Strategies for preventing the spread of fish and shellfish diseases

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SUMMARY: As the size and intensity of aquaculture enterprises increase in this millennium, so will the scale for potential loss from disease. Economies dependent on them for export earnings will be vulnerable to disastrous epizootics that must be avoided by implementation of appropriate safeguards. These should be based on a scientific determination of disease transfer risks and be designed to have minimum impact on international trade. The risk assessment process outlined by the WTO SPS agreement exemplifies this approach. We will illustrate the issues on preventing the spread of fish and shellfish diseases based on our experience with two shrimp viruses (IHHNV and WSSV). The greatest risk for disease spread lies with careless movement of living animals for aquaculture. Other risks will also be reviewed. Ultimately, intensive aquaculture will move towards closed recycle systems based on domesticated and genetically improved stocks. The impetus for this will come mostly as a result of pressure from national trade requirements concerning human and veterinary health risks, and from consumer groups concerned about the environment and animal welfare.

KEY WORDS: aquaculture, disease, risk, prevention

INTRODUCTION

Capture fisheries have reached their limits and the short-fall in future fish and shellfish demand for an ever increasing world population must come from aquaculture¹. As the size and intensity of aquaculture enterprises increase, so will the scale for potential loss from disease. Economies dependent on aquaculture for export earnings will be vulnerable to disastrous epizootics such as the staggering lost production due to white spot syndrome virus (WSSV) in Asia since 1993² and in Latin America since 1999. It will be increasingly important to avoid disease outbreaks by implementation of "appropriate safeguards" based on scientific determination of disease transfer risks and designed to have a minimum impact on international trade. The risk assessment process outlined by the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (SPS agreement) exemplifies this approach.

We contend that the greatest risk for spread of diseases lies by far with the careless, cross-boundary movement of living animals or fry destined directly for aquaculture facilities. There have been many examples of exotic species introductions according to the Food and Agricultural Organization statistics summarized in Table 1. This shows that about 65% of exotic species introductions

have been intentional, and that 69% of these (39% of the total) have been for aquaculture. The vast majority of introduced species (82%) have been finfish (Table 2), followed distantly by mollusks (9%) and crustaceans (6%). All would have the potential to carry pathogens. In our view, the most ominous would be viral and parasitic diseases that would otherwise have very little chance of being introduced.

Table 1. Causes of aquatic species invasions. Source: <http://www.fao.org/waicent/faoinfo/fishery/statist/fisoft/dias/statisti.htm>

Cause of import	No. of records	%
Known and intentional		
Aquaculture	1386	39
Fisheries	299	8
Ornamental	263	7
Research	104	3
Other reasons	272	8
Total intentional	2324	65
Known but unintentional		
Angling/sport	283	8
Diffused from other countries	139	4
Accidental	267	8
Bait	14	>1
Total unintentional	703	20
Unknown	552	15

Table 2. Species introductions by type. Source: <http://www.fao.org/waicent/faoinfo/fishery/statist/fisoft/dias/statisti.htm>

Group of species	# records	%
Fishes	2574	82
Molluscs	294	9
Crustaceans	191	6
Algae and plants	35	1
Other invertebrates	29	1
Other vertebrates	18	1

Because we are shrimp specialists, most of the examples given here will be for shrimp. However, the concepts and principles discussed could be equally applied to fish, mollusks and other aquatic species. We will focus on two shrimp viruses as recent examples of pathogens introduced with living shrimp for aquaculture, but other risks will also be reviewed. The strategy is to reduce these importation risks while also strengthening internal programs for direct disease control, including surveillance and (when appropriate) treatment, vaccination and confinement of outbreaks. In our opinion, intensive aquaculture operations will inexorably move towards closed recycle systems or otherwise bio-secure systems based on the cultivation of domesticated and genetically improved stocks. The impetus for this will come mostly as a result of pressure from international trade requirements concerning human health and import risks, and from consumer groups concerned about the environment and animal welfare.

