea, artisanal fisheries production is almost entirely derived from handline and gillnet methods, while the industrial fleet utilizes fish and shrimp trawl nets and purse seine nets. The industrial vessels operating in the Red Sea utilize trawl nets to target both demersal fish stocks and shrimp, with the majority of these vessels belonging to Saudi Fisheries Company and operating out of Jizan on the southern Red Sea coast.

Local species of marine turtles include green turtle (*Chelonia mydas*), loggerhead turtle (*Caretta caretta*), leatherback turtle (*Eretmochelys imbricata*), olive ridley turtle (*Lepidochelys oliacea*) and hawksbill turtle (*E. imbricata*).

Birds include Saunder's tern (*Sterna saundersi*), white-cheeked tern (*S. repressa*), great black-headed gull (*Larus icthyaetus*), pink-backed pelican (*Pelicanaus rufescens*), brown booby (*Sula leucogaster*), white-eyed gull (*Larus leucophtalmus*) and osprey (*Pandion haliaetus*).

Other species include dugong (*Dugong dugon*), Blainsville's beaked whale (*Mesoplodon desirostris*), white-tip reef shark (*Triaenodon obesus*), butterflyfishes (*Chaetodon* spp.), giant clams (*Tridacna* spp.), and several species of dolphins (Family Delphinidae). Seventeen percent of fish are endemic, with more than 90% endemic dottybacks (Family Pseudochromidae), and triplefins (Family Tripterygiidae).

Major threats include overfishing, spear fishing, souvenir collecting, scuba diving and the use of the coast for recreational activities, representing major disturbances to these coral reefs. Oil spills, sewage discharge, chemical pollution, industrial and urban development, extensive coastal development, land filling and coastal engineering pose further threats to the eco-region.

Aquaculture development has proceeded slowly in the countries bordering the Red Sea and Gulf of Aden, with the exception of Egypt, which has a significant proportion of its total fish supply being derived from pond culture of tilapia and carp species along the Nile River. Marine fish farming is not common in the Red Sea region.

Figure 5. Map showing are of proposed introduction for *L. vannamei* in KSA.

7.2 Climate and Weather Conditions

The general features of the climate and weather of KSA given below are from several sources, including from www.metoffice.gov.uk/media/pdf/j/m/Saudi Arabia, and UNEP (1994).

The Kingdom's lengthy coastline and proximity to water moderates the climate, but most of the country is desert with low and unreliable rainfall. Much of the country, with the exception of the mountains, is hot and arid, and water shortages and desertification pressing environmental problems. Significant seasonal and even diurnal fluctuations in temperature result in extreme climate conditions in many areas of the country. While in the past floods have affected certain parts of the country, the most recent one in 2009 (www.news.bbc.co.uk), tropical cyclones, flash floods, tidal surges, etc. are not common. In addition, KSA, which is a major part of Arabian Peninsula, has been affected by tsunamis in the past. The peninsula is bounded by the Persian Gulf on its northeast side, the Red Sea on its west side, and the Arabian Sea the Gulf of Aden, and the Indian Ocean to its east and south. According to Jordan (2008), each of these areas is very different geographically, tectonically and bathymetrically. And while the Red Sea is a geologically active area situated above the Red Sea Rift, only two localized tsunamis have been recorded in this region. The first major earthquake occurred in 1879, resulting in a tsunami that flooded the village of Tor on the Sinai Peninsula in the Gulf of Suez, and the second major earthquake occurred in 1884, causing multiple flooding between the localities known as Taulud and Edaga Barai (Jordon 2008). In addition, almost all other recorded tsunamis along the Arabian Peninsula have occurred on its eastern and southern edge, and the Indian Ocean is the most likely source area for future destructive tsunamis that would impact the Arabian Peninsula.

Following from above, the Red Sea is located in an arid region with an arid type of climate. Most of the region lies in a subtropical high pressure belt. The mean daily maximum temperature in January ranges from about 20°C in the far north to about 29°C in the far south and the corresponding figures for July are 35°C and 40°C, respectively. The southern region is considered to be among the hottest regions in the world. The air temperature in the northern region is slightly lower and ranges from 6-39°C at the Suez Canal compared to the southern region, where it ranges from 13-42°C along the Jeddah coast. Rainfall in the Red Sea region is extremely sparse and is usually localized in the form of short showers. Some specifics on climate and weather of the Red Sea include:

- *Rainfall* - Rain over the Red Sea is very sparse and is very often localized. A particular location may receive no rain for a period of time followed by a brief heavy fall that may not be repeated for a similar lengthy period. The rainy seasons are divided in two distinct periods: the winter rainfall (November to February) and the summer rainfall. The winter rainfall affects mainly the northern parts of the areas adjoining the Mediterranean region and occasionally reaches the northern Red Sea region. The frontal bands associated with these low pressure centers affect the weather of the northern and central parts, resulting in a drop in temperature, cloudiness and occasionally thunder and rain. The system affecting the weather and the climate of the central and southwestern parts is called the Sudan low and is responsible for the surface easterlies. The southeasterly winds coming from the Arabian Sea provide moisture for the rainy weather over the southern and central parts of the regions over the escarpments. The summer rainfall is mostly of convection type connected with the inter-tropical convergence zone which prevails during summer. It is governed by the humid southwesterly monsoon which causes rainfall on the southern parts of the Red Sea and the Gulf of Aden.

  Following from above, most of the rainfall occurs in the winter that lasts for short duration and is often associated with thunderstorms and dust-storms. The highest rainfalls (average of 109 mm/year at Port Sudan and 193 mm/year at Massawa) are generally recorded from the central Red Sea where the northern and southern Red sea air masses meet (UNEP 1994).

- *Water temperature* - The Red Sea is unique among deep water basins for having a stable water temperature of about 21.5°C throughout its deeper waters driven by winds and through density gradient. Due to evaporation and cooling, inflowing surface water becomes more dense as it flows northward and sinks at its northern end. The sinking water in the north during winter causes the water inside the Red Sea basin to ascend and flow out of the sea over the sill of Bab el Mandab. In summer, the northern Red Sea is characterized by its high temperature and low evaporation rate and hence is occupied by a surface layer of relatively low density, while in winter the water surface temperature at Suez is 17°C and increases to about 22°C at the entrance of the Gulf of Suez.

  Generally, surface water temperature in the Red Sea increases from north to south, showing wide variability (ranges between 22-32°C) between the different seasons. Also, the surface temperature generally declines towards the south due to the influx of cool water from the Gulf of Aden, and also gradually decreases towards the northern region. The deeper waters are stable throughout the region. Water renewal in the Red Sea is slow, and exchange with the ocean takes approximately 6 years for the 200m above the thermocline and 200 years for the entire sea.

- *Salinity* - Due to high evaporation rate (235 cm/year) and a complete lack of freshwater input, the Red Sea is considered as the most saline body of water in direct connection to the world oceans. From an average salinity of 36.5ppt at Bab el Mandab, the salinity increases to 40.5ppt at its northern end. These high salinities are due to intense evaporation, low renewal of the water mass and also due to the salt deposits in the Great Bitter Lakes of the Suez Canal and the presence of extensive salt layers in the Gulf of Suez region.

- *Tides and currents* - The tides of the Indian Ocean do not propagate into the Red Sea, i.e., there is no progressive tidal wave which moves through the Strait of Bab el Mandab and raises and lowers the water level within the Red Sea basin. Within the Red Sea, there is a local oscillatory tide of small amplitude which results in high water at one end of the sea when it is low water at the other end, i.e. the tidal ranges change widely from north (about 1.5-1.8m at Suez) to south (about 0.9m), with greatest values being at the two ends. There is no appreciable diurnal tide towards the centre near Port Sudan and Jeddah.

  Currents are generally weak and variable in the Red Sea except through the Straits and at its middle region. Generally, the movement of currents follows the winds, such that the northerly wind in summer drives surface water south for about 4 months, while in winter the flow is reversed, pushing water into the Red Sea from the Gulf of Aden. The net movement of water is greater in winter than in summer outflow, and the drift continues to the northern end of the Red Sea.

8 PROPOSED SOURCE OF STOCK AND NUMBERS OF ORGANISMS TO BE INTRODUCED

Source - For potential suppliers, refer to Annex 2.

9 ECOLOGICAL RISK ANALYSIS

9.1 Potential Invasiveness

Past experience with exotic penaeid species introduced for aquaculture elsewhere in the world indicates that escape of *L. vannamei* can be expected in different ways during harvest of ponds, water exchange, flooding events (Briggs *et al*. 2004), and ongoing escapes from ponds (Senanan *et al*. 2007), as well as from hatcheries and during transport (Wakida-Kusunoki *et al*. 2011). For example, anecdotal evidence suggested that fishermen in Thailand caught *L. vannamei* from both the Andaman and Gulf of Thailand coasts after significant floods in 2003 (Briggs *et al*. 2004). However, Senanan *et al*. (2007) did not find any evidence that the shrimp present in the Gulf of Thailand could reach maturation, and they were also not able to infer from their results that there is a self-sustaining population. Thus, there is still no available information regarding whether *L. vannamei* has established in the wild and if so, the effect of its interaction with existing crustacean species. This is further supported by Wakida-Kusunoki *et al*. (2011), who did not find any evidence for *L. vannamei* becoming established in the Mexican coast of the Gulf of Mexico.

According to Loebmann *et al* (2010), *L. vannamei* was imported to Brazil in the 1970s for aquaculture purposes and could have been accidently released from overflowed ponds and lakes into the marine environment. Based on Loebmann's research carried out during April and May 2009, four adult *L. vannamei* were collected using casting nets along with two native shrimp species. Loebmann *et al* (2010) concluded that the occurrence of this species in the wild may be large, and while its presence in Brazilian estuarine and coastal waters was also reported by Santos and Coelho (2002) and Barbieri and Melo (2005); whether invasion rates are actually increasing or are a result of more intensive research efforts in the recent past is still an open question (Ferreira *et al*. 2009).

Many marine communities have been invaded by non-native species (Steneck and Carlton 2001; Wiliams and Heck 2001), and there is concern that these species might reduce natural levels of biodiversity (Butman and Carlton 1995). However, in general, manipulative experiments rarely have been used to elucidate the ecological interactions between native and invading species (Kareiva 1996). As an exception to this, seagrass studies have contributed to the understanding that the ecological effects of invading species are complex and not always deleterious (Harrison 1987, Posey 1988). While many other studies and examples exist, the species cited or used in these experiments are not relevant here, as most are not related to penaieds or of commercial importance.

Following from above, currently the impacts of shrimp species (e.g. *P. monodon*) on the native fauna in areas where they have been introduced are uncertain. For example, according to Knott *et al*. (2012) about 300 *P. monodon* were collected off the coasts of South Carolina, Georgia and Florida in the three months after the August, 1988 accidental release of roughly 2,000 animals from an aquaculture facility in South Carolina. In September 2006, 18 years later, a single adult male was captured by a commercial shrimp fisherman near Dauplin Island, Alabama. A month later, five specimens were collected in Pamlico Sound, North Carolina, and a single specimen was caught in August, 2007 in Vermilion Bay, Louisiana and several more were reported from Florida and both Carolinas shortly thereafter. The first documented collections in Georgia, Mississippi and Texas occurred in 2008, 2009 and 2011, respectively. Although the number of reports is high (314 from 2006-2011), the number of individuals ranges from 1-15 and averages just slightly greater than one specimen per report. Introductions of *P. monodon* into the southeastern USA are most likely explained by escape from aquaculture facilities following flooding by storms and hurricanes or through migration from areas where *P. monodon* have previously become established in the wild; however, they are less probable, as other pathways for introduction (e.g. ballast water discharge) are also possible (Knot *et al*. 2012). The likelihood of floods, hurricanes and tsunamis to flood pond facilities in KSA is minimal (see Section 7.0), and available information with respect to the distribution, life cycle (see Sections 4.0 and 5.0) and other reports indicate that the chance of *L. vannamei* invading and establishing self-sustaining populations in the Red Sea is low.

Also, *P. monodon*, originally from Hawaii, were introduced to the Atlantic coast of the United States when they were accidentally released by the Waddell Mariculture Center in 1988. Commercial shrimpers have subsequently captured these prawns as far south as Florida, although the species is not believed to have become established (McCann *et al.* 1996). Similarly, *P. monodon*, *L. vannamei*, *L. stylirostris* and *M. japonicus* are all known to have escaped culture facilities in Hawaii, although none are known to be locally established (Brock 1992, Eldredge 1994). In the Pacific islands, *M. japonicus* has escaped culture facilities but has failed to establish, while *F. merguiensis* has become established in the wild in Fiji (Eldredge 1994). The effects, if any, that these exotic species have had on wild shrimp populations remain unknown (Briggs *et al.* 2004). There are no reports, however, that escapes of *L. vannamei* have led to any perceivable impact on wild shrimp populations in Thailand.

Despite the fact that *L. vannamei* has been widely introduced with some escapes into the wild, a comprehensive literature review did not find any substantial evidence for it becoming established in the wild outside its natural range, i.e., it may not become an easily "invasive" species. However, further research is needed on the ecology of this species in the wild and its impacts on fishermen's catches and native species (Briggs *et al.* 2004), including long-term monitoring to assess potential impacts of *L. vannamei* on the native shrimp species (Wakida-Kusunoki *et al*. 2011).

9.2 Potential Ecological Impacts

Rapid expansion of aquaculture and the escapes of *L. vannamei* to the natural environment may pose unintended ecological consequences. Ecological consequences may include introduction of non-native pathogens, predation, competition and alteration of local biotic communities. *Litopenaeus vannamei* is already present in marine ecosystems outside of its native range (Wenner and Knot 1991, Senanan *et al*. 2007, Loebmann *et al*. 2010). Although no one has yet documented widespread negative ecological consequences for *L. vannamei* in the region, the outbreak of TSV in cultured *L. vannamei* has been reported in China since 1999 (Tu *et al*. 1999) and in Thailand since late 2002 (Nielson *et al*., 2005). According to Molye and Light (1996), severity of ecological impacts at a local level depends greatly on the compatibility of the organism's physiological requirements with the new environment, as well as the resilience of local biotic communities. Ecological impacts, therefore, need to be assessed on a case-by-case basis. A prerequisite for many ecological interactions of an alien species and local biotic communities is an ability to sustain a population.

The concern is that *L. vannamei* may escape to the wild, displacing native shrimp populations through competition, hybridization and/or the transfer of serious pathogens (e.g., viruses) to native stocks. In the coastal waters adjacent to SAS farms, the perceived risk would be if *L. vannamei* were to occupy the same "ecological niche" as native species (e.g. *F. indicus*), competing for habitat (space) or feed or adversely interfering with the breeding success of native penaeid species. If *L. vannamei* occupies a "vacant" niche or if the abundance of other shrimp species is limited by other factors, it is possible that *L. vannamei* has the potential to add to shrimp catches. However, if *L. vannamei* does not breed and become established in the wild, any positive or negative impacts would likely be localized and limited in time.

9.2.1 Native species likely to be impacted

The escape of *L. vannamei* from culture ponds into the surrounding coastal environment can be expected as a result of accidental release during harvesting. The coastal areas adjacent to shrimp farms in KSA supports fisheries of native shrimp species, and the main risk would be that the escaped *L. vannamei* may cause competition or adversely interfere with the local *F. indicus.* While both *F. indicus* and *L. vannamei* have a typical penaeid life cycle in which the postlarval stages develop in coastal areas, *L. vannamei* has potential to add to shrimp catches. Studies in Thailand concluded that *L. vannamei* that escaped into the wild could not reach maturation and thus could not establish a self-sustaining population (Senanan *et al*. 2007). Thus, if *L. vannamei* does not breed and a large number were to accidently escape into the wild, any impacts are likely to be localizd and limited in scale. In addition, due to the large area of coastal waters available (adjacent to shrimp farms in KSA) to populations of penaeid prawns, it is unlikely that there will be a competition for habitat leading to loss of native species due to establishment of *L. vannamei* in the Red Sea. The potential for alterations to the trophic structure is not believed to be a problem, due to the omnivorous nature of the prawns and their naturally sparse occurrence.

9.2.2 Predation

Litopenaeus vannamei is omnivorous and is very efficient at utilizing the natural productivity of ponds, preying on small invertebrates; however, in the wild the expansive habitat (of the Red Sea) should preclude this behaviour. Prawns are generally often considered prey for larger predatory marine organisms including fish.

9.2.3 Competition

Due to similarities in some aspects of life history characteristics, competition for resources could potentially occur between *L. vannamei* and other native penaeid species, particularly *F. indicus*. While instances of introduced *L. vannamei* escaping and becoming established in the wild are known, for example, from culture facilities in Hawaii, Thailand, Fiji and Mexico, there is no documented evidence suggesting negative impacts due to interspecific interactions (Brock 1992, Eldridge 1995). While a recent study in Thailand concluded that *L. vannamei* could potentially compete with native shrimp species because it approaches food items faster and is more aggressive than some native shrimp species (Chavanich *et al*. 2008), it should be pointed out that this study does not represent a natural situation, as only two individuals were used in an aquarium; however, it may serve as a starting point for further ecological studies (Senanan *et al*. 2009). In addition, a recent study in Brazil concluded that all exotic species (including *L. vannamei*) pose potential risks to the environment because they are able to compete against native species for resources, i.e. *L. vannamei* sharing the same habitat and food items with native penaeids such as the pinkspot shrimp (*Farfantepenaeus brasiliensis*), the southern brown shrimp (*F. subtilis*), and the southern white shrimp (*L. schmitti*) (Loebmann *et al*. 2010). It should be pointed out that the number of individuals collected in the study period may be insignificant (only 4 adult specimens were collected during the "season fishery"); however, the data presented are of notable importance for future research.

9.3 Qualitative Ecological Risk Assessment

9.3.1 Results

The results of a qualitative ecological risk assessment are presented in Table 4. This table is based on the spreadsheet given in Appendix B of the ICES *Code of Practice on the Introductions and Transfers of Marine Organisms 2005* (ICES 2005) and ICES (2012), which has been modified to increase its applicability to this proposal. It outlines the parameters used for assessment, the supporting sections of this report, the assessment of risk for the parameter (estimated on a scale of H.M.L) and an estimate of the certainty for the parameter being assessed (estimated on a scale of VC-VU).

Table 4. Ecological risk assessment criteria for *Litopenaeus vannamei* (modified from ICES 2005 and ICES 2012).

Part 1*. Litopenaeus vannamei* Ecological Risk Assessment Process.