INFECTIOUS HYPODERMAL AND HEMATOPOIETIC NECROSIS VIRUS (IHHNV)

IHHNV was first discovered in the blue shrimp *Penaeus stylirostris* and white shrimp *P. vannamei* in the Americas in the early 1980's^{3,4}) where it is believed to have been introduced by importation of live experimental stocks of black tiger shrimp *Penaeus monodon* from Asia⁵). Work on this virus has been reviewed on several occasions^{6,7}). IHHNV has been reported in several species of wild and cultured penaeid shrimp throughout the world⁷), but causes acute epizootics and mass mortality only in *P. stylirostris*. By contrast, it causes reduced growth and deformities in *P. vannamei*. Shrimp that survive IHHNV epizootics may carry the virus for life and pass it on by vertical and horizontal transmission⁷). Infected adults seldom show signs of the disease or mortalities⁸). *P. monodon* appears to be relatively unaffected by IHHNV while *Penaeus indicus* and *Penaeus merguensis* appear to be refractory to infection^{6,7,9}).

It is important to realize that IHHNV was unknown before it jumped from exotic *P. monodon* to American *P. stylirostris* and *P. vannamei*. We now know that IHHNV is endemic and prevalent in Asian *P. monodon* where it rarely harms its host. Indeed, highly sensitive polymerase chain reaction (PCR) assays may be required to detect its presence¹⁰). This is the type of scenario that poses the most serious disease threat to present and future aquaculture operations: i.e., a relatively innocuous (and possibly unknown) endemic pathogen (particularly viral) is moved together with a living aquatic animal over a large geographical distance to a new location where the local aquaculture species (or even local varieties of the import species) may be much more severely affected. Crustaceans may be particularly problematic because of their apparent propensity to have persistent, single to multiple viral infections without gross or even histological signs of disease¹⁰). As far as we know, the total lost production from retarded growth and mortality due to IHHNV has not been estimated, but it must be substantial when taken over the whole shrimp aquaculture industry in the Americas since the early 1980's. Some indication of its impact can however be gained from work carried out with stocks of shrimp bred specifically to be free of IHHNV infection. In an intensive system in Hawaii, removal of IHHNV related "runting" increased crop value by 162% over infected stock¹¹).

WHITE SPOT SYNDROME VIRUS (WSSV)

Although WSSV initially caused serious shrimp production losses in Asia only²), it must now be considered the single most serious shrimp pathogen worldwide. It was first reported from farmed *Penaeus japonicus* in Japan in 1993^{12,16}) and called penaeid rod-shaped DNA virus (PRDV) or rod-shaped nuclear virus of *P. japonicus* (RV-PJ)^{12,13,15,17,18}). Similar rod-shaped viruses from elsewhere in Asia were called by various names²) but Lightner⁷) grouped them in a single white spot syndrome virus (WSSV) complex. WSSV is now considered to represent a new virus family¹⁹) tentatively called *Whispoviridae* or *Nimaviridae*²⁰). Captured broodstock and postlarvae used to stock rearing ponds are known to carry WSSV, as are numerous other crustaceans and perhaps even aquatic insect larvae, but massive mortality usually occurs with juvenile shrimp in rearing ponds, probably precipitated by environmental factors¹⁰). In Thailand, outbreaks of WSSV in shrimp culture ponds initially occurred in 1994^{21,22}) and caused a peak estimated lost production of 70,000 metric tons in 1996. The total, cumulative lost production for all Asian countries since 1993 must now amount to several hundred

thousand tons. However, losses were later reported from the USA²³⁾, Central America and South America and molecular biological techniques have shown that WSSV from these outbreaks is identical or closely related to that in Asia²²⁻²⁸⁾. Thus, by 2001 the cumulative global loss to WSSV must be in the order of 1 million metric tons or more.

The WSSV outbreaks in Japan were the first widely reported, but they actually followed Chinese outbreaks and apparently resulted from the import of living post larvae from China directly to aquaculture facilities in Japan¹⁴⁾. No one knows how the virus spread throughout Asia after that, but the common practice of moving broodstock and postlarvae freely amongst countries was probably the most rapid and effective means of spread. Almost certainly WSSV was spread from Thailand to Malaysia and India in this manner. In addition, WSSV was not reported from the Philippines until 2000²⁹⁾, probably because of an effective Philippine government ban on importation of broodstock and postlarvae. Anecdotal evidence suggests that the Philippine outbreaks in the late 1990's originated from illegal import of postlarvae from China. As in Asia, a good part of WSSV spread in the Americas probably resulted from international transport of live shrimp for aquaculture³⁰⁾.

We find it curious that WSSV had not been reported from China prior to the catastrophic disease outbreaks in 1993, in spite of the fact that shrimp aquaculture had been practiced there for many years. The nature of the outbreaks was reminiscent of the initial IHNV outbreaks for *P. stylirostris* in the Americas, suggesting that they may have originated from importation of a distant, exotic aquaculture species carrying a previously unknown pathogen. Although a possible link has never been investigated, it is interesting that red claw (*Cherax quadricarinatus*), an Australian freshwater crayfish, had been imported into China for aquaculture in the immediate period before the outbreaks. The possibility that it may have been the original source of WSSV in China should be investigated. Evidence from Ecuador may support this possibility. We have discovered and confirmed by *in situ* hybridization the presence of WSSV in archived slides and paraffin blocks of diseased *P. vannamei* from Ecuador from 1996 (Flegel and Alday de Graindorge, unpublished). Thus, it was actually present three years before the widespread WSSV epizootics in 1999 and approximately two years after the importation of Australian red claw for aquaculture in 1994. In addition, 10 healthy red claw specimens from 3 Ecuadorian freshwater areas, remote from the sea, tested

positive for WSSV by polymerase chain reaction (PCR) assay in 2000 (Alday de Graindorge, unpublished). Although WSSV can be lethal in experimental red claw infections³¹⁾, our field data from Ecuador suggest that it may carry the virus at low levels without gross signs of disease.

OTHER SHRIMP VIRUSES

Yellow-head virus was first reported from Thailand in 1990³²⁾, although the causative agent was not identified until two years later^{33,34)}. Viruses closely related to YHV have been reported from *P. monodon* in Australia^{35,36)}, where they have been named gill associated virus (GAV) and lymphoid organ virus (LOV). YHV is a rod shaped, enveloped, positive sense, ssRNA virus, probably belonging to a new family allied to the Coronaviridae^{37,38)}. It is capable of infecting several penaeid species, including those from the western hemisphere^{23,39)}. It can also infect planktonic shrimp species that are common residents of shrimp ponds as grossly normal carriers (e.g., *Palaemon styliferus* and *Acetes sp.*).

YHV caused serious losses in Thai production in the early 1990's but has been overshadowed by WSSV since 1994. Although severe YHV outbreaks have declined in frequency since the early 1990's, they still do occur and cause significant production losses. In spite of this, the virus has not spread as widely as WSSV, judging from the low frequency of reports from other countries in Asia and from the Americas^{23,40)}.

Taura syndrome (TS) was first described as a shrimp disease in Ecuador in 1992⁴¹⁾ and later found to be caused by a virus named Taura Syndrome virus or TSV^{42,43)}. It is a cytoplasmic, non-enveloped, icosahedral, positive-sense ssRNA virus and tentatively classified as a picornavirus^{44,45)}. TSV was a serious cause of shrimp mortality for reared *P. vannamei* in the Americas where it spread principally through regional and international transfer of live postlarvae and broodstock. More recently, it was reported from *P. vannamei* reared in Taiwan after importation of live shrimp stocks from the Americas⁴⁶⁾. Although TSV infects a number of penaeid species⁷⁾, it has caused serious commercial losses only for juvenile to adult stages of *P. vannamei*.

RISK ASSESSMENT

As with the international spread of disease for other aquaculture species, IHNV, WSSV and other shrimp viruses appear to have spread mainly through the move-

ment of infected broodstock and fry (postlarvae). However, other possible routes of disease introduction need to be considered. These include importation of green frozen shrimp, processed shrimp, shrimp head-meal, and crustaceans in released ballast water.

Import of live animals

Growing perception of environmental and disease threats has led to increased interest in uniform standards for importation of all living marine animals and plants. A Code of Practice Regarding Introductions of Non-indigenous Marine Organisms was proposed by the International Council for the Exploration of the Sea (ICES) in 1973. For introduction of animals already part of current commercial practice, the Code gave guidelines for reducing the risk of pathogen introduction⁴⁷. For shrimp the Code would recommend that:

- (a) An imported broodstock population should be established in an approved quarantine facility. The progeny, but not the original import, should be transplanted into the natural environment or farms only if no diseases or parasites became evident during the quarantine period. The quarantine period would provide opportunity for observation and its duration would be at least one complete life cycle, regardless of the stage at which the shrimp had been introduced.
- (b) All effluents from quarantine facilities would be sterilized in an approved manner, killing all living organisms.
- (c) If evidence of disease was obtained during the quarantine period, the introduced animals and their offspring would be destroyed immediately and the facility sterilized.