Step 1. Determining the Probability of Establishment

Element rating	**Supportive report sections**	**Probability of establishment (H, M, L)1**	**Level of certainty (VC to VU)**
Estimate of probability of the organism successfully colonising and maintaining a population in the intended area of introduction 3	Section 9.0, 9.1, 9.2, 5.1, 4.9.2	**L**	**RC**
If the organism escapes from the area of introduction, estimate the probability of its spreading 4	Section 9.1, 9.2, 7.0, 5.1	**L**	**RC**
Final rating 5,6		**L**	**RC**

Step 2 Determining the consequences of establishment of *Litopenaeus vannamei*

Element rating **Estimate of magnitude of environmental impacts, if established**	**Supportive report sections**	**Consequences of Establishment (H, M, L)7**	**Level of Certainty (VC to VU)8**
Ecological impact on native ecosystems both locally and within the drainage basin.9	Section 9.0, 9.1, 9.2, 4.1	**L**	**RC**
Genetic impacts on local self-sustaining stocks or populations 10	Section 9.2	**L**	**VC**
Final rating11,12		**L**	**RC**

Step 3 Estimating *Litopenaeus vannamei* risk potential

Component rating	**Supportive report sections**	**Element Rating (H, M,L)**	**Level of Certainty (VC-VU)**
Probability of establishment estimate13	Section 4.1, 9.1, 9.2	L	RC
Consequences of establishment14	Section 9.1, 9.2, 5.0	L	RC
FINAL RISK ASSESSMENT15,16		L	RC

9.4 Conclusions

Litopenaeus vannamei has been widely introduced around the world, and based on our comprehensive literature review we did not find any evidence for it becoming established in the wild outside of its natural range, i.e. it may not become an easily "invasive" species, and also no one has yet documented widespread negative ecological consequences for *L. vannamei.* While transfer of exotic pathogens is potentially a major concern, the effects of escape and establishment of self-sustaining populations of *L. vannamei* are still unknown but considered unlikely.

The life cycle of *L. vannamei* occurs in coastal waters that include open bays and shelves, habitat that is extensive in KSA (shelf area of approximately 95,040km^2 and a 2,640km coastline). The adult phase is more offshore, while the larvae are found in near-coastal detritus beds and in estuaries. The variety of food sources available within this environment makes competition for food also unlikely, as evidenced by the existence of native prawn populations including *F. indicus*.

Although Eldredge (1994), Senanan *et al*. (2007), Loebmann *et al*. (2010) and Wakida-Kusunoki *et al*. (2011) note that escapes of *L. vannamei* and other penaeid shrimps due to pond flooding and other factors have occurred, they are not known to have led to the establishment of large local populations. Thus, although *L. vannamei* has escaped from sites, the extent that such escapes will be able to establish self-sustaining populations in the natural environment is still an open question (Ferreira *et al*. 2009). The fact that previous introductions of *L. vannamei* to other areas over a long period of time (since 1970 and 1980) have not led to the establishment of large local populations indicates a low ecological risk.

Although the escape of *L. vannamei* at some point in time is probable, this risk analysis indicates that if a wild population is established, its impacts are more likely to be beneficial due to its potential to increase local fisheries resources, than detrimental, due to adverse ecological impacts. While lack of data is always a problem in considering the likely ecological impacts of an exotic species introduction, the existing information indicates that the introduction of *L. vannamei* to KSA is unlikely to be detrimental to the local ecosystem.

10 RECOMMENDATIONS

Based on this review, the overall estimated risk potential is LOW, however it is recommended that SAS and its partners adopt an appropriate level of protection (ALOP) that is "very conservative" and with an acceptable level of risk that is "very low'.

In order to reduce the disruption of the production process and enhance profitability, SAS and its partners must be alert at all times to the meteorological conditions at the farm site and the climatic conditions at the locality, including the chances of certain events (including flooding, king/high tides), and implement preventive measures to reduce the numbers of escapees from farms and hatcheries.

SAS and its partners should establish a monitoring program for the presence of *L. vannamei* in the wild to allow detection of the geographic spread of escapees and to assess their impacts, and to communicate the risks associated with any alien species to SAS and its partners and fishermen to help prevent future escapes.

SAS and other relevant parties should support relevant research on *L. vannamei*.

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Pathogen Risk Analysis for the Introduction of Whiteleg Shrimp (*Litopenaeus vannamei*) to the Kingdom of Saudi Arabia

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Executive Summary

In summary, the proposal to introduce whiteleg shrimp to KSA is characterized by the high level of risk management measures that are proposed by the Saudi Aquaculture Society. While there are many serious pathogens of penaeid shrimp, the proposed risk management measures are considered to be sufficient to remove all of these pathogens from consideration as potential hazards.

This risk analysis examines the pathogen risks associated with the proposed importation of adult broodstock whiteleg shrimp (*Litopenaeus vannamei*) to the Kingdom of Saudi Arabia (KSA) for aquaculture development. The risk analysis is conducted at the request of the Saudi Aquaculture Society (SAS), Jeddah and is a component of a *Proposal for the Introduction of whiteleg shrimp* (*Litopenaeus vannamei*) *to the Kingdom of Saudi Arabia* for aquaculture development that is being prepared by the SAS for consideration by the Government of KSA.

There have been only a few dozen comprehensive risk analyses conducted globally on the pathogen risks posed by the introduction of a live aquatic animal for aquaculture development. This is the first such risk analysis conducted in the Middle East region and its commissioning, along with the preparation of a formal proposal for species introduction and the commissioning of associated genetic and ecologic/environmental risk assessments demonstrates an extremely high level of social responsibility by the SAS.

The commodity to be imported is specific pathogen free (SPF) broodstock whiteleg shrimp which will be sourced from a list of Approved Suppliers.

The risk analysis has highlighted the high level of risk management measures that are proposed by the SAS. These include: (i) use of specific pathogen free (SPF) shrimp; (ii) establishment of a list of Approved Suppliers of SPF broodstock *L. vannamei* to KSA; (iii) agreement in principle of suppliers to on-site inspection of facilities; (iv) list of Approved Importers; (v) limited time period during which importation will be permitted; (vi) high security quarantine of imported broodstock; (vii) monitoring and diagnostics testing of broodstock while in quarantine and Biosecure Breeding Centers (BBCs); (viii) release of only F1 postlarvae to grow-out ponds; (ix) monitoring and diagnostics testing of shrimp in grow-out ponds and (x) contingency planning in case of disease caused by an exotic pathogen. These risk management measures represent world`s best practice for biosecurity arrangements following translocation of aquatic animals.

The risk analysis considers 30 pathogens/pathogen groups (possible hazards) that have been reported globally from *L. vannamei* or other penaeid shrimp. While many of these possible hazards are serious pathogens of penaeid shrimp, the analysis suggests that the risk management measures proposed by SAS are very likely to be sufficient to remove all of these pathogens from consideration as hazards that could be released into the environment of KSA.

In addition, the following recommendations are made:

1. The Government of KSA should recognize and endorse the suggestion that the national appropriate level of protection (ALOP) should be "high" or "very high" and with an acceptable level of risk (ALOR) that is "low" or "very low".

2. As the risk assessment is highly dependent upon the risk management measures proposed by SAS, monitoring systems must be established to ensure that all risk management measures are fully and effectively implemented.
3. To minimize the risk of WSSV, TSV and other pathogens already present in KSA gaining entry, it is recommended that the initial high security quarantine facility and the Biosecure Breeding Centers (BBCs) be located as far away as possible from existing shrimp farms.
4. Saudi shrimp growers should strive to become self-sufficient in broodstock and postlarval production as soon as possible by setting up breeding and genetic improvement programs for *L. vannamei*, as this will further reduce the risk of pathogen introduction.
5. To better understand the potential for pathogen transfer between cultured and wild stocks, baseline studies of diseases of decapod crustaceans in the vicinity of aquaculture facilities should be conducted. Such monitoring will also help to detect any transfer of introduced exotic pathogens from *L. vannamei* to wild crustacean populations.
6. The SAS should conduct susceptibility testing of local penaeids to check for the presence of cryptic or unknown pathogens in the imported broodstocks.

Acronyms and Abbreviations

ADMA	Aquacuture Department of the Ministry of Agriculture (Kingdom of Saudi Arabia)
AHNS	Acute hepatopancreatic necrosis syndrome
ALOP	Appropriate level of protection
ALOR	Acceptable level of risk
BBC	Biosecure breeding center
BMN	Baculoviral midgut gland necrosis
BMNV	Baculoviral midgut gland necrosis virus
BP	Baculovirus penaei
EMS	Early mortality syndrome
FCR	Food conversion ratio
FAO	Food and Agriculture Organization of the United Nations
F1	First generation following broodstock (F0)
GATT	General Agreement on Tariffs and Trade
GAV	Gill associated virus
HH	High health
HPV	Hepatopancreatic parvovirus
ICE	Integrase contraining element
ICES	International Council for the Exploration of the Sea
IHHNV	Infectious hypodermal and hematopoietic necrosis virus
IMNV	Infectious myonecrosis virus
IRA	Import risk analysis
KSA	Kingdom of Saudi Arabia
LOVV	Lymphoid organ vacoulization virus
LSNV	Laem-Singh virus
MBV	Monodon baculovirus
MCMS	Mid-crop mortality syndrome
MOV	Mourilyan virus
MrNV	Macrobrachium rosenbergi nodavirus
MSGS	Monodon slow growth syndrome
NHP	Necrotizing hepatopancreatitis
OIE	World Organisation for Animal Health
PL	Postlarvae
PMB	Penaeus monodon-type baculovirus
PRA	Pathogen risk analysis
PvNV	Litopenaeus vannamei nodavirus
RDS	Runt deformity syndrome
RLB-MHD	Rickettsia-like bacteria-Milky hemolymph disease
RNA	Ribonucleic acid
RPS	Rhabdovirus of penaeid shrimp
SAS	Saudi Aquaculture Society
SMVD	Spawner-isolated mortality virus disease
SOPs	Standard operating procedures
SPF	Specific pathogen free

SPR	Specific pathogen resistant
SPS Agreement	Sanitary and Phytosanitary Agreement
SPT	Specific pathogen tolerant
TAADs	Transboundary aquatic animal diseases
TS	Taura syndrome
TSV	Taura syndrome virus
USMSFP	United States Marine Shrimp Farming Program
WFS	White feces sysdrome
WSD	Whitespot disease
WSSV	White spot syndrome virus
WTD	Whitetail disease
WTO	World Trade Organization
XSV	Extra small virus
YHD	Yellowhead disease
YHV	Yellowhead virus

LIST OF ANNEXES

1.0 Introduction

1.1 Purpose

This pathogen risk analysis (also termed an import risk analysis, IRA) is commissioned by the Saudi Aquaculture Society (SAS) as a component of a proposal to introduce broodstock whiteleg shrimp (*Litopenaeus vannamei*)[3] sourced from approved specific pathogen free (SPF) facilities into the Kingdom of Saudi Arabia (KSA) for aquaculture development in approved shrimp culture facilities along the Red Sea coast.

1.2 Terms of Reference

Terms of Reference (TOR) for the risk analysis are as follows:

The consultant team for the pathogen risk analysis will:

1. Conduct a comprehensive analysis of the pathogen risks associated with the proposed introduction of *P. vannamei* into Saudi Arabia.

2. The risk analysis will follow the general methods outlined in FAO Fisheries and Aquaculture Technical Paper No. 519 (in particular, the paper by Arthur and Reantaso, 2008), in No. 519/1 (in particular, section 4.1: Overview of the pathogen risk analysis process) and will conform to the import risk analysis process as outlined in the *Aquatic Animal Health Code* of the World Organisation for Animal Health (OIE, 2012) and, as far as possible, with the guidelines given in the *Code of Practice for the Introductions and Transfers of Marine Organisms* (ICES, 2005) and the procedures outlined in Annex B: Risk Review of the ICES Code (as given in ICES, 2012, Annex 6 , Appendix B: Risk Review, pp. 356-262).

3. The risk analysis will be based on a commodity description formulated with the proponents that will involve the use of specific pathogen free (broodstock) of *P. vannamei* derived from pre-approved suppliers, and the use of other risk mitigation measures (diagnostics testing, stringent quarantine, etc.) as developed in the draft proposal framework.

4. The consultants will deliver a draft version in Microsoft Word (electronic format) of the risk analysis to the Saudi Aquaculture Society (SAS) by 1 December 2012, and will provide a final version in similar format addressing any comments or corrections resulting from independent review.

[3] The genus *Penaeus* was revised by Pérez Farfante and Kensley (1997), who raised the Subgenus *Litopenaeus* (and other subgenera) to generic rank. Although some authors are reluctant to accept this change (e.g. Flegel, 2007), their arguments do not appear to hold taxonomic validity (see McLaughlin *et al.*, 2008). We thus encourage readers to become accustomed to using these new taxonomic combinations.

1.2 Background

Past experience has amply demonstrated that the international movement of live penaeid shrimp of unknown or uncertain health status is a high risk activity that has been responsible for the spread of many serious shrimp diseases to new geographical areas, often with serious economic consequences (see for example, Briggs *et al*., 2004; Bondad-Reantaso and Subasinghe, 2005; Bondad-Reantaso *et al*., 2005a,b; Flegel, 2006b; Biosecurity Australia, 2009; Rodgers *et al*., 2011). It is therefore clear that any proposal to introduce an exotic species of penaeid shrimp to the Kingdom of Saudi Arabia (KSA) must include rigorous guarantees and risk management measures to ensure that the stocks to be imported are free from serious transboundary aquatic animal diseases (TAADs).

However, it is also clear that the application of risk management measures as proposed by the SAS (see Section 8.0) will reduce the risk of introducing new pathogens to a level that is compatible with the risk tolerance of the majority of countries. It is therefore possible to introduce whiteleg shrimp to KSA in a manner that most would consider "prudent and cautious", and which would remain within the national appropriate level of protection (ALOP), which for KSA remains undefined, but is likely to be either "high" or "very high" level (i.e. a "low" or "very low" risk tolerance).

The diseases of penaeid shrimp are relatively well known thanks to their importance as aquaculture species and have been considered in detail in several previous risk analyses (e.g. Sukrakanchana *et al*., 2005; Bondad-Reantaso *et al*., 2005a; Biosecurity Australia, 2009) and are further discussed in this report. It is sufficient to state that penaeid shrimp are infected by a number of untreatable serious diseases (mainly of viral etiology), most of which are listed by the World Organisation for Animal Health (OIE, 2012a) and whose introduction into KSA can be avoided by use of appropriate biosecurity measures (in particular, sourcing of broodstocks from approved specific pathogen free (SPF) facilities (see Section 8.1).

With regard to the introduction of shrimp to new geographic areas for aquaculture development, Briggs *et al.* (2004) recommended that:

- all facilities exporting shrimp should have a minimum two-year disease-free status, are certified as such and can submit independent, qualified certification of their status;
- properly collected samples of imported shrimp should be submitted to certified disease diagnostic laboratories for confirmation of disease-free status, while maintaining shrimp in biosecure quarantine facilities before release into the environment; and
- cohabitation trials of all imports should be conducted with indigenous shrimp.

Flegel (undated, 2006b) also stressed that countries considering the introduction of *L. vannamei* or any other crustacean should follow the full International Council for the Exploration of the Sea (ICES) protocol for introductions and transfers of marine organisms (see ICES, 2005, 2012), with the addition of cohabitation tests employing important native crustacean species. Other authors (e.g. Andrade, 2011) have proposed that more rigorous schemes combining the use of SPF stocks and ICES-like quarantine and testing protocols should be used for the development of new SPF lines and genetically improved stocks of shrimp, and *inter alia*, the introduction of new species for aquaculture development. Such an approach is being proposed by SAS for the introduction of whiteleg shrimp to KSA.

2.0 Benefits to be Derived from the Introduction of *Litopenaeus vannamei*

Litopenaeus vannamei offers advantages over the presently cultured Indian white prawn (*Fenneropenaeus indicus*) that include:

- immediate availability of broodstock of known health history from SPF facilities that are certified to be free from certain major pathogens;
- a readily closed life cycle that will allow a broodstock development and genetic improvement program free of serious pathogens to be established in KSA;
- a reliable supply of postlarvae (PL) free from serious pathogens;
- ability to use broodstock for more than a single reproductive cycle;
- wide acceptability in international markets;
- possibility of better and faster growth than *F. indicus*; and
- existence of some white spot syndrome virus (WSSV) tolerant stocks, which may allow for improved survival should it prove impossible to eradiate existing whitespot syndrome virus (WSSV) infections from grow-out ponds.

Following successful pilot testing under local culture conditions, the longterm goal of the SAS is to develop SPF lines of hatchery broodstock within the country within the context of breeding and genetic improvement programs. This will minimize the need for further importations of broodstock or postlarvae (PL) from abroad with the inherent risks of pathogen translocation.

A summary of the advantages and disadvantages of culturing *L. vannamei* is given in Table 1.

Table 1. Summary of advantages and disadvantages of the culture of *Litopenaeus vannamei*.

Characteristic	Advantages	Disadvantages
Breeding and Domestication	• Closed life cycle permits breeding and genetic selection programs to be readily established, eliminating problems associated with use of wild broodstock and/or PL collection. • Can be spawned up to 8 times. • Females produce extremely high numbers of nauplii, greatly reducing	• SPF animals often have high mortality in disease-laden environments.

	the number of broodstock needed as compared to *F. indicus.*	
Growth rate	• Rapid grow up to 20 g	• Growth rate slows after reaching 20 g, making production of large-sized shrimp slower.
Stocking density	• Easier to culture in very high densities (60-150/m^2, but up to 400/m^2) • Not as aggressive as *P. monodon* or *L. stylirostris*.	• Very high stocking densities require high control over pond/tank management practices and are high-risk strategies.
Salinity tolerance	• Tolerant of a wide range of salinities (0.5-45 ppt) and more amenable to inland culture sites than *P. monodon* or *L. stylirostris*.	• None
Temperature tolerance	• Highly tolerant of low temperatures (to 15 °C), enabling culture in cold season.	• None
Dietary protein requirements	• Requires lower protein feed (20-35%) than *P. monodon* or *P. stylirostris* (36-42%), resulting in reduced operational costs and amenability for closed, heterotrophic systems. • Food Conversion Ratios (FCRs) are lower at 1.2 compared to 1.6.	• None
Disease tolerance	• Some WSSV-tolerant SPF stocks available.	• Highly susceptible to and a carrier of TSV, WSSV, IHHNV and YHV/LOVV.
Larval Rearing	• Higher survival rates in hatchery (50-60%) as compared to *P. monodon* (20-30%).	• None
Post-harvest characteristics	• If treated with ice, are resistant to melanosis.	
Marketing	• Generally preferred in the United States due to taste. Strong demand in Asia. • Meat yield is higher (66-68%) than for *P. monodon* (62%)	• *P. monodon* and *L. stylirostris* can grow to larger size, commanding higher price than *L. vannamei*. • High competition on international markets for *L. vannamei* as production is world-wide.
Origin	• SPF, SPR and SPT stocks are readily available, greatly reducing the likelihood of pathogen introduction.	• Exotic to KSA, and thus risks due to pathogen, genetic and ecological/environmental impacts must been considered.

3.0 Alternate Strategies

The primary alternate strategy available to the SAS is to continue culture of *Fenneropenaeus indicus*. However, the fact that broodstocks of *F. indicus* are now infected with WSSV would necessitate "cleaning" of broodstocks or their complete re-establishment from wild populations, a costly and time-consuming undertaking that would require up two or more years to achieve. At the same time, this would not address the current problems of high susceptibility to WSSV, inability to spawn broodstock more than once, and continued vulnerability of breeding programs to WSSV infection from enzootic infections in grow-out ponds and the natural environment.

Another alternate strategy would be the use of blue shrimp (*Litopenaeus stylirostris*), a less widely cultured exotic species for which broodstock from SPF facilities are available. However, such SPF facilities are few in number, stocks generally have less history under SPF conditions, and health guarantees may therefore be less reliable.

The use of giant tiger prawn (*Penaeus monodon*), a species native to the Red Sea and previously widely cultured in the Asia-Pacific, is also possible. However, this would also involve importations of SPF stocks (again, with a more limited number of potential suppliers) and would be more difficult to implement, as a closed life cycle for *P, monodon* is more difficult to achieve than for *L. vannamei* or *L. stylirostris*. Attempts were made during the early years of aquaculture development in KSA to culture *P. monodon* and *P. semisulcatus* (green tiger prawn), was but were abandoned due to the generally higher salinities found in the country (source: http://www.fao.org/fishery/countrysector/naso_saudiarabia/en).

The importation of whiteleg shrimp offers many technical and marketing advantages to the SAS that are not met by alternative species (see Section 2.0). At the same time, SAS members will continue to attempt to "clean" WSSV infections from existing broodstocks of Indian white shrimp. This approach will allow aquaculturists to continue to culture Indian white shrimp for specialized markets (e.g. the market for large-size prawns in Japan), if they so desire, while at the same time providing the opportunity to access the new markets and culture advantages offered by SPF whitleg shrimp.

4.0 Summary of Risk Management Measures Proposed by the Saudi Aquaculture Society

The proposed importation is characterized by a high level of risk management that is designed to ensure that (i) serious pathogens are not present in imported broodstocks of *L. vannamei*; and (ii) that in the unlikely event a serious pathogen does enter the country with imported broodstock, it will not gain access to aquaculture grow-out ponds or the natural environment where it could possibly establish in wild crustaceans.

The risk mitigation measures to be applied are (these are described in more detail in Section 8):

- Sourcing of whiteleg shrimp only from SPF facilities that meet minimum standards to ensure reliability of claims of freedom from specific pathogens, as well as high probability of freedom from certain non SPF-listed pathogens (establishment of a list of Approved Suppliers).
- Allowing importation only to approved aquaculture facilities in KSA that meet minimum standards for biosecurity and the use of standard operating procedures (SOPs) (establishment of a list of Approved Importers).
- Allowing importation to occur only for a limited time period (3 years), after which further importations will be considered only if necessary to allow further development of breeding and genetic improvement programs.
- Initial holding of imported broodstock (F0) in high-security quarantine facilities that meet minimum standards of construction and operation. Construction and operating standards will also minimize the possibility of diseases present in the external environment gaining entry to the facility. Upon satisfactory completion of diagnostics testing, broodstock will be moved to Biosecure Breeding Centers (BBCs) having a similar level of biosecurity.
- Monitoring and diagnostics testing of broodstock while in quarantine facility and BBCs.
- Only F1 postlarvae (PL) to be stocked in grow-out ponds. Imported broodstock will be destroyed and disposed of in a sanitary manner once they are no longer useful for breeding.
- Monitoring of health status of F1 shrimp while in grow out.
- Preparation of contingency plans to limit or eradicate any exotic pathogen that escapes quarantine through vertical transmission from broodstock to F1 generation.

5.0 Examination of Pathogen Risks

5.1 Commodity Description

Table 2 defines the precise nature of the commodity to be imported.

Table 2. Commodity description for the proposed introduction of whiteleg shrimp (*Litopenaeus vannamei*) to the Kingdom of Saudi Arabia.
Species to be introduced*:Litopenaeus vannamei* (whiteleg shrimp)
Proposed date of importation: beginning January 2013, for a period of 3 years
Life cycle stage to be imported: Broodstock only
Importers: Participating members of the Saudi Aquaculture Society, Jeddah, Saudi Arabia (List of Approved Importers)
Exporter: Approved SPF facilities (list of Approved Suppliers to be developed)
Source*:* High security SPF culture facilities (List of Approved Suppliers)
Proposed number of shipments: as required

***Volume*:** as required
Proposed destination: participating shrimp farms along the Red Sea coast, KSA

5.2 International and Regional Context of the Risk Analysis

With the liberalization of international trade through the General Agreement on Tariffs and Trade (GATT), the establishment of the World Trade Organization (WTO) and its *Agreement on the Application of Sanitary and Phytosanitary Measures* (SPS Agreement), WTO member countries are now required to use the risk analysis process as a means to justify any restrictions on international trade beyond those specified by the Aquatic Animal Health Code (OIE, 2012a) based on risks to human, animal or plant health (see WTO 1994, Rodgers 2004). Risk analysis is thus an internationally accepted standard method for assessing whether trade in a particular commodity (e.g. a live aquatic animal or its product) poses a significant risk to human, animal or plant health, and if so, what measures could be adopted to reduce that risk to an acceptable level.

The general framework for import risk analysis for live aquatic animals and their products is laid out in the World Organization for Animal Health's (OIE) *Aquatic Animal Health Code* (OIE, 2012a), and the Kingdom of Saudi Arabia, as a member of both the OIE and the WTO, is obligated to follow OIE and WTO procedures.

Although not obligatory to KSA, the International Council for the Exploration of the Sea's (ICES) *Code of Practice for the Introductions and Transfers of Marine Organisms 2005* (ICES 2005, 2012) is widely accepted globally as the key framework for assessing proposals to introduce exotic aquatic species to new environments outside their native range. Among others, the ICES Code addresses the evaluation of potential genetic, ecologic and pathogen risks associated with the translocation of aquatic organisms. Conformation with the recommendations of the ICES Code can thus considered best practice when introducing new species for aquaculture development.

5.3 Aquatic Animal Biosecurity Framework and Biosanitary Requirements of the Kingdom of Saudi Arabia

Aquatic biosecurity in the Kingdom of Saudi Arabia is the responsibility of the Aquaculture Department of the Ministry of Agriculture (ADMA). The ADMA convenes the recently established Biosecurity Committee, comprised of representatives of key aquaculture producers and relevant government staff to consider proposals for the introduction of new aquatic species. The Biosecurity Committee is also implementing a program of actions to upgrade farm-level biosecurity against serious pathogens

5.4 Appropriate Level of Protection

The appropriate level of protection (ALOP, also referred to as the "acceptable level of risk", ALOR), is the level of protection deemed appropriate by a country in establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory (see WTO, 1994). As such, establishing an ALOP is a political, rather than a scientific decision, and must be made at the highest level of government. Where no formal statement of ALOP exists, a country's ALOP may often be defined by its practices in protecting its human, animal and plant life from hazards, as reflected in its legislation and other official documents, policies and procedures (see Wilson, 2000). Although the Government of the Kingdom of Saudi Arabia has not issued a formal statement as to ALOP, it is clear that its general policy towards risk for other (non-fisheries) commodities is rather stringent. Thus, a conservative approach to protecting KSA's aquatic animal health status is suggested (i.e. a "high" or "very high" ALOP) and the level of risk considered acceptable for KSA is characterized as "low" or "very low" (see AQIS, 1999).

5.5 Precautionary Approach

The concept of the precautionary approach is widely used in fisheries management and elsewhere where governments must take action based on incomplete knowledge (see Garcia, 1996). The *Code of Conduct for Responsible Fisheries,* Section 7.5.1 (FAO, 1995) states that:

> "States should apply the precautionary approach widely to conservation, management and exploitation of living aquatic resources in order to protect them and preserve the aquatic environment. The absence of adequate scientific information should not be used as a reason for postponing or failing to take conservation and management measures."

In the assessment of potential pathogen-related risks associated with the proposed introduction or transfer of a live aquatic animal species, a precautionary approach requires that both the importing and exporting nations act responsibly and conservatively to avoid the introduction of potential "pest" species and the spread of serious pathogens (see Arthur *et al.* 2004).

6.0 Risk Analysis Methods

This risk analysis was undertaken by Drs J. Richard Arthur (International Consultant on Risk Analysis, Canada) and Victoria Alday-Sanz (International Shrimp Health Expert, Barcelona, Spain).

Background information and scope for the risk analysis was developed as part of a proposal to introduce whiteleg shrimp (*L. vannamei*) to KSA for aquaculture development that has been prepared by a project team formulated by the SAS. In addition to Drs Arthur and Alday-Sanz, the team includes Dr. Rodger W. Doyle, Genetic Computation Ltd., Canada (genetics risk analysis) and Drs Peter B. Mather, David Hurwood and Satya Nandal, Queensland University of Technology, Australia, (ecologic/environmental risk analysis).

Following completion of the risk analysis, the draft document was submitted to independent external expert review by Dr Ben Diggles, Digsfish Services, Pty Ltd., Queensland, Australia. While the comments and suggestions of Dr Diggles have, where possible been addressed, the conclusions and recommendations presented herein, and any errors, remain solely those of the consultants. A copy of Dr. Diggles's comments and the authors' responses is appended as Annex 1.

The results of this pathogen risk analysis were incorporated, along with the results of genetic and ecologic/environmental risk assessments, into the proposal, was be presented to the Government of the Kingdom of Saudi Arabia on 11-12 December for consideration of approval.

6.1 General Approach

The general approach used in the pathogen risk analysis follows that outlined by the OIE (2012a), AFFA (2001), Arthur e*t al*. (2004, 2009) and Arthur and Bondad-Reantaso (2012).

The outstanding feature highlighted by this risk analysis is the high level of risk mitigation measures being proposed by the proponent (the Saudi Aquaculture Society, SAS) (see Section 8). These measures meet (and in some cases exceed) the recommendations for introductions and transfers developed by the International Council for the Exploration of the Sea (ICES, 2005, 2012) and include:

- Use of SPF broodstock sourced from a list of Approved Suppliers who have been screened to meet a rigorous set of criteria with regard to assuring guarantees of freedom from specific pathogens (Sections 8.1-8.3).
- Establishment of a list of Approved Importers (Section 8.4).
- Limited time period during which importation will be permitted (Section 8.5)
- Quarantine of imported broodstock in high security quarantine facilities and Broodstock Breeding Centers (BBCs), with associated diagnostics testing and health monitoring (Sections 8.6 and 8.7).
- No release of imported broodstock from quarantine or BBCs - only F1 postlarvae will be released to grow-out ponds (Section 8.8).
- Monitoring and diagnostics testing of F1 shrimp during growout. (Section 8.9).
- Contingency plan should any serious pathogen resulting from the introduction appear in broodstock or growout ponds (Section 8.10).

The risk analysis process as outlined by OIE (2012a) includes:

- hazard identification
- risk assessment
- risk management
- risk communication

The current risk analysis will address hazard identification and, if any potential hazards are identified from among the list of possible hazards, will continue with risk assessment for these hazards, and if necessary due to an unacceptable level of risk, will examine additional risk management measures above those outlined in Section 8. Risk communication will be the responsibility of the Saudi Aquaculture Society and/or the Government of the Kingdom of Saudi Arabia.

6.2 Risk Analysis Methods

6.2.1 Hazard Identification

A *hazard* is any pathogenic agent that could produce adverse consequences upon the importation of a commodity (a live aquatic animal or its product), while *hazard identification* is the process of identifying pathogens that could potentially be introduced in the commodity considered for importation. In this analysis, the hazard identification process involves consideration of all pathogens/pathogen groups that could plausibly infect whiteleg shrimp (*Litopenaeus vannamei*) or other penaid species on a global basis and evaluating whether these "possible hazards" are identified hazards that would require risk assessment. In considering each possible hazard, the risk management measures proposed by the SAS are taken into consideration at the start of the risk analysis, rather than added (if needed) during the risk management stage of the risk analysis to reduce the risk posed by hazards that pose unacceptably high risk. If no "possible hazards" are identified as hazards, the risk analysis is completed.

6.2.2 Risk Assessment

Risk assessment is the process of identifying and estimating the risks associated with the importation of a commodity and evaluating the consequences of taking those risks. It consists of:

- *Release assessment* - The process of describing the biological pathway(s) necessary for an importation activity to "release" (that is, introduce) a hazard into a particular environment, and estimating the likelihood of that complete process occurring.[4] Important factors that need to be considered in release assessment include: (a) biological factors, such as the susceptibility of the animals from which the commodity is derived to their potential hazards and their infectiousness; the means of transmission of the potential hazards; the infectivity, virulence and stability of the potential hazards; and the routes of infection; and (b) country factors, such as an evaluation of the exporting country's aquatic animal health services, the incidence and/or prevalence of the disease, farming and husbandry practices, and geographical and environmental characteristics; and (c) commodity factors, such as the ease of contamination; relevance of any processes and production methods; the effect of processing, storage and transport; and the quantity of commodity to be imported.
- *Exposure assessment* - The process of describing the biological pathway(s) necessary for exposure of humans and aquatic and terrestrial animals in the importing country to the hazards and estimating the likelihood of the exposure(s) occurring, and of the spread or establishment of the hazards. The factors to be considered include those

[4] Most countries consider that the "release" pathways terminate and the "exposure" pathways begin at the importing country's border, a practice that is followed in this risk analysis.

considered for the release assessment. Additional factors include: (a) country factors such as the presence of potential intermediate hosts or vectors, customs and cultural practices; and (b) commodity factors, such as the intended use of the imported animals and waste disposal practice.

- *Consequence assessment* - When an exposure assessment determines that there is more than a negligible risk of introduction of a disease agent, a consequence assessment will consider the possible biological, environmental and economic consequences that could result from the disease agent being released into the natural environment. Information required for consequence assessment includes estimation of the potential biological, environmental and economic consequences associated with the entry, establishment and spread of the hazard. These include both direct consequences such as outcome of infection in domestic and wild animals and their populations (morbidity and mortality, production losses, animal welfare) and public health consequences; and indirect consequences, such as economic considerations (control and eradication costs, surveillance costs, potential trade losses (such as embargoes, sanctions and lost market opportunities)), and environmental considerations (amenity values, social, cultural and aesthetic conditions).

6.2.3 Risk Management

Risk management is the process evaluating the estimated risk to determine if it is significant to the importing country, and if it is, of identifying, documenting and implementing measures that can be applied to reduce or eliminate the level of risk. Risk management measures for a given hazard (risk mitigation) are only considered when the estimated level of risk for the hazard exceeds the country's ALOR. The level of unmitigated risk, the ALOR and the individual nature of the hazard will determine what risk management measures, if any, can be applied to reduce the risk to an acceptable level.

In the present risk analysis, the proponents have proposed extensive risk management measures to reduce the likelihood that serious pathogens will be introduced and become established in KSA. *The risk analysis takes these measures into consideration during the hazard evaluation process* and will only examine additional risk management measures should the proposed measures be insufficient to reduce risk to an acceptable level (i.e. a risk estimate that exceeds "low" or "very low").

6.2.4 Risk Communication

Risk communication is the process by which information and opinions regarding hazards and risks are gathered from potentially affected and interested parties during a risk analysis, and by which the results of the risk assessment and proposed risk management measures are communicated to the decision makers and interested parties in the importing and exporting countries. It is a multidimensional and iterative process and should ideally begin at the start of the risk analysis process and continue through out (OIE, 2012a). Good risk communication is thus an essential component of any risk analysis; however, it is not an activity of the current PRA, which is itself a component of the larger risk analysis process. Information on achieving good risk communication is given in Arthur *et al.* (2004, 2009).

6.2.5 Terms used to describe the probability of an event occurring

In assessing the likelihood of an adverse advent occurring, the descriptive definitions for qualitative likelihoods used in this risk analysis follow the six-category system given by AFFA (2001):

- High: The event would be very likely occur
- Moderate: The event would occur with an even probability
- Low: The event would be unlikely to occur
- Very low: The event would be very unlikely to occur
- Extremely low: The event would be extremely unlikely to occur
- Negligible: The event would almost certainly not occur

6.2.6 Terms used to describe the consequences of an event occurring

The terms used to describe the consequences of an adverse event occurring follow those outlined by AQIS (1999):

- Catastrophic: Establishment of disease would be expected to cause significant economic harm at a national level, and/or cause serious and irreversible harm to the environment.
- High: Establishment of disease would have serious biological consequences (e.g., high mortality or morbidity) and would not be amenable to control or eradication. Such diseases could significantly harm economic performance at an industry level and/or may cause serious harm to the environment.
- Moderate: Establishment of disease would have less pronounced biological consequences and may be amenable to control or eradication. Such diseases could harm economic performance at an industry level and/or may cause some environmental effects, which would not be serious or irreversible.
- Low: Establishment of disease would have mild biological consequences and would normally be amenable to control or eradication. Such diseases may harm economic performance at an industry level for a short period and/or may cause some minor environmental effects, which would not be serious or irreversible.
- Negligible: Establishment of disease would have no significant biological consequences and would require no control or eradication. Such diseases would not affect economic performance at an industry level and would cause negligible environmental effects.

6.2.7 Combining Likelihood Estimates

Matrices used for risk combining qualitative estimates of likelihood of events occurring, combining probability of likelihood or release and likelihood of exposure, and for combining likelihood of release x exposure with estimates of consequence follow those given in Arthur and Bondad-Reantaso (2012) and are presented below in Tables 3 and 4.

Table 3. Matrix for Combining Risk Likelihoods. This matrix is used for (i) combining likelihoods of completing steps along a release or exposure pathway and (ii) combining likelihood of release and likelihood of exposure.

Estimated Likelihood of Event 2 ↓ / Estimated Likelihood of Event 1 →	**Negligible**	**Very Low**	**Low**	**Moderate**	**High**
High	Negligible	Very Low	Low	Moderate	High
Moderate	Negligible	Very Low	Low	Low	
Low	Negligible	Very Low	Very Low		
Very Low	Negligible	Negligible			
Negligible	Negligible				

Table 4. Matrix for Estimating Total Risk Posed by a Hazard.