Given the experience with IHNV in *P. monodon*, it would seem prudent to add to clause (a) the requirement that local species of shrimp, and especially economically important ones, be included as co-habitants in the quarantine phase. This would guard against the unintentional transfer of any well-tolerated, unknown pathogen from the exotic host to local species that might be more vulnerable and more seriously affected. Doing this would have avoided the release of IHNV in the Americas, WSSV in Japan and TSV in Taiwan. However, to make the guidelines work, standard protocols for inspection, disease diagnosis, and certification of shipments of live marine animals are required and the infrastructure for this is not always in place. More than anything, increased

awareness of the threats among aquaculture practitioners is needed.

In addition to the ICES code, signatories to the GATT and WTO are bound by the Agreement On The Application of Sanitary and Phytosanitary Measures (the "SPS Agreement"). The SPS Agreement applies to all sanitary and phytosanitary measures which may, directly or indirectly, affect international trade, including trade in live organisms. It permits sanitary and phytosanitary measures necessary to protect human, animal or plant life or health, but only if they are based on scientific principles. They cannot be maintained without sufficient scientific evidence, and must not arbitrarily or unjustifiably discriminate between members where identical or similar conditions prevail. In particular, it states that measures cannot be applied in a manner that would constitute a disguised restriction on international trade.

The risk of introducing exotic pathogens can be reduced significantly by using specific pathogen-free (SPF) stocks. However, in addition to possible deficiencies in SPF technology, other disease problems may develop from unknown pathogens in the imported populations. SPF stocks may also vary in the specific pathogens for which they are designated "free", so the excluded pathogens must be clearly identified.

Another approach with viral diseases is to develop resistant shrimp strains or to use shrimp species that are "specific pathogen resistant" (SPR), regardless of their pathogen status. However, "resistant" shrimp may sometimes carry the relevant virus as a persistent infection and be capable of introducing it to naïve populations. Even if a virus of the same name is already present in a native population, new strains may be introduced with SPR stocks and their prevalence might be maintained or increased. In addition, stress during farming can trigger increased viral replication in shrimp, resulting in disease outbreaks. There is also a danger that an exotic virus could mutate into a more pathogenic strain. This is particularly important for TSV and YHV, since RNA viruses are known to mutate and evolve rapidly⁴⁸. Even with SPF or SPR shrimp, first importation should involve co-habitation tests with local species and varieties to guard against introduction of unknown pathogens.

Fortunately, excellent diagnostic probes are available for all of the major shrimp pathogens⁴⁹ and they can be applied for screening broodstock and fry before importation and stocking. For example, PCR screening of shrimp

Figure 1. Flow chart showing risk factors associated with frozen shrimp.

postlarvae before stocking is now a routine practice for many Asian shrimp farmers, and it has been credited with reducing the risk of WSSV outbreaks^{50,51}. As a corollary, the risk of introducing IHNV, WSSV and other viruses with unscreened, living, captured animals or their fry from infected areas is extremely high.

Import of processed shrimp

Importation of cooked, dried and salted shrimp pose no threat of pathogen introduction, as the conditions used in their processing regime would inactivate the viruses.

Import of green shrimp

Green shrimp are fresh/frozen raw shrimp. Careful assessment shows that the possible risks from green shrimp vary greatly with product target use and the likelihood that viable viral particles present in frozen carcasses will reach a susceptible host. Pathogen viability can be affected by factors such as processing time, freezing and thawing after processing. The usage pattern and disease risks for various types of green shrimp are shown in Fig. 1 and discussed in the following paragraphs.

Before proceeding, however, we would like to caution against the use of PCR analysis to detect virus infection in commodity shrimp (i.e., processed or frozen shrimp). PCR is a very sensitive method for the detection of pathogens. However, it is based on detection of relatively small nucleic acid fragments and so can give positive results with non-viable viral particles or even degraded DNA (i.e., un-infectious material). Due to the high risk of dis-

ease transfer associated with living stocks destined for aquaculture, it is generally accepted as a valid test for screening live animals (OIE, Diagnostic Manual for Aquatic Animal Diseases), and we can accept that some rejected animals will be assay positive but un-infectious. However, PCR assays and even bioassays to evaluate the disease status of frozen shrimp carcasses destined for human consumption is, in our view, inappropriate since the relative risk of disease transfer from this source is likely to be low, as described in the following paragraphs.

Green shrimp for bait

Green bait shrimp are generally inexpensive and small (under 10-15g) and may be a potential route for dissemination of shrimp pathogens. Meyers⁵² has suggested that the European strain of virulent viral haemorrhagic septicaemia virus (VHSV) arose through the habit of feeding frozen bait to hatchery fish, and laboratory experiments have shown that frozen, Asian shrimp from supermarkets can result in WSSV disease when thawed and fed directly to test shrimp in an aquarium^{30,53}.