Estimated Likelihood of Release and Exposure ↓ / Estimated Consequence of Release and Exposure →	**Negligible**	**Low**	**Moderate**	**High**	**Catastrophic**
High	Negligible Risk	Low Risk	Moderate Risk	High Risk	Extreme Risk
Moderate	Negligible Risk	Low Risk	Moderate Risk	High Risk	Extreme Risk
Low	Negligible Risk	Very Low Risk	Low Risk	Moderate Risk	High Risk
Very Low	Negligible Risk	Negligible Risk	Very Low Risk	Low Risk	Moderate Risk
Negligible	Negligible Risk	Negligible Risk	Negligible Risk	Negligible Risk	Negligible Risk

7.0 Diseases of Penaeid shrimp

7.1 Overview of Diseases of Penaeid Shrimp

Penaeid shrimp are hosts to a wide range of pathogens and parasites (e.g. see Table 6). With regard to aquaculture development, the most important of these are viruses, many of which are listed by the World Organisation for Animal Health (OIE 2012a) as having the potential to cause diseases having major economic, environmental and/or social impacts. Many of these viruses have been spread domestically, regionally and internationally (through both legal and illegal channels) through the ill-considered introduction and transfer of live broodstock and PL to fuel the rapid expansion of aquaculture, often with disastrous consequences. As noted by (Lightner, 2011a), the global spread of serious shrimp viruses such as IHHNV, TSV and WSSV was to some extent due to the emergence of "new" shrimp diseases and their spread to new countries and regions prior to their recognition by the industry and the subsequent development of reliable diagnostic methods for them. Shrimp populations in the new areas where such pathogens are introduced are often totally naive, lacking in innate resistance, and thus much more susceptible than are populations having a long history of exposure. Regional pandemics in Asia and the Americas due to viruses such as WSSV, TSV, IHHNV, IMNV and YHV have collectively cost the industry at least 10 billion dollars in losses (Table 5), as well as having serious impacts on the social well being of rural populations.

Table 5. Estimated losses due to certain OIE-listed viral diseases of penaeid shrimp (modified from Lightner, 2011a)

Virus	Region	Year of Emergence	Estimated losses to industry (US$)
IHHNV[1]	Americas	1981	0.5-1 billion
YHS	Asia	1991	0.5 billion
TSV	Americas	1991/1992	1-2 billion
	Asia	1999	0.5-1 billion
WSSV[2]	Americas	1992/1993	6 billion
	Asia	1999	1-2 billion
IMNV	Americas	2004	100-200 million
	Asia	2006	?

[1] Includes the Gulf of California fishery for *Litopenaeus stylirostris*.
[2]More recently estimated to be closer to US$ 15 billion (see Lightner *et al.*, 2012)

7.2 Status of Knowledge of Pathogens and Parasites of *Litopenaeus vannamei*

As *L. vannamei* is by far the most widely cultured penaeid shrimp globally and has been the subject of extensive efforts to develop specific pathogen free (SPF), specific pathogen resistant (SPR) and specific pathogen tolerant (SPT) stocks, its pathogens and parasites are probably the most extensively documented among all penaeid shrimp species. A listing of pathogens known to infect *L. vannamei* includes at least 30 viruses, bacteria and parasites and several emerging diseases whose exact etiology have not yet been elucidated (see Table 7).

7.3 Specific Pathogen Free stocks of *Litopenaeus vannamei*

The world's first population of SPF shrimp was developed by the United States Marine Shrimp Farming Program in 1989 (USMSFP, undated).

7.3.1 Definitions of SPF, SPR and SPT shrimp

There is much confusion among aquaculturists as to the meaning of specific pathogen free (SPF), specific pathogen resistant (STR) and specific pathogen tolerant (SPT) as applied to penaeid shrimp. The following definitions follow those given by the United States Marine Shrimp Farming Program (USMSFP, undated).

Specific pathogen free (SPF) - denotes shrimp that have a documented history (at least two years long) of being free from all pathogens specified on a supplier' listing. The status of the shrimp changes depending on the level of biosecurity under which they are maintained (they are only SPF while inside a Nucleus Breeding Center, NBC). Note that:

- Broodstock supplied by different facilities may have differing lists of pathogens for which freedom is guaranteed.
- To be included on an SPF list, a pathogen must be reliably diagnosed, physically excluded by the facility, and a significant threat to the industry.
- SPF shrimp have the possibility of being infected by other, non-listed organisms, some of which may have the potential to become pathogenic under different culture situations or in different species.[5]
- The health status of an SPF shrimp changes as soon as it leaves the supplying SPF facility. It is now known as having *high health* (HH) status due to the lowered biosecurity. HH shrimp can regain SPF status if transferred directly to a high biosecurity facility and a new health record is established based on absence of disease and rigorous diagnostics testing for specified pathogens over a period of at least two years.
- SPF shrimp do not necessarily display improved resistance to or tolerance of infection.

[5] The special problem of the potential for cryptic pathogens to be present in SPF stocks due to the tendency for crustaceans to carry life-long, persistent viral infections without any gross or histological signs of infection (due to the phenomenon of viral accommodation in crustaceans) has been discussed by Flegel (2006b), who notes that at least three major pathogens (IHHNV, TSV and WSSV) have been translocated globally because this characteristic has not been widely recognized by aquaculturists who have moved grossly normal broodstock and PL. Because of the additional danger from previously unknown viruses or variants of known viruses for which no assay methods exist, Flegel (2006b) considered that it is not sufficient to certify the exotic animals as free (SPF) for a list of known pathogens with available diagnostic tests. However, the possibility that such unknown pathogens may be present in SPF stocks cannot easily be evaluated by risk analysis, and there has been no documented instance of the movement of broodstocks originating from SPF facilities having been responsible for the spread of such pathogens.

- High biosecurity facilities that are designed to produce SPF shrimp are often termed *Nucleus Breeding Centers.* Shrimp derived from such centers may be transferred to medium biosecurity facilities (*Multiplication Centers*) where they are bred to produce PL that are transferred to the grow-out ponds of commercial farms having low biosecurity (*Commodity Farms*). The movement of shrimp for production is thus always from higher to lower biosecurity.

Specific pathogen resistant (SPR) - denotes shrimp that display resistance to infection to specified pathogens. Note that:

- Shrimp may be SPF to a wide list of pathogens, and also SPR to one or more of these pathogens.

Specific pathogen tolerant (SPT) - denotes shrimp that have been selected for increased tolerance to a given pathogen or pathogens. Note that:

- Shrimp may be SPF to a wide list of pathogens, and also SPT to one or more of these pathogens.

In considering a supplier of SPF shrimp, a purchaser should determine:

- the specific pathogens listed;
- the diagnostics methods used to screen shrimp;
- the date of last screening, the name of the diagnostician who conducted it and the supplier's willingness to supply a copy of the results;
- the disease surveillance program used to monitor the stocks; and
- the nature of the certificate issued by the supplier.

8.0 Details of Proposed Risk Management Measures

8.1 Use of Specific Pathogen Free Shrimp

Only broodstock sourced from a list of Approved Suppliers of SPF shrimp will be used. Table 6 presents a summary of changes in the United States Marine Shrimp Farming Program (USMSFP) working list of "specific" and excludable pathogens for penaeid shrimp for the period 1990-2010.[6] Note that the listing presented in Table 6 is indicative of pathogens which meet the requirements of exclusion from SPF stocks, i.e.: (i) they are biological agents, (ii) they cause serious disease in penaeid shrimp; and (iii) reliable diagnostics methods are available for screening of broodstock and other lifecycle stages. In addition to the 15 pathogens/pathogen groups listed in Table 6 , one additional disease, whitetail disease (WTD), caused by Macrobrachium rosenbergii nodavirus (MrNV) and extra small virus (XSV) meets these criteria. In practice, due to the emerging nature of some pathogens or their restricted geographic distributions, in conjunction with the known histories of their SPF stocks, SPF suppliers do not routinely screen for all of these 16 pathogens/pathogen groups (the Oceanic Institute, for example, screens for 15 pathogens/pathogen groups (all those listed in Table 6) but not for MrNV (see Annex 2).

[6] Funding to USMSFP was discontinued in 2011 (S. Moss, Oceanic Institute, Hawaii, pers. comm.).

Table 6. United States Marine Shrimp Farming Program (USMSFP) working list of ‘‘specific’’ and excludable pathogens for penaeid shrimp for 1990, 2000 and 2010 (modified from Moss *et al.*, 2012).

Pathogen	**1990**	**2000**	**2010**
Viruses			
Infectious hypodermal and hematopoietic necrosis virus (IHHNV)	X	X	X
White spot syndrome virus (WSSV)		X	X
Yellow head virus complex (YHV, GAV, LOV)		X	X
Taura syndrome virus (TSV)		X	X
Baculovirus penaei (BP)	X	X	X
Monodon baculovirus (MBV)		X	X
Baculoviral midgut gland necrosis (BMN)		X	X
Hepatopancreatic parvovirus (HPV)	X	X	X
Infectious myonecrosis virus (IMNV)		X	X
Litopenaeus vannamei nodavirus (PvNV)			X
Prokaryotes			
Necrotizing Hepatopancreatitis (NHP)		X	X
Rickettsia-like bacteria-Milky hemolymph disease (RLB-MHD)			X
Protozoans			
Microsporidians	X	X	X
Haplosporidians	X	X	X
Gregarines	X	X	X
Number of pathogens/pathogen groups	**6**	**13**	**15**

8.2 Establishment of a List of Approved Suppliers of SPF Broodstock

Importations of broodstock will only be permitted through a list of Approved Suppliers. The following criteria must be met for a company to be listed as an Approved Supplier:

- Current stock has been held under SPF conditions for at least two years.
- During this period, no outbreaks of serious disease have occurred.
- During this period, diagnostics testing for listed pathogens has been conducted by an independent laboratory at least four times (at six-month intervals or more frequently) with no positive results (testing methods and results of screening for all diseases are submitted; testing for OIE-listed diseases has been done to specifications given in the OIE Manual (OIE, 2012b)).
- Supplier attests that no purchaser of SPF stocks originating from his facility has complained of receiving diseased animals or has initiated legal action against the supplier for this reason.
- Supplier agrees in principle to inspection of his facility by experts designated by SAS to verify statements regarding stock history and biosecurity.
- The stock(s) must be certified as free from the following 12 pathogens/pathogen groups:
    - Infectious hypodermal and haematopoietic necrosis virus (IHHNV)
    - White spot syndrome virus (WSSV)
    - Yellow head virus (YHV)

    - Taura syndrome virus (TSV)
    - *Baculovirus penaei* (BP)
    - Monodon baculovirus (MBV)
    - Hepatopancreatic parvo-like virus (HPV)
    - Infectious myonecrosis virus (IMNV)
    - Necrotising hepatopancreatitis (NHP)
    - Microsporidians
    - Haplosporidians
    - Gregarines
- Additionally, freedom from the following four additional pathogens/pathogen groups must be demonstrated based on either (i) SPF status for these diseases or (ii) stock history and production records, supplemented by additional diagnostics testing as specified:
    - Whitetail disease (WTD)
    - Baculovirus midgut gland necrosis virus (BMNV)
    - Litopenaeus vannamei nodavirus (PvNV)
    - Rickettsia-like bacteria-Milky hemolymph disease (RLB-MHD)

An initial listing of known suppliers *L. vannamei* broodstock produced in SPF facilities that can be screened as potential Approved Suppliers is presented in Annex 2. This list will need to be further developed and screened using the criteria listed above and, if deemed necessary, by on-site inspection (see Section 8.3, below).

8.3 On-site Inspection of Suppliers

Prospective suppliers must agree in principle to an on-site inspection of their facilities by a team of experts appointed by SAS to confirm that the required biosecurity measures are in place.

8.4 List of Approved Importers

The SAS will establish a list of Approved Importers who will agree to meet all specified standards for risk management measures and to allow independent verification of same.

8.5 Limited Time Period During which Importation will be Permitted

To facilitate pilot testing of *L. vannamei* to local culture conditions and to reduce the risk of pathogen entry with translocation of broodstock, importations from approved suppliers will be terminated once sufficient broodstocks have been established. SAS will conduct an annual assessment of the program and future needs to determine if importations can be terminated.

8.6 High Security Quarantine of Imported Broodstock

Upon entry into KSA, imported broodstock will be held in high security quarantine facilities that will prevent the escape of broodstock and any larval (F1) stages and any pathogens that may be present. Quarantine facilities will meet minimum standards of construction and standard operating procedures (SOPs) appropriate to such high containment facilities (i.e. as outlined in Section 4 of Arthur *et al.,* 2007 and in Annex 6 of ICES, 2012; See Annex 3 of this proposal). Construction and operating standards will also minimize the possibility of diseases present in the external environment gaining entry to the facility. Upon satisfactory completion of diagnostics testing, broodstock will be moved to Biosecure Breeding Centers (BBCs) having a similar level of biosecurity.

8.7 Monitoring and Diagnostics Testing of F0 Broodstock While in Quarantine Facility and BBCs

Broodstock in quarantine facilities or BBCs will be monitored for health on a daily basis and will be tested for specified pathogens upon arrival, before leaving quarantine and at termination, so that if pilot testing is successful, BBCs may eventually achieve SPF status. Diagnostics testing will also be conducted should any unexplained mortalities occur.

8.8 Release of only F1 Shrimp to Grow-out Ponds

Only postlarvae (F1 generation) will be released from BBCs. Imported broodstock will be destroyed and disposed of in a sanitary manner once they are no longer useful for breeding.

8.9 Monitoring and Diagnostics Testing of Shrimp in Grow-out Ponds

PL (F1) stocked in grow-out ponds (commodity shrimp) will be monitored daily and prior to harvest, samples taken for diagnostics testing. In the event of any unusual condition or mortality, disease investigations will also be conducted.

8.10 Contingency Planning in Case of Disease caused by an Exotic Pathogen

Each participating farm will develop a contingency plan to deal with a disease emergency due to an exotic pathogen. Emergency preparedness will allow rapid response, restricting pathogen spread and increasing the possibility that the pathogen can be contained and eradicated. Contingency planning will follow the recommendations given in Arthur *et al.* (2005), OIE (2012a) and ICES (2005, 2012).

9.0 Disease Status of the Kingdom of Saudi Arabia

The national aquatic animal disease status of KSA is poorly known. In 2010 and 2011, white spot syndrome virus (WSSV) and Taura syndrome virus (TSV), two viral diseases of penaeid shrimp listed by the World Organsation for Animal Health (OIE, 2012a) were detected in cultured Indian white prawn (*Fennerpenaeus indicus*) (CEFAS, 2012; *Tang et al.,* 2012a, b).

Based on molecular genetic studies, Tang *et al.* (2012a,b) concluded that both viruses were likely to have become established in aquaculture facilities in KSA through the use of infected broodstock of *F. indicus* originating from the Red Sea, and not introduced via the importation of exotic penaeid species, as has often been the case in other shrimp-growing countries. The above observation does not, however, explain how these two exotic pathogens could have become established in wild populations of *F. indicus*.

Other countries bordering the Red Sea include Israel (Sinai Peninsula), Djibouti, Egypt, Eritrea, Ethiopia, Sudan and Yemen. The only crustacean disease listed by the International Aquatic Animal Disease Database (CEFAS, 2012) for these countries is hepatopancreatic parvo-like virus (HPV), which is listed as occurring in Israel based on OIE data. However, the distribution of HPV within Israel is unknown (OIE, 2007a), and thus this report may not pertain to the Red Sea.

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10 Hazard Identification

Table 7 presents a list of possible hazards (pathogens/pathogen groups) reported from penaeid shrimp. In order for a possible hazard to be given further consideration in the risk analysis, the following criteria must be fulfilled:

- the pathogen must have been reported to infect, or is suspected of being capable of infecting broodstock of *L. vannamei*;
- the agent must be an obligate pathogen (i.e., it is not a ubiquitous free-living organism that is capable of becoming an opportunistic pathogen of *L. vannamei* under certain environmental or culture conditions);
- the agent must cause significant disease outbreaks and associated losses in populations of *L. vannamei* or, if not a significant pathogen of *L. vannamei*, it must cause serious disease outbreaks in populations of other species of decapod crustaceans; and
- it must be plausible that that the agent might be present in specific pathogen free (SPF) populations of *L. vannamei* that will be approved for importation to the KSA using the criteria outlined in Section 8.

The results of the hazard identification are presented in Table 8.

Table 7. Results of hazard identification for pathogens of penaeid shrimp (Y=Yes, N=No, P=Plausible, X = not applicable) (all pathogens are known biological entities unless otherwise indicated).

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Viruses										
White spot syndrome virus (WSSV)	Y	Y	Y	Y	Y	Y	X	N	Wongteerasupaya *et al.*, 1995; Lo *et al.*, 1996; Flegel, 1997, 2006; Tapay *et al.*, 1997; Lightner, 2011; Lightner *et al.*, 2012; CEFAS, 2012; Tang *et al.*, 2012a,b; OIE, 2012b	Significant pathogen that is potentially lethal to most of commercially cultivated penaeid shrimp species. Extremely wide host range (see Table 1 in Flegel, 2006). Originated in Asia but now has near global distribution due to movements of infected live shrimp and their products. Whitespot disease (WSD) has been reported from most shrimp farming countries in Asia, the Middle East, and North, South and Central America. Only Australia, Africa and some specific zones or compartments can be considered free of WSD according to the guidelines laid out in the OIE *Aquatic Animal Health Code*. The impact of WSD was recently estimated as being close $15 billion (see Lightner *et al.*, 2012). Epizootics occur most often in the cooler seasons in most shrimp farming regions. Reported to be present in the Kingdom of Saudi Arabia (Tang *et al.* 2012a,b; CEFAS, 2012) Risk can be mitigated by sourcing of broodstock from SPF facilities.

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Infectious hypodermal and haematopoietic necrosis virus (IHHNV)	Y	Y	Y	P	Y	Y	X	N	Lightner *et al*., 1983a,b, 1995, 2012; Bell and Lightner, 1984, 1987; Bonami *et al*., 1990; Lightner, 1996a,b, 2011; Pantoja *et al*., 1999; Flegel, 2006, 2012; OIE, 2012b	Significant pathogen of penaeid shrimp; infects a wide range of penaeids, occurring in both wild and cultured shrimp. A major pathogen of *Litopenaeus stylirostris*, causing epizootics and mass mortalities in juveniles and subadults. Also infects *L. vannamei*, causing "runt deformity syndrome", resulting in production losses due to poor growth and deformity. *Penaeus monodon* rarely show signs of IHHN or RDS (Lightner, 2011, Lightner *et al*., 2012, Flegel, 2012). Introduction to Mexico in 1990 led to collapse of the wild fishery for *L. stylirostris* in the northern Gulf of California. Widely distributed in the Americas (with the exception of the USA and Panama, which are free). Occurs throughout much of East and SE Asia in wild and cultured *P. monodon*, but does not seem to cause production losses in this species (see Lightner, 2011; Lightner *et al*., 2012). Infected shrimp may become carriers for life, passing infections via vertical and horizontal transmission. Risk can be mitigated by sourcing of broodstock from SPF facilities.
Infectious myonecrosis virus (IMNV)	Y	P	Y	N	Y	Y	X	N	Lightner, 2011; Lightner *et al*., 2012; OIE, 2012b; Flegel, 2012	Pathogen currently has a limited host and geographic distribution in the Americas and Asia, where its only known host is *L. vannamei*. First described in cultured shrimp in northeast Brazil in 2003; subsequently reported from Indonesia in 2006. Causes significant disease and mortalities in juvenile and subadult pond-reared stocks of *L. vannamei*. IMN presents as a disease in *L. vannamei* with an acute onset of gross signs and elevated mortalities, but it progresses with a more chronic course accompanied by persistent moderate mortalities. Risk can be mitigated by sourcing of broodstock from SPF facilities.

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Taura syndrome virus (TSV)	Y	Y	Y	Y	Y	Y	X	N	Hasson *et al.*, 1995, 1999; Lightner 1996a,b, 2011; Bonami *et al.*, 1997; Brock *et al.*, 1997; Nunan *et al.* 1998;Flegel, 2006; Tang *et al.*, 2012a,b; OIE 2012b; Lightner *et al.*, 2012; CEFAS, 2012	Principal host is *Litopenaeus vannamei*, with cumulative mortalities due to epizootics ranging from 40 to >90% in cultured populations of PLs, juveniles & subadults. Other species can be also infected and present disease (e.g. *L. stylirostris*, *L. setiferus*, & *L. schmitti*). Experimental infections in *Farfentepenaeus aztecus*,*F. duorarum*, *Fenneropenaeus chinensis*, *Penaeus monodon* and *Marsupenaeus japonicus*, but these species do not appear to develop clinical disease. Survivors may carry infections for life. Horizontal transmission by cannibalism & contaminated water; vertical transmission from infected broodstock to offspring is strongly suspected. Disease due to TSV has been reported from most shrimp farming countries in Asia, the Middle East, and North, South and Central America. Only Australia, Africa and some specific zones or compartments can be considered free of TS according to the guidelines laid out in the OIE *Aquatic Animal Health Code* (Lightner *et al.*, 2012). TSV has been moved with live shrimp transfers to many of the shrimp-growing countries of the Americas. While wild PL with TSV infections were reported near shrimp farms with ongoing TSV epizootics, infections in wild shrimp have not been further documented, suggesting that TSV does not have a discernable impact on wild shrimp populations. After WSD, TS is the second most costly disease in terms of lost production to the global shrimp farming industry. The development and global distribution of TSV-resistant domesticated lines of *L. vannamei* is among the reasons why this species has become the dominant shrimp species famed globally (Lightner *et al.*, 2012). Reported to be present in the Kingdom of Saudi Arabia (Tang *et al.*, 2012a,b; CEFAS, 2012) Risk can be mitigated by sourcing of broodstock from SPF facilities.

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Whitetail disease (WTD)	P	P	Y	P	N	Y	X	N	Sudhakaran *et al*., 2006; Ravi *et al*., 2009; Bonami & Widada, 2011; OIE, 2012b	A disease of giant freshwater prawn (*Macrobrachium rosenbergi*) that has recently been reported to infect penaeid shrimps. Natural infections reported from postlarval *F. indicus* and *P. monodon* in India reared in proximity to *M. rosenbergii* culture (Ravi *et al*., 2009). Experimental infections in PL of *F. indicus, P. monodon* and *M. japonicus* did not produce disease; however, marine shrimp are believed to act as reservoirs for Macrobrachium rosenbergii nodavirus (MrNV) and extra small virus (XSV), and their virulence is maintained within marine shrimp tissues (see Sudhakaran *et al*., 2006; Bonami & Widada, 2011). Adults *of M. rosenbergii* and penaeid shrimp appear to be resistant (or tolerant of) infections. However, OIE (2012b) notes that infected *M. rosenbergii* may be lifelong carriers. Risk can be mitigated by sourcing of broodstock from SPF facilities &/or diagnostics testing to OIE standards (OIE 2012b).

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Yellowhead virus (YHV) and associated viruses : Lymphoid organ vacuolization virus (LOVV) and Gill-associated virus (GAV)	Y	Y	Y	P	Y	Y	X	N	Boonyaratpalin *et al.*, 1993; Wongteerasupaya *et al.*, 1997; Nadala *et al.*, 1997). Bondad-Reantaso *et al.*, 2001; Flegel, 2006, 2012; OIE, 2012b; Lightner, 1996b, 2011; Lightner *et al.* 2012	Natural infections in *P. monodon*, lethal experimental infections in *L. stylirostris* and other species. First described from Thailand, YHV is now present throughout much of Asia and also in the Americas (Mexico). The disease remains enzootic in SE Asia, but severe losses are infrequently reported (Lightner *et al.*, 2012). Disease due to YHV has largely been limited to SE Asia and India (Lightner *et al.*, 2012). Other low-virulence genetic variants have been reported in wild and farmed penaeids in other regions of the world. No confirmed reports of actual YHV-caused disease outbreaks in the Americas (Lightner, 2011). Not a major disease in cultured stocks of *L. vannamei* in East and SE Asia where YHV is enzootic and highly prevalent in wild and farmed stocks of *P. monodon*. Recent work has shown that there are five or six geographical types of YHV and that the most virulent type (YHV-1) has been reported to cause serious disease outbreaks only in Thailand. In Asia, serious disease outbreaks (i.e., high and rapid mortality) caused by YHV Type-1 have been reported sporadically only from Thailand. YHV Type-1 is subdivided into Type-1a (the first to appear in *P. monodon* in Thailand) and Type-1b (a variant that appeared later in *L. vannamei* in Thailand) ((see Flegel, 2012). YHV, GAV and LOVV are closely related single stranded, positive sense RNAviruses that have now been included in a new genus *Okavirus* in a new family Ronivirdae. LOVV and GAV share approximately 95% DNA sequence identity and 100% amino acid identity, establishing that they are the same virus type, while GAV and YHV share approximately 85% DNA sequence identity and 96% amino acid identity indicating that they are different types (see Flegel, 2006). Bondad-Reantaso *et al.* (2001) note that GAV can occur in healthy and diseased shrimp and was previously called LOVV when observed in healthy shrimp. Risk can be mitigated by sourcing of broodstock from SPF facilities.

Pathogen	**Reported from *Litopenaeus vannamei***	**Infects broodstock**	**Causes significant disease**	**Present in Saudi Arabia**	**SPF stocks exist**	**Negligible risk of release**	**Negligible risk of exposure**	**Further consideration required**	**References**	**Comments**
Baculovirus penaei (BP) (Tetrahedral baculovirosis)	Y	Y	Y	P	Y	Y	X	N	Lightner 1983, 1988; Lightner *et al*. 1989; Bondad-Reantaso *et al.*, 2001; OIE, 2012b	Causes serious disease in *Farfentepenaeus duorarum*, *F. aztecus*, *L. vannamei* and *Merlicertus marginatus*. Also reported from *Penaeus penicellatus*, *Litopenaeus schmitti*, *F. paulensis* and *F. subtilis* (Bondad-Reantaso *et al.*, 2001). All penaeid species may be potential hosts (OIE, 2012b) Enzootic in wild penaeids in the Americas and Hawaii, BP has not been reported in wild or cultured penaeid shrimp in the eastern hemisphere despite numerous introductions of American penaeids to Asia and the Indo-Pacific (OIE, 2012b). Risk can be mitigated by sourcing of broodstock from SPF facilities.

Pathogen	Reported from ***Litopenaeus vannamei***	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Hepatopancreatic parvo-like virus (HPV)	Y	Y	Y	P	Y	Y	X	N	Bonami *et al.*, 1995; Lightner 1996b; Flegel, 2006, 2012; OIE, 2007a; CEFAS, 2012	Natural infections in *Fenneroenaeus chinensis, Penaeus esculentus, F. indicus, Marsupenaeus japonicus, P. monodon, F. merguiensis, P. penicellatus, Litopenaeus schmitti, P. semisulcatus* , *L. stylirostris* and *L. vannamei*. Its geographical distribution includes the Indo-Pacific area (PR China, Taiwan, Korea, Philippines, Malaysia, Singapore, Australia, Indonesia and Thailand), Africa (Kenya), Middle East (Israel and Kuwait) and the Americas (Hawaii, Ecuador, Mexico and Brazil) (see Bonami *et al.*, 1995). HPV infection in cultured shrimp has been linked to chronic mortalities during early larval or postlarval stages and may result in stunted growth during early juvenile stages. The effect of HPV infection on adult shrimp is unknown, however, it may compromise their survival if the infection is severe and the shrimp is in a highly demanding metabolic state (i.e. during gonad maturation). Although suspected, no epizootics of HPV disease have been confirmed and documented (CEFAS, 2012) HPV can be removed easily by washing eggs and nauplii in the hatchery (Flegel, 2012). Risk can be mitigated by sourcing of broodstock from SPF facilities.

Pathogen	**Reported from *Litopenaeus vannamei***	**Infects broodstock**	**Causes significant disease**	**Present in Saudi Arabia**	**SPF stocks exist**	**Negligible risk of release**	**Negligible risk of exposure**	**Further consideration required**	**References**	**Comments**
Penaeus monodon-type baculovirus (PMBV) (Spherical baculovirosis)	N	Y	Y	P	Y	Y	X	N	Bondad-Reantaso *et al.*, 2001; Flegel, 2006, 2012; OIE, 2012b; CEFAS, 2012	Natural infections in *Metapenaeus ensis, Feneropenaeus indicus, F. meuguensis, P. mondon, P. penicellatus, Melicertus plebejus* (CEFAS, 2012). Enzootic in wild penaeids in the following regions bordering on the Indo-Pacific: East and SE Asia, Indian subcontinent, Middle East, Australia, Indonesia, New Caledonia, East Africa and Madagascar. Outside the normal geographical range of *P. monodon*, MBV has not been reported in wild penaeids. However, MBV has been reported from sites where introduced *P. monodon* has been cultured in the Mediterranean, West Africa, Tahiti and Hawaii, as well as several sites in North and South America and the Caribbean, but only in the introduced *P. monodon* stocks (OIE, 2012b). Despite the simultaneous culture of MBV-infected *P. monodon* in a number of farms in various countries in the Americas (Ecuador, Brazil, Puerto Rico, and the States of Texas, South Carolina and Hawaii of the USA), and the consequent direct exposure of certain penaeids from this region (i.e. specifically *L. vannamei*, *L. stylirostris* and *Farfentepenaeus californiensis*) to MBV, the virus did not produce infections in these species, nor has it become established in the shrimp farms or in wild stocks of exposed regions (OIE, 2012b). Infected animals may become lifelong carriers (OIE, 2012b). PMBV can be removed easily by washing eggs and nauplii in the hatchery (Flegel, 2012). Risk can be mitigated by sourcing of broodstock from SPF facilities.

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Baculovirus midgut gland necrosis virus (BMNV)	N	P	Y	P	Y	Y	X	N	Bondad-Reantaso *et al*., 2001; Flegel, 2006	Natural infections observed *in Marsuenaeus japonicus*, *P. monodon* and *Melicertus plebejus*; experimental infections in *Fenneropenaeus chinensis* and *P. semisulcatus* (see Bondad-Reantaso *et a*l., 2001). Distribution includes Japan. BMN-like virus has also been reported from Korea RO, the Philippines and possibly Indonesia and Australia (see Bondad-Reantaso *et a*l., 2001). A serious pathogen of larval *M. japonicus* in Japan in the early period of shrimp culture development but was excluded from the cultivation system after the mode of transmission from infected broodstock was established, and thorough washing of the eggs or nauplii as a routine preventative measure. Risk can be mitigated by sourcing of broodstock from SPF facilities and/or additional diagnostics testing.

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Litopenaeus vannamei nodavirus (PvNV)	Y	P	Y	N	Y	Y	X	N	Tang *et al.*, 2007, 2011	PvNV is a nodavirus causing whitish muscle (whitetail disease) in *Litopenaeus vannamei* (Tang *et al*., 2007) . Gross clinical signs and histopathogy are very similar to those for IMNV, both viruses targeting primarily the skeletal muscle and causing white or opaque tail. Histologically, the diseases are almost indistinguishable, both being characterized by muscle necrosis and the formation of prominent lymphoid organ spheroids. PvNV is distinct from IMNV of penaeid shrimps and MrNV of giant freshwater prawn. The virus had 69% sequence similarity to the capsid gene of MrNV of *M. rosenbergii*. PvNV has so far only been reported as affecting cultured *L. vannamei* in Belize, infection resulting in a 50% reduction in production in some affected ponds. By laboratory injection and per os challenge, *P. monodon* was also shown to be susceptible to PvNV infection. However, injected *P. monodon* did not display white tails or muscle necrosis. Although PvNV did not cause mortality of *L. vannamei* in laboratory bioassays, it appears to affect survival in grow-out ponds; mortality is sporadic and is often associated with environmental stress such as crowding and high temperature. PvNV has been detected in mosquitofish, seabird feces, barnacles and zooplankton, suggesting that the virus can be spread via these carriers (Tang *et al.*, 2011). A commercial diagnostic kit (IQ 2000^{TM} PvNV Prevention and Detection System) is available through the University of Arizona (http://www.iq2000kit.com/products_2.php?bgid=1&gid=1&sgid=8). Risk can be mitigated by sourcing of broodstock from SPF facilities &/or additional diagnostics testing.

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Rhabdovirus of penaeid shrimp (RPS)	Y	Y	N	N	N	N	Y	N	Lightner 1996b	RPS may use penaeid shrimp as carrier hosts; its morphology is very similar to certain fish rhabdoviruses, it replicates in a fish cell line and may not replicate in shrimp. Does not cause disease in shrimp following massive challenge and causes no distinctive histopathology (other than minor changes to the lymphoid organ) in challenged shrimp. RPS has been isolated only from *L. vannamei* and *L. stylirostris* from Hawaii and Ecuador. RPS is a poorly understood virus found in penaeid shrimp, and it is not known if it is a true pathogen of penaeid shrimp or if it uses shrimp as carrier hosts while having finfish as its principal hosts.
Mourilyan virus (MOV)	N	P	N	N	N	Y	X	N	Flegel, 2006	Appears to be endemic in populations of *Penaeus monodon* from Queensland, Australia, Malaysia and Thailand. Also infects *M. japonicus* in Australia. Pathogenicity not clearly demonstrated. Risk can be mitigated by sourcing of broodstock from SPF facilities &/or additional diagnostics testing.
Spawner-isolated mortality virus disease (SMVD)	N	P	P	N	N	Y	X	N	Bondad-Reantaso *et al*., 2001; CEFAS, 2012	Infection and disease due to SMV has only been reported from cultured or captive wild adult *P. monodon* and cultured *Cherax quadricarinatus*. Experimental infections in *P. esculentus*, *Melicertus japonicus*, *Fenneropenaeus merguiensis* and *Metapenaeus eisis*. Reported from Queensland, Australia, Philippines and Sri Lanka. SMV is one of several viruses associated with mid-crop mortality syndrome (MCMS), which caused significant mortalities among juveniles and subadults of *P. monodon* cultured in Australia from 1994 to 1996.
Bacteria										

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Necrotising hepatopancreatitis (NHP)	Y	Y	Y	N	Y	Y	X	N	Frelier *et al*., 1993; Lightner *et al*., 1992, 2012; Lightner 1996b; Flegel 2012	Reported only from American penaeids (*Litopenaeus vannamei*, *Farentepenaeus. aztecus*, *L. stylirostris*, *L. setiferus* and *F. californiensis*). Apparently not been introduced nor established in SE Asia, probably due to the nature of the disease and its requirement for high water temperatures and high salinity (from a prolonged dry season). NHP was introduced to an arid, hot location in northeast Africa with a careless introduction of *L. vannamei* and became temporarily established in cultured shrimp stocks. Other regions of south central Asia (e.g. India, East Africa and the Middle East) have extended dry seasons with high water temperatures and are beginning to import *L. vannamei*, raising the risk of introduction (Lightner *et al*. 2012; Flegel, 2012). After WSD, TS and vibriosis, NHP is the most significant disease in the Americas in terms of production losses and management cost. NHP causes significant production losses in shrimp farms, which may approach 100% if not correctly diagnosed and treated. The occurrence of NHP disease seems to be dependent upon a combination of high temperature and high salinity, with the disease most often occurring in regions where it is enzootic during the dry season when water temperatures and salinity are near or greater than 30 ^{o}C and 30 ppt, respectively (Lightner *et al.*, 2012). Risk can be mitigated by sourcing of broodstock from SPF facilities.
Spiroplasma penaei	Y	P	Y	N	N	Y	X	N	Nunan *et al.*, 2005; Heres & Lightner , 2010	Described from the hemolymph of *L. vannamei* from the Caribbean coast of Columbia. Heres & Lightner (2010) characterized this bacterium from a shrimp farm near Cartagena, Colombia that was suffering from high mortalities in ponds with very low salinity and high temperatures.

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Streptococcus spp.	Y	P	Y	P	N	N	Y	N	Lightner *et al.*, 2012	*Streptococcus* spp. have caused severe mortalities in *Penaeus monodon* farmed in the Indo-Pacific (Madagascar and East Africa) and in *L. vannamei* farmed in Central and South Americas (French Guyana). Disease occurs during the rainy season when water temperatures are persistently high and salinity is near 0‰ for lengthy periods of time. In Madagascar, wild *Macrobrachium* sp. affected with the disease were collected from one of the affected farms's supply canals, suggesting that this freshwater crustacean might be a reservoir host, or alternatively, that it also becomes infected from another source (e.g. freshwater fish). Sequencing of the generated amplicons from some samples have identified *Streptococcus iniae* and *S. porcinus*, with 98% homology to the reference species in GenBank. *Streptococcus* spp. are ubiquitous bacteria that can occasionally become facultative pathogens of aquatic animals, usually in situations when the host is compromised by adverse environmental conditions.
Vibriosis *Vibrio harveyi* *V. mediteraneanei* *V. nigripulcritudo* *V. parahaemolyticus* *V. vulnificus*	Y	Y	Y	Y	N	N	Y	N	Lightner and Redman, 1985; Lightner, 1988, 1996a; Alvarez *et al.*, 1998; Flegel 2012	Vibriosis affects all penaeid species; mortality ranges from inconsequential to 100%; worldwide distribution. Serious *Vibrio* infections are usually the result of mismanagement. Disease outbreaks caused by *V. penaecida* and *V. nigripulcritudo* can be initiated by unmanageable weather conditions; a new species, *V. mediteraneanei*, has recently been reported from diseased shrimp in Thailand (see Flegel, 2012). Ubiquitous in aquatic systems; potentially infectious to all penaeids.