The use of green shrimp as commercial fishing bait or as a feed for live aquaculture stocks could allow viable viral particles to reach a susceptible host. Feeding them to hatchery stocks would be particularly dangerous, since it could contaminate fry destined for widespread stocking in culture ponds. Since bait shrimp are small and of low value, they are not usually farmed shrimp. On the other hand, emergency harvested shrimp from farms may be sold as bait if they are of small size. Since emergency harvests are frequently related to viral outbreaks, then

bait shrimp that originate from shrimp aquaculture ponds may entail higher risk than those from natural sources. However, this does not mean that bait shrimp captured in the wild pose no risk. Indeed, it is known that natural infections of WSSV and YHV do occur in wild populations^{24,54,55} although the frequency is probably variable with season and source.

The risk of shrimp disease transfer via sport fishing bait is a hot issue of debate in Australia where recreational fishing is a popular sport. In our opinion, the risk from this is very low when one considers all of the necessary factors. For example, Australia bans the import of bait shrimp, so that most bait shrimp used is of local origin. This is almost always heads-on (H-ON) shrimp. However, it is possible that a small percentage of more expensive imported table shrimp or shrimp illegally labeled as bait shrimp might be used for fishing. Using data for 1998 Thai exports to Australia (Table 3), 320 tons of H-ON frozen green shrimp were imported into Australia. If we assume all of this was used as bait, dividing this amongst the 92,000 recreational fishermen in the Northern Territories alone (not all of Australia) who spend a total of 2.2 million hours fishing annually (A survey of recreational fishing in the Northern Territory; <http://www.nt.gov.au/dpif/fisheries/recfish/fishfacts.shtml>), would give a negligible amount of 1.6 mg of bait per fisherman per fishing hour per year. This suggests a relatively low potential risk of transmission from this source. If this amount were divided amongst all Australian fishermen for a year it would result in an even lower risk of exposure.

Even if an infection was by chance successfully achieved by this route, we would have to consider that the infection would have to become established and that only a portion of the infected animals could potentially lead to permanent establishment of the pathogen in the local environment. Further transmission from the environment to aquaculture facilities would reduce the probability still further. Although many of the proportions given are guesses, a recent publication has shown that the infection coefficient from highly viremic whole shrimp in an aquarium is 0.6⁵⁶ (i.e., of every 100 exposures, only 60 result in infection). In spite of our belief that there is low risk from recreational fishing bait, we do however, support the Australian Quarantine Inspection Service (AQIS) recommendation (Prawn product import risk analysis: technical issues paper, Australian Quarantine and Inspection Service, Canberra) that it be further reduced by a ban on the import of green bait shrimp origi-

nating from WSSV endemic areas. This belief is based on a consideration of the ease of implementation, the potential impact of the disease on Australian farmers, and equally importantly the relatively minor impact on international trade in this commodity.

It has also been recommended that the practice of feeding shrimp broodstock with green shrimp and other crustaceans be discontinued in Taiwan and in Thailand^{24,54} because of the risk of transferring WSSV and other viruses. This is true whether the feeds used are local or imported. Indeed, the practice of feeding raw crustaceans should be completely discontinued in shrimp aquaculture facilities given the potential risk of pathogen transfer.

Table 3. Shrimp exports from Thailand to Australia, 1997-1998.

Product Type	1997		1998	
	Wt (Kg)	%	Wt (Kg)	%
Frozen Raw H-ON	33,036	1.1	320,297	6.9
Frozen Raw H-SO	5,511	0.2	13,800	0.3
Frozen Raw Peeled	607,617	20.0	542,187	11.6
Sub-Total	648,161	21.3	878,284	18.9
Frozen Cooked H-ON	255,530	8.4	831,537	17.8
Frozen Cooked H-SO	-	-	14,208	0.3
Frozen Cooked Peeled	2,140,394	70.3	2,937,285	63.1
Sub-Total	2,395,924	78.7	3,783,030	81.2
Total	3,044,085	100.0	4,661,314	100.0

H-ON = heads on; H-SO = heads off

Green shrimp for the food processing industry

There is a possibility that viable viral particles could reach a susceptible host from improperly handled waste of processing plants. Processing water could be a source of viable viral particles, depending on a number of complex factors, but certainly requiring a rather direct mixing with water containing shrimp or other susceptible crustaceans. However, in normal practice, processing water from peeling and heading operations is rather high in BOD and is not allowed to be discharged directly into open waters. After passing through treatment ponds and reaching quality standards acceptable for discharge, it is very doubtful that any viable virus particles would be present.