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Vibrio penaeicida	Y	P	Y	P	N	Y	X	N	Ishimaru *et al.*, 1995; de la Pena *et al.*, 1993, 1995, 1997; Costa *et al.*, 1998; Saulner *et al.*, 2000; Aguirre-Guzmán *et al.*, 2005; Flegel, 2012	De la Pena *et al.* (1993) isolated this distinct *Vibrio* from diseased *M. japonicus.* Ishimaru *et al.* (1995) also detected it in apparently healthy prawns and water samples obtained from aquaculture ponds associated with diseased shrimp. *Vibrio penaeicida* has been considered as a true pathogen rather than an opportunistic invader as are other *Vibrio* spp. Costa *et al.* (1998) also reported that *V. penaeicida* causes a seasonal vibriosis (also known as "Syndrome 93") affecting juvenile and broodstock of *L. stylirostris* in New Caledonia. Aguirre-Guzmán *et al*. (2005) experimentally infected juvenile *L. vannamei*. Disease outbreaks caused by *V. penaecida* and *V. nigripulcritudo* can be initiated by unmanageable weather conditions (Flegel, 2012). Probably ubiquitous in marine aquatic environments. PCR-based diagnostics test available (see Saulnier *et al.* (2000).
Shrimp tuberculosis *Mycobacterium marinum* *M. fortuitum* *Mycobacterium* sp.	P	P	N	P	N	N	Y	N	Lightner 1988, 1996b	Ubiquitous; potentially infectious to all penaeids.

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Rickettsia-like bacteria - Milky hemolymph disease (RLB-MHD)	P	P	Y	N	Y	Y	X	N	Brock,1988, Lightner, 1996b; OIE, 2007b; Nunan *et al*., 2010	Rickettsia or rickettsia-like microorganisms have been described in wild penaeid shrimp from Hawaii and in cultured penaeids from Mexico and SE Asia. *Melicertus marginatus*, *Fenneropenaeus merguiensis* and *Penaeus monodon* have shown infections by rickettsial-like bacteria in the epithelial cells of the hepatopancreatic tubules. *Litopenaeus stylirostris* was experimentally infected by rickettsia of *M. marginatus.* Milky hemolymph disease (MHD) affects spiny lobster (*Pandilurus* spp.) in Viet Nam (OIE 2007b). Very similar diseases, with similar gross and histopathological lesions, primarily in connective tissues, have been reported in farmed *P. monodon* and in captive-wild European shore crab (*Carcinus maenas*).(OIE, 2007b, Nunan *et al*., 2010). Risk can be mitigated by sourcing of broodstock from SPF facilities.
Protozoa										
Microsporidians *Enterocytozoon hepatopenaei* *Agmasoma penaei*	Y	P	Y	P	Y	Y	X	N	Flegel, 2012	*Enterocytozoon hepatopenaei* originally discovered in *Penaeus monodon* but was not associated with serious disease. Recent outbreaks of white feces syndrome (WFS) in *P. monodon* in Viet Nam and *L. vannamei* in Viet Nam and Thailand have been associated with serious infections by a morphologically similar microsporidian. The parasite shares 95% rRNA gene sequence identity with that of *E. hepatopenaei* and thus is probably a variant of the same species or a new species in the genus (see Flegel, 2012). Fish have been identified as probable carriers of *Agmasoma penaei* in Thailand (Flegel, 2012). Risk can be mitigated by sourcing of broodstock from SPF facilities.

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Haplosporidians *Haplosporidium* sp. Unidentified haplosporidians	Y	Y	Y	P	Y	Y	X	N	Lightner, 1996b; Utari *et al*., 2012; Flegel, 2012	In cultured and wild penaeid shrimp including *L. stylirostris.* Infections by unidentified haplosporidians were reported in cultured *L. vannamei* imported from Nicaragua into Cuba from 1985 to 1986 and more recently in Belize, Central America. Haplosporidians believed to be conspecific with those described from Central America were also reported from juvenile *L. vannamei* in Indonesia, where since 2007 high mortalities in hatcheries and grow-out ponds in Sumatra and Java Islands have occurred at less than one month in pond culture (see Utari *et al.* 2012; Flegel, 2012). Risk can be mitigated by sourcing of broodstock from SPF facilities.
Gregarines (e.g.) *Nematopsis* spp. *Paraophioidina scolecoides* *Cephalolobus penaeus* *C. petiti*	Y	Y	N	P	Y	Y	X	N	Bower, 1996	All penaeids are potential hosts to these protistans. Reported from *L. vannamei* and numerous other species of shrimp (including *Farfentepenaeus. duorarum*) in which pathology has not been reported. Hosts include *F. aztecus, Solenocera membranacea*, *P. semisulcatus.* Global distribution, but individual species may have limited geographic or host distribution. Trophozoites and gametocytes occur in the lumen and are often attached to the lining of the intestine. In most cases, the reduced absorption of food from the gut lumen or occasional intestinal blockage by the gregarines is thought to be of little pathological importance for the host. Risk can be mitigated by sourcing of broodstock from SPF facilities.
Fungi										
Lagenidium spp.	P	P	N	P	N	N	Y	N	Lightner, 1988, 1996b	Larval mycosis may affect all penaeid species; opportunistic pathogen.
Sirolpidium spp.	P	P	N	P	N	N	Y	N	Lightner, 1988, 1996b	Affects all penaeids; opportunistic pathogen.

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Fusarium spp. *F. oxysporum* *F. moniliforme* *F. solani*	Y	P	N	P	N	N	Y	N	Lightner 1996b; Souheil *et al.*, 1999	Opportunistic pathogen; isolated form both cultured and wild crustaceans. All penaeids probably susceptible, the most susceptible being *Melicertus japonicus* and *Farfantepenaeus californiensis*; *L. stylirostris and L. vannamei* are considered moderately susceptible, while *P. monodon* and *Fenneropenaeus merguiensis* are relatively resistant. *Fusarium solani* was reported to cause high losses among juvenile and adult penaeid shrimp cultured in Japan, America, Israel and France. *M. japonicus* is highly susceptible to *F. solani* infections and can also be infected by *F. moniliforme* in Japan (see Souheil *et al.*, 1999). *F. oxysporum* is reported to cause gill blackening disease in *M. japonicus* (Souheil *et al*., 1999). *F. solani* is an opportunistic pathogen of penaeids that is capable of establishing infections only in shrimp that have been compromised by other infectious diseases, exposure to chemical irritants or certain heavy metals, or excessive crowding. Commonly isolated from both cultured and wild crustaceans, *Fusarium* spp. are also common pathogens of plants and occasionally of terrestrial animals.
Emerging Diseases of Unknown Etiology										
Acute hepatopancreatic necrosis syndrome (AHNS)/Early Mortality Syndrome (EMS)	Y	P	Y	N	N	N	Y	N	NACA, 2012; Flegel, 2012	Since 2009, causes mass mortalities and severe economic losses in *Penaeus monodon* and *Litopenaeus vannamei*. Reported from China, Malaysia, Thailand and Viet Nam (NACA, 2012; Flegel, 2012). While the apparent spread of AHNS suggests that an infectious or at least biological agent may be involved, thus far, laboratory challenge tests have failed to demonstrate that the disease is transmissible and no infectious agent or toxin has been identified.
Infectious muscle necrosis	Y	P	Y	N	N	Y	X	N	Melina *et al.*, 2012	Reported from *L. vannamei* from grow-out ponds in Ecuador. Suggested to be due to a new infectious agent or a different strain of IMNV.

Pathogen	Reported from *Litopenaeus vannamei*	Infects broodstock	Causes significant disease	Present in Saudi Arabia	SPF stocks exist	Negligible risk of release	Negligible risk of exposure	Further consideration required	References	Comments
Monodon slow growth syndrome (MSGS)	N	P	Y	N	N	Y	X	N	Flegel, 2006; 2012	Known only from *Penaeus monodon* in Thailand; etiological agent(s) not yet identified. Laem-Singh virus (LSNV), has been found in shrimp from MSGS ponds, but its role has not yet been clearly established. A second agent called an integrase containing element (ICE) was subsequently found in shrimp from MSGS ponds. Although LSNV and ICE occur together in shrimp from MSGS ponds but not in shrimp from normal ponds, a direct causal link between the dual infection and MSGS has yet to be proven. LSNV and ICE should be added to the list of pathogens to be excluded from SPF stocks. MSGS was the main reason that Thai shrimp farmers switched from rearing *P. monodon* to rearing *L. vannamei* derived from SPF stocks. Sritunyalucksana *et al*. (2006) reported on an apparently innocuous virus associated with MSGS in Thailand.

10.2 Pathogens Not Considered Further

As shown in Tables 7 and 8, none of listed pathogens (possible hazards) meet all requirements necessary to be considered hazards meriting further consideration by risk assessment.

Viruses

Viruses are clearly the most serious threats to penaeid shrimp culture. The following eight viruses can be excluded from further consideration due to their inclusion in the SPF-list of pathogens required from Approved Suppliers and the availability of reliable diagnostics tests that can been applied during quarantine:

- Whitespot syndrome virus (WSSV)
- Infectious hypodermal and haematopoietic necrosis virus (IHHNV)
- Infectious myonecrosis virus (IMNV)
- Taura syndrome virus (TSV)
- Yellowhead virus and closely related viruses (YHV/GAV/LOVV)
- *Baculovirus penaei* (BP)
- Hepatopancreatic parvo-like virus (HPV)
- Penaeus monodon-type baculovirus (PMB) (Spherical baculovirosis)

The remaining five viruses of possible concern can be excluded from further consideration for the following reasons:

- Whitetail disease (WTD): reliable diagnostics test available; has been reported only from stocks of *L. vannamei* raised in close proximity to cultured *Macrobrachium rosenbergii*.
- Penaeus vannamei nodavirus: (PvNV): some SPF stocks available; reliable diagnostics test available; not likely to infect stocks of *L. vannamei* available from Approved Suppliers, as it is known only from a very limited geographical area (Belize).
- Rhabdovirus of penaeid shrimp (RPS): does not cause significant disease; not likely to infect stocks of *L. vannamei* available from Approved Suppliers as it has a limited geographical distribution (Hawaii, Ecuador).
- Mourilyan virus (MOV): some SPF stocks available; reliable diagnostics test available; pathogenicity not clearly demonstrated; not likely to infect stocks of *L. vannamei* available from Approved Suppliers, as it is has not been reported from this species and has a restricted geographical distribution (Australia and parts of SE Asia)
- Spawner-isolated mortality virus disease (SMVD): not likely to infect stocks of *L. vannamei* available from Approved Suppliers as it is not reported as a pathogen of this species and has a geographical distribution limited to the Asia-Pacific region.

Bacteria

Most of the bacteria causing disease in penaeid shrimp culture are opportunistic species present in marine environments and having broad geographic distributions. Two serious bacterial diseases can be excluded from further consideration due to their inclusion on the SPF-list of pathogens and the availability of reliable diagnostics tests:

- Necrotising hepatopancreatitis (NHP)
- Rickettsia-like bacteria - Milky hemolymph disease (RLB-MHD)

The following three pathogen groups can be excluded from further consideration because they are widely distributed in aquatic environments and are opportunistic pathogens in shrimp culture facilities where then can be controlled by good husbandry practices:

- *Streptococcus* spp.
- *Vibrio* spp.
- Shrimp tuberculosis (*Mycobacterium* spp.)

The remaining bacterial pathogens can be excluded from further consideration for the following reasons:

- *Spiroplasma penaei:* not likely to infect stocks of *L. vannamei* available from Approved Suppliers, as it is known only from a very limited geographical area (Caribbean coast of Columbia)
- *Vibrio penaeicida*: reliable diagnostics test available; not likely to infect stocks of *L. vannamei* available from Approved Suppliers as it is known only from a very limited geographical area (Japan, New Caledonia)

Protozoan parasites

Three groups of parasites are of concern to penaeid shrimp culture; as they all appear on the SPF list, they can be excluded from further consideration:

- Gregarines
- Haplosporidians
- Microsporidians

Fungi

All fungi of concern are ubiquitous, opportunistic pathogens of shrimp and can thus be excluded from further consideration:

- *Lagenidium* spp.
- *Sirolpidium* spp.
- *Fusarium* spp.

Other emerging diseases of unknown etiology

These three possible hazards can be excluded from further consideration for the following reasons:

- Acute hepatopancreatic necrosis syndrome (AHNS)/Early mortality syndrome (EMS): not demonstrated to be caused by a biological agent; geographic distribution restricted to East and SE Asia. As no reliable diagnostic tests are available., it is difficult for SPF suppliers to be sure this agent is not present in their facilities. Although a calculable risk of disease translocation remains in the absence of disease agent identification, exposure is highly unlikely due to the screening of SPF suppliers and the use of stringent quarantine.

- Infectious muscle necrosis: not likely to infect stocks of *L. vannamei* available from Approved Suppliers as it is known only from a very limited geographical area (Ecuador).
- Monodon slow growth syndrome (MSGS): not likely to infect stocks of *L. vannamei* available from Approved Suppliers as it is not known in affect this species and is reported only from a very limited geographical distribution (Thailand).

11.0 Conclusions

The risk analysis considered a total of 30 pathogens/pathogen groups as possible hazards for the introduction of whiteleg shrimp from Approved Suppliers of SPF broodstock to the Kingdom of Saudi Arabia. The risk management measures proposed by the proponents (see Section 8) were considered to be sufficient in all cases to allow the importation to proceed with an acceptable level of risk to the KSA.

12.0 Recommendations

The following additional recommendations are made:

1. The Government of the Kingdom of Saudi Arabia should confirm that the suggestion that the national appropriate level of protection (ALOP) should be "high" or "very high" and with an acceptable level of risk (ALOR) that is "low" or "very low".

2. The risk assessment made in this risk analysis is highly dependent upon the risk management measures proposed by the proponents being fully implemented. Monitoring systems must be established to ensure that all risk management measures are effectively implemented.

3. To minimize the risk of WSSV, TSV and other pathogens gaining entry, it is recommended that the initial high security quarantine facility and the BBCs be located as far away as possible from existing shrimp farms.

4. Saudi shrimp growers should strive to become self-sufficient in broodstock and PL production as soon as possible by setting up breeding and genetic improvement programs for *L. vannamei*, as this will further reduce the risk of pathogen introduction.

5. To better understand the potential for pathogen transfer between cultured and wild stocks, baseline studies of diseases of decapod crustaceans in the vicinity of aquaculture facilities should be conducted. Such monitoring will also help to detect any transfer of introduced exotic pathogens from *L. vannamei* to wild crustacean populations.

6. The Saudi Aquaculture Society should conduct susceptibility testing of local penaeids to check for the presence of cryptic or unknown pathogens in the imported broodstocks (see Flegel, 2006b).

Table 8. Summary of reasons for exclusion of possible hazards of *Litopenaeus vannamei* as actual hazards.

Reason for Not Being Considered a Hazard							
	Pre-border measures ensuring negligible risk of release into KSA				Post-border measures ensuring negligible risk of exposure		
Possible Hazard	**SPF Listed**	**Reliable Diagnostics Test Available**	**Pathogen Not Likely to Infect *Litopenaeus vannamei***	**Pathogen has Highly Restricted Geographical Distribution**	**Organism Unlikely to Escape Quarantine**	**Ubiquitous Organism**	**Not a Significant Pathogen**
WSSV	X	X			X		
IHHNV	X	X			X		
IMNV	X	X		X	X		
TSV	X	X			X		
WTD		X	X		X		
YHD/LOVV/GAV	X	X			X		
BP	X	X			X		
HPV	X	X			X		
PMB	X	X	X	X	X		
BMNV	X	X	X	X	X		
PvNV	X	X		X	X		
RPS					X		X
MOV	X	X	X	X	X		X
SMVD			X	X	X		?
NHP	X	X			X		
Spiroplasma penaei				X	X		
Streptococcus spp.					X	X	
Vibriosis					X	X	
Vibrio penaeicida		X		X	X	?	
Shrimp tuberculosis					X	X	
RFLB-MHD	X	X		X	X		
Gregarines	X				X		
Haplosporidians	X				X		
Microsporidians	X				X		
Lagenidium spp.					X	X	
Sirolpidium spp.					X	X	
Fusarium spp.					X	X	
AHNS/EMS					X		
Infectious muscle necrosis				X	X		
SSGS			X	X	X		

13.0 Summary of Results of Completion of ICES Protocol, Annex 6, Annex A, Part 2 (ICES, 2012)

Table 9 summarizes the results of completion of the ICES risk assessement process for pathogens, parasites and fellow travelers:

Table 9. Results of completion of the CES Annex 6, Appendix A, Part2: Pathogen, Parasites and Fellow Traveler Risk Assessment Process.

Step 1 Determining the Probability of Establishment		
Element Rating	**Probability of Establishment (H,M,L)**	**Level o(f Certainty VC to VU)**
Estimate the probability that a pathogen, parasite or fellow traveler may be introduced along with the species proposed for introduction.	Low	VC
Estimate the probability that the pathogen, parasite or fellow traveler will encounter susceptible organisms or suitable habitat	High	VC
Final Rating	**Low**	**VC**
Step 2 Determining the Consequence of Establishment of a Pathogen, Parasite or Fellow Traveler		
Element Rating - Impacts of establishment of a parasite, pathogen or fellow traveler on native species and/or aquaculture in a watershed	**Consequences of Establishment (H,M,L)**	**Level of Certainty**
Ecological impacts on native ecosystems both locally and within the drainage basin including disease outbreak, reduction in reproductive capacity, habitat changes, etc.	High	RU
Genetic impacts on local self-sustaining stocks or populations (i.e. whether the pathogen, parasite or fellow traveler affects the genetic characteristics of native stocks or species.	Low	VC
Final Rating	High	RU
Step 3 Estimating Pathogen, Parasite or Fellow Traveler Risk Potential		
Component Rating	**Element Rating (H,M,L)**	**Level of Certainty (VC to VU)**
Probability of Establishment estimate	Low	RC
Consequence of Establishment estimate	High	RU
Final Risk Estimate	**Medium**	**RU**

The above example analysis is made using a highly serious pathogen (e.g. one of the OIE-listed diseases) to complete the table (as with PRA, all pathogens considered a threat (i.e. a potential hazard, in PRA terms) would need to be evaluated separately). A "medium" risk, using the ICES system would indicated that risk management measures are required. Note that the ICES system does not permit an estimate of "Probability of Establishment" of a pathogen lower than "low". In the present risk analysis for whiteleg shrimp, the risk management measures proposed by SAS will reduce the probably of pathogen introduction to a level where all pathogens are not considered to be potential hazards. As a general observation, the use of the ICES protocol for evaluation of pathogen risk is considered to be less rigorous than applying import risk analysis (IRA) using the OIE (2012a) framework.

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Tang, K.F.J., Pantoja, C.R., Redman, R.M. & Lightner, D.V. 2007. Development if in situ hybridization and RT-PCR assay for the detection of a nodavirus (PvNV) that causes muscle necrosis in *Litopenaeus vannamei*. *Dis. Aquat. Org.*, 75, 183–190.

Tang, K.F.J., Pantoja, C.R., Redman, R.M., Navarro, S.A. & Lightner, D.V. 2011. Ultrastructural and sequence characterization of *Litopenaeus vannamei* nodavirus (*Pv*NV) from Belize. *Dis. Aquat. Org.*, 94, 179–187.

Tapay, L.M., Lu, Y., Gose, R.B., Brock, J.A. & Loh, P.C. 1997. Infection of white-spot baculovirus-like virus (WSBV) in two species of penaeid shrimp *Penaeus* *stylirostris* (Stimpson) and *P. vannamei* (Boone). p. 297–302. *In* T.W. Flegel & I.H. MacRae, eds. *Diseases in Asian Aquaculture III.* Fish Health Sect., Asian Fish. Soc., Manila.

USMSFP. Undated. *INDUSTRY BULLETIN! USMSFP clarifies SPF standards*. US Marine Shrimp Farming Program, 2 pp. (available at: http://www.broodstock.com/Hawaiian_SPF_Shrimp_Broodstock/home_files/spf_flyer.pdf)

Utari, H.B., Senapin, S., Jaengsanong, C. Flegel, T.E. & Kruatrachue, M. 2012. A haplosporidian parasite associated with high mortality and slow growth in *Penaeus* (*Litopenaeus*) *vannamei* cultured in Indonesia. *Aquaculture*, 85-89.

Wilson, D. 2000. The appropriate level of protection. p. 159164. *In* Quarantine and Market –Access – Playing by the WTO Rules, a forum held 6-7 September 2000, Canberra, Australia

Wongteerasupaya, C., Boonsaeng, V., Panyim, S., Tassanakajon, A., Withyachumnarnkul, B. & Flegel, T.W. 1997. Detection of yellow-head virus (YHV) of *Penaeus monodon* by RT-PCR amplification. *Dis. Aquat. Org.,* 31: 181–186.

Wongteerasupaya, C., Vickers, J.E., Sriurairatana, S., Nash, G.L., Akarajamorn, A., Boonsaeng, V., Panyim, S., Tassanakajon, A., Withyachumnarnkul, B. & Flegel, T.W. 1995. A non-occluded, systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and causes high mortality in the black tiger prawn, *Penaeus monodon. Dis. Aquat. Org.*, 21:69–77.

WTO. 1994. Agreement on the Application of Sanitary and Phytosanitary Measures. p. 69-84. *In* The results of the Uruguay Round of multilateral trade negotiations: the legal texts. General Agreement on Tariffs and Trade (GATT), World Trade Organization, Geneva.

Authors' Qualifications

J. Richard Arthur, M.Sc., Ph.D.

Dr J. Richard Arthur has more than 40 years experience in aquatic animal health. He received his M.Sc. (parasitology) and Ph.D. (fisheries parasitology) from the University of Calgary and completed a US Academy of Sciences Scientific Exchange Visit to the Soviet Union and Czechoslovakia, and Department of Fisheries and Oceans Post-doctoral and NSERC Industrial Research Fellowships in Canada before serving as a Project Advisor to the Bureau of Fisheries and Aquatic Resources, Manila, Philippines and the International Development Research Center's Fish Health Network Coordinator. After serving briefly as IDRC's Program Officer (Fisheries and Aquaculture) based in Singapore, he returned to Canada in 1989 as Head of the Parasitology Section of DFO's Maurice Lamontagne Institute, where he conducted research on the taxonomy of fish parasites and their use as biological tags for stocks of commercially important fish.

Since 1997 Dr Arthur as been an independent international consultant on aquatic animal health based in Barriere, British Columbia. He has working experience in more than 50 countries through contracts with the Food and Agriculture Organization of the United Nations, the Network of Aquaculture Centres in Asia-Pacific, the Secretariat of the Pacific Community and the Asian Development Bank. His consultancy work has focused mainly on risk analysis for aquaculture, biosecurity, and national aquatic animal health policy development and strategic planning.

He is the author or editor of more than 100 scientific publications, including several recent volumes on the application of risk analysis to the aquaculture sector.

Victoria Alday-Sanz, D.V.M., M.Sc., Ph.D.

Dr Victoria Alday-Sanz received her DVM from the University of Zaragoza and her M.Sc. and Ph.D. from the Institute of Aquaculture, University of Stirling. She has over 20 years professional experience, working mainly on aquatic animal health, including shrimp and fish diseases, diagnostics, health management, sanitary legislation and biosecurity.

Dr Alday-Sanz has collaborative projects with international organizations such as the European Union, the Food and Agriculture Organization of the United Nations, FESA, the World Organization for Animal Health and the Asian Development Bank. She is a frequent consultant to the shrimp farming industry and to insurance and financial companies. She has worked on R&D for INVE Technologies and is currently Director for Aquaculture Animal Health of Pescanova.

Dr Alday-Sanz is the author of over 80 publications and one patent, including two books, five book chapters, 32 refereed journal papers and more than 50 popular articles. She has also written numerous reports for international agencies.

Dr Alday-Sanz`s career has involved extensive international travel for research, consultancy and advisory work, project management and training in Southeast Asia, Latin America, the Middle East and Europe. She has directed a diagnostic service laboratory and research teams, coordinated multidisciplinary projects, and designed and implemented workshops.

Annex 1(A)

Independent Review by Dr Ben Diggles

Annex 1(B)

Response to the Independent Review of Dr. Ben Diggles

Prepared by Richard Arthur

We thank Dr Diggles for his thoughtful review which contains many useful comments. We have generally adopted all the major comments and corrections suggested by Dr Diggles, as well as the majority of the editorial suggestions marked directly on the draft MS. Our specific comments to individual points raised by the reviewer follow:

2.2.2 - the term "Import Risk Analysis" is only used for pathogen risk analyses involving international trade and within the OIE framework. It is not applied in other risk sectors (e.g. genetics, ecologic). However, in FAO documents we have often used the term "Pathogen Risk Analysis" to encompass both IRA (whose use is restricted to international movements) and non-international movements (domestic transfers). I would thus prefer to use Pathogen Risk Analysis in the title, with a brief explanation inserted in the introduction.

2.2.3 - "Glossary" refers to a section giving definitions of terms. I think what is meant here is an "Acronyms and Abbreviations" section - this has been inserted.

2,2,4 "For the sake of completeness, a summary of why the proposed undertaking is the one preferred by industry could be included at the end of the relevant section (Section 3.0)." - added.

2.2.5 "Given that the hazard identification step excludes several pathogens based on the risk mitigation methods used, presentation of additional detail on the risk mitigation methods used would be useful."

Risk management methods are presented only in broad terms, laying out the general principles and/or guidance that will be followed. The SAS will play a key and essential role in assuring that management standards are fully developed (e.g. infrastructural requirements, SOPs) based on the referenced international guidance but adopted to local conditions and the species being introduced. SAS, along with the relevant government agency (Ministry of Agriculture) will ensure monitoring and audit of all processes.

2.2.6 Commodity description - More detailed reference to the commodity has been added in the Executive Summary and the Introduction.

2.2.7 Biosanitary arrangements in the KSA - some available information has been added. I have asked colleagues in KSA to see if more information can be obtained. I expect that both the SAS and the Biosecurity Committee will play key roles in ensuring that all risk management measures are correctly and fully implemented.

2.2.8 Disease status of the KSA - The reviewer is correct that the lack of information on national pathogen status has caused us to adopt a very conservative approach (in such situations a "precautionary approach" is widely advocated). We have thus assumed that if it is "plausible" that a pathogen could be present in KSA (based on its known geographical distribution and host range) that it should be considered. Risks due to all such pathogens are rendered "negligible" by the use of SFP boodstocks and stringent quarantine

2.2.10 Hazard identification

" However, because the risk assessment process is effectively being performed for each potential hazard within Tables 7 and 8 as part of the hazard identification process, in my opinion additional detail is needed in both tables to ensure that the risk assessment process remains transparent. This is because for most of the hazards, the likelihood of release into KSH appears **negligible**, due to risk mitigation steps that occur **pre-border** (e.g. use of SPF broodstock, additional testing and so on)."

Agree - the modifications to Table 7 as suggested in the marked copy provided by the reviewer have been adopted.

"In this proposal, because of the comprehensive, worlds best practice risk mitigation measures proposed to be employed **post-border**, the likelihood of exposure of aquatic organisms in KSA to all the hazards (including unknown diseases) appears **negligible**. In essence, because the proposed risk mitigation measures rely upon **both** pre-border and post-border steps, I consider that it is important that the IRA recognises this in some way. I have made suggestions using track changes outlining a few changes to Tables 7 and 8 that could assist with ensuring this process remains transparent, and the IRA better highlights at which stage the chosen risk mitigation steps will be employed against each hazard. "

Agree - this is a very useful change. We have adopted the reviewer's suggested changes to Table 7 & 8. However, we have retained the use of "P" (plausible), rather than "?" (undefined by the reviewer) to indicate the possibility that the pathogen might be present in KSA (however, unlikely), based on the range of host species infected and the known geographical distribution.

Annex 2

Preliminary List of Suppliers of Specific Pathogen Free *Litopenaeus vannamei* Broodstock

State of Hawaii

The following suppliers of *Litopenaeus vannamei* SPF broodstock are listed by the State of Hawaii, Department of Agriculture (updated 13 September 2012) as having been in operation for over 24 months with continuous negative PCR surveillance testing (http://hawaii.gov/hdoa/adp/shrimpstock):

Keawa Nui Farms LLC. (Molokai)
Contact: Mr. John Austin
HC 1-479
Kaunakakai, HI 96748
Telephone: 808-558-8931
FAX: 808-558-8934
E-mail: kiwi1961@mac.com
Website: http://keawanui.nexcess.net/
Relevant Company Information:

- P. *vannamei* SPF and SPR certified broodstock are free of WSSV, TSV, YHV, IHHNV, HPV, MBV, IMNV, microsporidians, haplosporidians, gregarines, BP/MBV, NHP.
- Facilities have been disease-free for over 12 years.
- Each shipment is accompanied by A Health Status Report, Certificate of Origin, US Fish & Wildlife permit, Invoice, Packing Information, Export declaration, Airway bill

Kona Bay Marine Resources – Waimea Aquatic Laboratory (Kauai)
Contact: Mr. Jim Sweeney
7550 Kaumualii Hwy.
Kekaha, HI 96752
Phone: 808-338-0331
FAX: 808-338-0332
E-mail: info@konabaymarine.com
Website: http://www.konabaymarine.com
Relevant Company Information:

- World's leading provider of SPF and SPR *P. vannamei*. Supplies broodstock to customers around the world and specializes in shipping throughout Asia. Kona Bay GSR-Taura™ is the world's leading line of Taura virus resistant shrimp ; developed by Kona Bay to be highly resistant to TSV and to be very high growth.

- Kona Stock of *P. vannamei* is from the SPF population at the Oceanic Institute in Hawaii. Weight of males ranges between 40 to 45 g and 45 to 50 g for females. Age of shrimp broodstock is from 9 to 12 months from postlarval stage.
- Commercial hatcheries in Asia, Latin America and the US that use Kona Bay GSR-Taura™ are assured of having the finest white shrimp available. Kona Bay GSR-Taura™ broodstock are highly efficient producers of both nauplii and postlarvae.
- Certified SPF by the State of Hawaii, Kona Bay GSR-Taura™ is raised in an advanced bio-secure facility. All shipments include a Health Status Report prepared by the State of Hawaii certifying its disease-free status.

Molokai Sea Farms International (Molokai)
Contact: Mr. Steve Chaikin
P. O. Box 560
Kaunakakai, HI 96748
Phone: 808-553-3547
FAX: 808-553-5216
E-mail: shrimp@broodstock.com
Website: http://www.broodstock.com
Relevant Company Information:

- Specializes in the production of SPF and SPR broodstock. Domesticated families of broodstock have a stock origin from the SPF/SPR populations of the USDA Marine Shrimp Farming Program at the Oceanic Institute in Hawaii.
- SPR broodstock are resistant to TSV. The Oceanic Institute refined these families from extensive research and development.
- The State of Hawaii Aquaculture Disease Prevention Program's Veterinarian Medical Officer lll Allen C. Riggs DVM, MS routinely samples our population for WSSV, IHHNV, HPV, TSV, YHV, INMV MVB and other serious pathogens of marine shrimp. Shrimp are tested using state of the art technology by D.V. Lightner at the Aquaculture Pathology Laboratory, University of Arizona.
- Facilities have remained free of serious shrimp pathogens since the inception of shrimp operations in 1984. Molokai Sea Farms International is the longest running aquaculture operation in the State of Hawaii.
- A Health Status Report, Certificate of Origin, US Fish & Wildlife permit, Invoice, Packing Information, export declaration and airwaybill accompany each shipment. We can request special documents such as Disease History Reports or Sanitary Certificates from the Disease Prevention Program if required.

The Oceanic Institute – Makapu'u Facility (Oahu)
Contact Mr. Steve Arce
41-202 Kalanianaole Highway
Waimanalo, HI 96795
Phone: 808-259-7951
FAX: 808-259-9762

E-mail: sarce@oceanicinstitute.org
Website: www.oceanicinstitute.org
Relevant information:

- Oceanic Institute (OI) is primarily a "not-for-profit" research and development organization that makes limited quantities of SPF *P. vannamei* broodstock available to private industry when there are excess stocks from research/breeding activities.
- OI typically only makes stocks available 3 times per year in 1-2 month windows (when the stocks are between 8-9 months of age), based on their research/production schedule. The next window of availability will begin in January 2013. After January, OI anticipates having stocks available in April/May.
- OI offers genetically improved *P. vannamei* broodstock from its selective breeding program for sale to select, successful companies which have sound management and biosecurity practices.
- The broodstock are SPF and will be accompanied by a State of Hawaii health certificate and certificate of origin.
- Disease screenings are conducted by the University of Arizona's Aquaculture Pathology Laboratory (an OIE Reference Laboratory) in conjunction with the State of Hawaii's Shrimp Surveillance and Certification Program. All OI shrimp stocks are screened for TSV, WSSV, YHV/GAV/LOV, IHHNV, MBV, BP, BMN, IMNV, HPV, PvNV, MoV, NHP, RLB-MHD, microsporidians, haplosporidians and gergarines.
- The broodstock are from a select group of OI's top-performing families selected for rapid growth and high growout survival. In research trials conducted at OI, the families were stocked at a mean size of 2.0 g, evaluated for growth at a stocking density of 209 shrimp/m^2 and were harvested at 20.4 g. At these high stocking densities, mean growth of the selected families was 0.29 g/day (2.0 g/wk) and mean survival was 88%.
- There is a minimum order of 600 broodstock and the price per broodstock is USD$25 excluding materials and freight charges. Broodstock are offered on a first come, first served basis, and interested buyers can secure their order by forwarding a 50% deposit to OI. The remainder of the balance will be due at least 7 days prior to shipment. (source, S. Arcie, Oceanographic Institute, pers. comm.)

Shrimp Improvement Systems - Hawaii LLC (Big Island)
Contact: Mr. Kenneth Tay
73-4460 Kaahumanu Highway Suite #108
Kailua-Kona, HI 96740
Phone: +65 6397 0555
E-mail: kenneth@shrimpimprovement.com
Relevant Company Information: see below (note: recently expanding in Hawaii via purchase of High Health Shrimp; also see: http://hawaiitribune-herald.com/sections/news/local-news/shrimp-farm-expanding-big-island.html)

State of Florida

Shrimp Improvement Systems (LLC SIS)
88081 Old Overseas Hwy
Islamorada FL 33036
Phone: (305) 852-0872
Fax: (305) 852-0874
Website: http://www.shrimpimprovement.com/
Relevant Company Information:

- Since the start of operations at Plantation Key, representative samples of all SIS stocks have been routinely monitored and have been found to be free of WSSV, TSV, IHHNV, YHV and NHP.
- On an ongoing basis, representative tissue and/or hemolymph samples from all stocks are submitted to an independent outside shrimp disease specialist to confirm the disease-free status of the NBC.
- All shrimp stocks in the NBC are tested twice a year by PCR for WSSV, TSV, IHHNV, YHV, IMNV and NHP using OIE approved methodologies and primers. Samples are collected from larval tanks, hatching tanks, PL tanks, maturation tanks and broodstock raceways using sample size statistical guidelines that assure a 95% confidence interval.
- In addition to routinely monitoring the disease status of the shrimp stocks within the NBC, wild-caught indigenous shrimp and crab species from the Plantation Key area are also monitored twice yearly by PCR for WSV, TSV, and IHHNV as a means of assessing thy potential for possible contamination of the facilities by local crustacean species.

Asia-Pacific

Aquaculture Promotion Co., Ltd.
22nd Floor, CP Tower, 313 Silom Road
Bangrak, Bangkok, Thailand 10500
Mr. Suravut Bavornvipat
E-mail : cpbroodstock@gmail.com
Tel. 668-4465-3022, 662-625-8300-7 Fax. 662-638-2737
Website: http://www.cpbroodstock.com
Relevant Company Information:

- CP SPF SPR broodstock has been developed over the past 10 years for Asian culture systems. The broodstock has a proven track record in producing superior results in maturation units, hatcheries, and farms. No other broodstock is comparable in providing both hatcheries and farms with the animal performance to deliver superior profits.
- CP introduced SPF *P. vanamei* into Asia in 2002, with the importation of certified disease free SPF shrimp from Hawaii. Subsequently, over 10 independent populations of SPF shrimp have been added to the CPF breeding program. Only shrimp that were cleared of a strenuous quarantine process were put into the

nuclear breeding center. The breeding center is where all selection takes place and new founding shrimp have not been added since 2005. Only select juveniles are passed out of the center to closed system, biosecure broodstock farms where the final broodstock for hatcheries are produced.
- Both nuclear breeding centers and broodstock farms are monitored for disease, and are certified by the government of Thailand as SPF for TSV, WSSV, HPV, IHHNV, MBV, YHV, GAV, IMNV, BP and NHPB.

Saipan Aquaculture
As Falipe Saipan
Commonwealth of Northern Mariana Islands 96950
USA
Tel. No. (670) 233-4770
E-mail: inquiry@saipanaquaculture.com
Website: http://www.saipanaquaculture.com/
Relevant Company Information:

- Our breeding programs are derived from large number of families, with a broad genetic base and incorporate intense selection on each generation using combination of family selection, mass selection (WFS) and marker assisted selection. The programs maintain population inbreeding at less than 1% per year to enable sustainable long term genetic improvement. The broodstock are offspring from a select group of top performing families selected for rapid growth, TSV resistance, and high pond survival.
- TSV resistance is determined by bioassay laboratory challenge tests. Families are exposed to TSV isolates from US (USTX95), Belize (BH01), Thailand (TH04) and Venezuela (VE05). Between and within family selection Genetic selection is undertaken from data collated and analysed from raceway, pond and bioassay trial.
- Product Guarantees include: Produced in Premium Health SPF facilities, Produced using no antibiotics, Produced using non-GMO feeds and non-GMO technology. Certified negative to class I viruses (WSSV, TSV, IHHNV, YHV)
- Our production system also complies with Best Aquaculture Practices (BAP) and all existing government and environmental laws on protection and conservation.
- To maintain our High Health Status, samples from on going cultures are periodically sent to the world renowned Shrimp Pathology Laboratory of University of Arizona. The facility has already established more than 3 years of SPF status. In addition, all incoming shrimp are certified SPF and quarantined until established as high health animals.
- Our production system allows for full traceability of the genealogy of the animal. Full production parameters are also recorded for each tank and culture batches, for reference if the need arises.
- The farm is certified SPF facility and has been under the disease surveillance of the University of Arizona Pathology Laboratory for more than 3 years now. The farm is located 220 feet above sea level, 2 miles from the nearest ocean and water sourced from a deep well 260 feet down. We have no neighboring shrimp farm.

The closest shrimp farms to us are the small farms in Guam more than 200 kilometers away.

Other potential suppliers

The following additional suppliers were recently approved by the Government of India (list dated 12.04.2011) to supply SPF *P. vannamei* to Indian shrimp growers (http://aquaculture.tn.nic.in/pdf/attn_importspfbroodstock-020109.pdf):

M/s. SyAqua
159, Serm – Mit Tower 11th FlK
Sukhumvit 21 (Asoke) Road
North Hlongtoey, Wattana
Bangkok – 10110, Thailand
Phone No: 66- 2- 661-7607- 9
Email: syaqua@syaqua.com

M/s. Vannamei 101 Co.Ltd., with joint venture
Partner Sibsaen Aqua Marine
178/5 Moo I, Paklok, Thalang
Phuket – 83110, Thailand
Phone No: (66)76- 529724
Email: mattbriggs101@gmail.com; david@ Vannamei 101.com
sibsaenshrimp@hotmail.com

M/s. Charoen Pokphand Foods Public Co. Ltd.
Shrimp Genetic Improvement Center
313, CP Tower, Silom Road, Bangrak
Bangkok – 10500, Thailand.
Phone No: (638) - 2000
Email: kungrankij@yahoo.com; cpudomask@yahoo.com

Shrimp Improvement Systems Pte. Ltd.
No.90, Lim Chu Kang Lane,
6F SINGAPORE 718873.
Tel. No.: (65) 6397 0555.
Fax No.: (65) 6397 0880.
E-Mail: sis_shrimp@singnet.com.sg
Website: www.shrimpimprovement.com
Relevant Company Information: see above.

INDEPENDENT TECHNICAL ASSESSMENT: IMPORT RISK ASSESSMENT FOR THE INTRODUCTION OF WHITELEG SHRIMP (*Penaeus vannamei*) TO THE KINGDOM OF SAUDI ARABIA

DigsFish Services Report: DF 12-03
14 December 2012

INDEPENDENT TECHNICAL ASSESSMENT: IMPORT RISK ASSESSMENT FOR THE INTRODUCTION OF WHITELEG SHRIMP (*Penaeus vannamei*) TO THE KINGDOM OF SAUDI ARABIA

Prepared by:

Ben Diggles PhD

Prepared for:

Saudi Aquaculture Society

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Disclaimer:

Summary

DigsFish Services was commissioned to provide an independent assessment of technical aspects of an Import Risk Assessment (IRA) conducted to assess the risk of pathogen introduction via proposed importations of whiteleg shrimp (*Penaeus vannamei*) (the commodity) into the Kingdom of Saudi Arabia (KSA). The assessment found the risk analysis is of a high calibre and its structure conforms with international guidelines. Both the pre-border and post-border risk mitigation measures proposed for the translocation represent worlds best practice in biosecurity arrangements following translocation of aquatic animals. Because of this, the simplified risk assessment process used has arrived at technically correct assessments of the risks posed by each potential hazard. Because of the comprehensive pre-border and post-border risk mitigation measures proposed, the likelihood of exposure of aquatic organisms in KSH to the hazards (including unknown diseases) appears **negligible**.

Some suggestions which may help clarify the document, include:

- As the scope of the IRA is limited solely to assessment of pathogen risk (as opposed to genetic, ecological or other risks), it may be appropriate to indicate this by including the word "pathogen" in the title.

- It would be useful if the document contained a glossary so that readers unfamiliar with shrimp diseases can better access relevant information on acronyms if required.

- The document could benefit from a few minor changes, particularly in the hazard identification step, which could be elaborated slightly to clarify which of the potential hazards are being mitigated by the risk reduction measures applied pre-border, as opposed to those being mitigated by post-border risk reduction measures.

- Several recommendations are also presented which, if adopted, would further improve the level of risk mitigation surrounding the proposed translocation. All of these recommendations are important and ideally should be adopted as part of KSHs biosecurity and zoosanitary procedures if possible.

- Please refer to the annotated version of the draft IRA for editorial suggestions.

1.0 Introduction

DigsFish Services was commissioned by the Saudi Aquaculture Society to provide an independent assessment of the technical aspects of an Import Risk Assessment (IRA) conducted by JRA Consulting. The subject of the IRA was to assess the risk of pathogen introduction via proposed importations of whiteleg shrimp (*Penaeus vannamei*) (the commodity) into the Kingdom of Saudi Arabia (KSA).

2.0 Assessment

2.1 Summary of IRA

The risk analysis provides an assessment of the risks of introduction of pathogens of *Penaeus vannamei* via proposed translocation of broodstock adult shrimp from specified approved suppliers of Specific Pathogen Free (SPF) *P. vannamei*. After introductory information (Section 1.0) the analysis defines the benefits to the KSA likely to be derived from the proposed undertaking (Section 2.0), as well as a number of alternative development strategies that could be employed by the industry (Section 3.0).

Section 4.0 then details the proposed risk mitigation methods which are to be used to control the risk of pathogen introduction. Section 5.0 contains information on the commodity in question, the regional and international contexts within which the IRA is presented, and promises to contain details on the biosecurity and zoosanitary arrangements within the KSA (though these were unavailable in the draft version that I assessed). Also included in this section were comments on other requirements for the analysis, including an estimation of the KSA's Appropriate Level of Protection (ALOP).

The methodology used in the IRA is explained in Section 6.0, while Section 7.0 contains a brief overview of diseases of penaeid shrimp with an emphasis on *P. vannamei*. The comprehensive risk mitigation measures proposed are outlined in Section 8.0, while the disease status of the KSA is briefly described in Section 9.0. The risk assessment proper begins in Section 10.0 with the hazard identification step, which identifies a comprehensive list of disease agents (hazards) which are known to infect the commodity. These are detailed in Table 7 (which was mislabelled as Table 8 in the draft).

As discussed in Section 6.2.3 on page 10, *the risk analysis takes the proposed risk management measures outlined in Section 8.0 into consideration during the hazard evaluation process*. This simplifies the assessment process because none of the potential hazards are considered as actual hazards that require further assessment. In effect, the comprehensive range of risk mitigation measures proposed leads to the risk assessment process being effectively performed for each potential hazard within Tables 7 and 8 as part of the hazard identification process. Section 11.0

summarises the outcomes and conclusions of the assessment process. Finally, Section 12.0 contains several recommendations for management of the translocation process.

2.2 Evaluation of IRA

2.2.1 Methodology

Dr Arthur is recognised worldwide as an expert in risk analysis for aquatic animals. He has published a large amount of literature on the subject and has contributed to guidelines used by regulatory authorities worldwide, including FAO, OIE and NACA. Because of this, the risk analysis is a high calibre document. The structure of the IRA follows international guidelines and the methodology used (see Section 6.0) conforms with recommendations by the OIE (OIE 2012) as used by other IRAs (e.g. AFFA 2001, Biosecurity Australia 2009, Diggles *et al*. 2009), and thus is entirely appropriate for the undertaking at hand.

2.2.2 Title and Scope

As the scope of the IRA is limited solely to assessment of pathogen risk (as opposed to genetic, ecological or other risks), it may be appropriate to indicate this by including the word "pathogen" in the title.

2.2.3 Glossary

A large number of acronyms are used in shrimp aquaculture. For the benefit of laypeople who may read the document, the acronyms used all need to be contained in one glossary at the front of the document, for ease of reader access if required. I have include an example glossary in Appendix 1 at the end of this document as a prototype which may be adapted if need be to help expedite this process, if there are critical time constraints imposed on the production of the final IRA.

2.2.4 Alternative strategies

After introductory information the analysis defines the benefits to the KSA likely to be derived from the proposed undertaking, as well as a number of alternative strategies that could be employed by the industry. It appears to be assumed (but not stated) that the alternative strategies are considered by the industry not to provide the same or higher level of industry and national benefit that could be expected from the proposed undertaking. For the sake of completeness, a summary of why the proposed undertaking is the one preferred by industry could be included at the end of the relevant section (Section 3.0).

2.2.5 Proposed risk mitigation methods

The various risk mitigation methods proposed in Section 4.0 and presented again in more detail in Section 8.0 are adopted from Briggs *et al.* (2004), Flegel (2006), Andrade (2011), OIE (2012) and the International Council for the Exploration of the Sea (ICES) protocols for introductions and transfers of marine organisms (see ICES, 2005, 2012). Together, these protocols basically represent what is internationally recognised as world best practice for biosecure translocation of aquatic organisms. However, many details of the risk mitigation methodologies proposed for use are left to other references, such as Arthur *et al.* 2007 and ICES 2012). Given that the hazard identification step excludes several pathogens based on the risk mitigation methods used, presentation of additional detail on the risk mitigation methods used would be useful.

2.2.6 Commodity description

Section 5.0 contains information on the commodity in question, however this is 6 pages into the IRA. For the benefit of readers or laypeople who may be unaware of the background to the proposal, the document could benefit from mentioning exactly what the commodity is (broodstock, rather than PL or other life stages) earlier, such as in the executive summary introduction and/or background sections. Some suggestions for this have been included in the annotated version of the draft.

2.2.7 Biosanitary arrangements in the KSA

Section 5.0 also promises to contain details on the biosecurity and zoosanitary arrangements within the KSA. However, this information was unavailable in the draft version that I assessed, but should be included when available because of the important relationships that would need to be developed between the KSA authorities and the proponents for activities such as monitoring of compliance with proposed risk mitigation arrangements, disease testing, potential zoning or compartmentalisation within the KSA, and ongoing disease surveillance.

2.2.8 Disease status of the KSA

The disease status of the KSA is briefly described in Section 9.0. It is noted that the disease status of penaeid shrimp in the KSA is not well known, and that the status of many important diseases remains undetermined at this time. To assist readers, the hazard identification table (Table 7) should contain details of country status for each disease (present/absent, or unknown). This has not hindered the IRA process though, as it appears that it has been assumed that absence of evidence equates to evidence of absence, meaning the IRA is more conservative than it otherwise could have been. Furthermore, the occurrence of WSSV and TSV in native shrimp populations in the KSA has not been used as an excuse to ignore these diseases in the IRA. The analyst rightly assumes that the industry and the KSA intends to control these diseases at a national level, through methods such as risk analysis, risk mitigation, and possibly other methods such as zoning or compartmentalisation.

2.2.9 ALOP for the KSA

Also included in Section 9.0 were comments on other requirements for the analysis, including an estimation of the KSA's Appropriate Level of Protection (ALOP). I note that the ALOP chosen is highly conservative, and indeed similar to that adopted by countries such as Australia and New Zealand, both of which are recognised internationally as having world leading biosecurity standards.

2.2.10 Hazard identification

The hazard identification step (Section 10.0) identifies a comprehensive list of disease agents (hazards) which are known to infect the commodity. These are detailed in Table 7 (mislabelled as Table 8 in the draft). As discussed in Section 6.2.3 on page 10, ***the risk analysis takes the proposed risk management measures outlined in Section 8.0 into consideration during the hazard evaluation process***. This step was useful in that it reduces the need for further (and unnecessary) assessment of risks associated with release and exposure for the majority of hazards that were identified in the hazard identification process. This greatly reduces the length and complexity of the risk assessment process, without necessarily compromising the quality of the outcome.

Indeed, I consider that the simplified risk assessment process has arrived at technically correct assessments for each potential hazard (as summarised in Sections 10.2 and 11.0 and in Table 8). However, because the risk assessment process is effectively being performed for each potential hazard within Tables 7 and 8 as part of the hazard identification process, in my opinion additional detail is needed in both tables to ensure that the risk assessment process remains transparent. This is because for most of the hazards, the likelihood of release into KSH appears **negligible**, due to risk mitigation steps that occur **pre-border** (e.g. use of SPF broodstock, additional testing and so on).

However, for some of the hazards (e.g. ubiquitous organisms and unknown or emerging diseases such as EMS/AHNS for which no reliable diagnostic methods are available at this time, and/or no SPF stocks exist), there is no reliable way to ensure that pre-border risk mitigation steps can completely exclude a pathogen (if present), meaning that in essence, the risk of introduction (release into KSH) remains **non-negligible**. As mentioned in Section 7.1, Lightner (2011) noted that the global spread of serious shrimp viruses such as IHHNV, TSV and WSSV was to some extent due to the emergence of "new" shrimp diseases and their spread to new countries and regions **prior to their recognition** by the industry and the subsequent development of reliable diagnostic methods for them. Thus the same can still be said today for EMS and other unknown or emerging diseases of shrimp (and for that matter, diseases of all other aquatic organisms too). One flaw in conventional risk analysis is that the risk of disease translocation remains in the absence of disease identification (Gaughan 2002). Because of this, absence of identification of known pathogens is not sufficient grounds in itself to consider all pathogen risks have been completely mitigated. This is why it is important to employ the precautionary principle (as mentioned in Section 5.5), and ensure that risk mitigation for proposed translocations does not rely entirely on pre-border measures.

In this proposal, because of the comprehensive, worlds best practice risk mitigation measures proposed to be employed **post-border**, the likelihood of exposure of aquatic organisms in KSH to all the hazards (including unknown diseases) appears **negligible**. In essence, because the proposed risk mitigation measures rely upon **both** pre-border and post-border steps, I consider that it is important that the IRA recognises this in some way. I have made suggestions using track changes outlining a few changes to Tables 7 and 8 that could assist with ensuring this process remains transparent, and the IRA better highlights at which stage the chosen risk mitigation steps will be employed against each hazard.

2.2.11 Recommendations

Section 12.0 contains several recommendations which, if adopted, would further improve the level of risk mitigation surrounding the proposed translocation. I agree that all of these recommendations are important and should be adopted as part of KSHs biosecurity and zoosanitary procedures if possible. Instead of being presented as dot points, I consider that the recommendations should instead be numbered to simplify matters if and when specific recommendations need to be referred to at a later date.

2.2.12 Other editorial matters

Some additional editorial suggestions are contained in a version of the document annotated using track changes.

Conclusion

The risk analysis conforms to international guidelines and I consider that the simplified risk assessment process used has arrived at technically correct assessments of the risks posed by each potential hazard. Because of the comprehensive, worlds best practice risk mitigation measures planned to be employed for this proposed translocation, the likelihood of exposure of aquatic organisms in KSH to the hazards (including unknown diseases) appears **negligible**. However, the document could benefit from a few minor changes, as outlined above. In particular, additional details could be included in the hazard identification step, mainly to clarify which of the potential hazards are being mitigated by the risk reduction measures applied pre-border, as opposed to those being mitigated by post-border risk reduction measures.

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Appendix 1 - Example of a glossary used for a shrimp IRA

ABBREVIATIONS AND ACRONYMS

AHNS	Acute Hepatopancreatic Necrosis Syndrome
ALOP	Appropriate level of protection
ALOR	Acceptable level of risk
ASDD	Abdominal Segment Deformity Disease
BMNV	Baculoviral Midgut Gland Necrosis Virus
BP	*Baculovirus penaei*
BW	Body weight
DNA	Deoxyribonucleic Acid
EMS	Early Mortality Syndrome
FAO	Food and Agriculture Organization of the United Nations
GATT	General Agreement on Tariffs and Trade
GAV	Gill Associated Virus
GNS	Gut and Nerve Syndrome
HH	High Health
HPV	Hepatopancreatic Parvovirosis
ICES	International Council for the Exploration of the Sea
IHGS	Idiopathic Hyaline Granulomatous Syndrome
IHHNV	Infectious Hypodermal and Haematopoietic Necrosis Virus
IMNV	Infectious Myonecrosis Virus
IRA	Import Risk Analysis
LOVV	Lymphoid Organ Vacuolization Virus
LPV	Lymphoid Parvo-like Virus
LSNV	Laem Singh Virus
LSS	Loose Shell Syndrome
MBV	(= PMB, PVB) Monodon Baculovirus
MCMS	Mid Crop Mortality Syndrome
MCRV	Mudcrab Reovirus
MHD-SL	Milky haemolymph disease of spiny lobsters
MOV	Mourilyan Virosis
MrNV	*Macrobrachium rosenbergii* nodavirus
MSGS	Monodon Slow Growth Syndrome
NACA	Network of Aquaculture Centres in Asia-Pacific
NHP	Necrotising Hepatopancreatitis
OIE	Office International des Epizooties, the world organisation for animal health
PaV1	*Palinurus argus* Virus
PCR	Polymerase Chain Reaction
PHRV	Penaeid Haemocytic Rod Shaped Virus
PL	Postlarvae
PvNV	*Penaeus vannamei* Nodavirus
RDS	Runt Deformity Syndrome
RLB	Rickettsia-like bacterium
RPS	Rhabdovirus of Penaeid Shrimp
REO	Reo-like Viruses
RNA	Ribonucleic Acid
SBV	*Scylla* baculovirus
SMV	Spawner-isolated Mortality Virus
SPF	Specific Pathogen Free
SPR	Specific Pathogen Resistant
SPS	Sanitary and Phytosanitary Agreement
SPT	Specific Pathogen Tolerant
TEM	Transmission electron microscopy
TGAV	Tegumental Gland Associated Virus
TSV	Taura Syndrome Virus
WSSV	White Spot Syndrome Virus
WTO	World Trade Organization

YHLV	Yellow Head Like Viruses
YHV	(= YBV) Yellow Head Virus