For solid wastes, Lightner³⁰ has proposed that an outbreak of WSSV in Texas may have originated from a factory processing imported, Asian shrimp and discard-

ing residues in an open dump from whence birds dropped infected carcass residues in nearby shrimp ponds. Although the suggestion remains unsubstantiated, frequent reference to this publication has given it the weight of fact in some forums and led to the argument that imported, exotic, green shrimp present a serious risk to aquaculture with native shrimp. We accept that improperly discarded solid wastes may entail greater risks of disease transfer than discharge water, and that both materials would present an extreme risk if introduced directly into receiving waters with susceptible crustacean hosts. However, determining the degree of risk from solid waste would also be complex and depend upon volume of material, viral loads, time in the dump before transfer, relative susceptibility of local species, virus viability, etc. As with bait shrimp, assessment of these risks and their inclusion in a full risk assessment would probably indicate a relatively low risk compared with more direct means of virus transfer. A simple solution to this problem would be to locate processing plants away from shrimp farming areas and to refrain from using open dumps. Because WSSV has many hosts¹⁰⁾, processing plants dealing with the fresh water prawn (*Macrobrachium rosenbergii*)⁵⁷⁾ and other crustaceans imported from WSSV infected areas should also regard these precautions.

Processing plants are generally very hygienic and use chlorinated water or other disinfectants in processing and cleaning. WSSV has been inactivated by exposure to sodium hypochlorite and povidone-iodine^{58,59)}. It is also inactivated by heating at 50°C for 20 min, by drying at 30°C and by treatment with ethyl ether. In 12.5% sodium chloride solution the virus is inactivated in 24 h at 25°C, although at high concentration it survives in sterile sea water at low temperature (4°C) for up to 120 days. By contrast, water from outbreak ponds appears to be infectious in the open environment for not more than 4 days⁵⁴⁾.

Two practical and easily implemented measures can be recommended to reduce the risk of infection from processing plants to negligible levels. For exporting countries, it should be possible to introduce simple, rapid, and inexpensive procedures to prevent shrimp arising from WSSV outbreak ponds being used in export production lines. This would reduce the already low risk of transfer. These procedures could be quickly adopted by voluntary inclusion in existing HACCP routines. For importing countries, appropriate waste disposal regulations should be introduced for re-processors of imported

shrimp, and if possible, they should not be located near shrimp aquaculture operations. As with bait shrimp, the cost associated with the impact of any disease and the relative ease of implementation of control measures would have to be taken into account. However, the volumes of material are much larger and this would also have to be considered on a disease by disease basis to establish a clear cost benefit.

Green shrimp for direct human consumption

We believe that the risk of viral transfer to shrimp aquaculture via shrimp destined for human consumption is very low whether the shrimp are whole, head-off or de-veined. Any perceivable risk can be easily eliminated by implementation of simple risk reduction procedures, such as those outlined in the previous section. Our contention is based on the fact that green shrimp have been traded globally in the order of one million metric tons per year without any proven link to the transfer of disease to aquaculture. For example, IHNV is endemic and highly prevalent in Asian *P. monodon*, yet the virus did not cause difficulties in the Americas until imported with living stocks for aquaculture. In another example, Japan has a resident shrimp aquaculture industry for *P. japonicus*. It is also one of the world's biggest per capita importers of shrimp with a good portion of this being frozen shrimp from Southeast Asia. In addition to frozen shrimp, Japan also imports live *P. japonicus* and *P. monodon*. From 1991 to 1998 during which time YHV was continuously reported, Japan imported 360,000 metric tons of fresh and frozen shrimp from Thailand alone. Approximately 1,000 metric tons was imported as live shrimp directly to the restaurant trade. There have been no reports of YHV outbreaks in Japan, in spite of this very large quantity, the very small geographic area of Japan, the relative proximity of most food processing plants to the sea and the lack of any specific risk reduction measures. Similarly, the USA imported 326,000 tonnes of fresh and frozen shrimp from Thailand over the same period with no evidence of YHV transfer by this route. Even for WSSV, the "evidence" for transfer by this route remains highly circumstantial and, given the volume of shrimp treated annually, outbreaks of the disease have been negligible in importing countries. There have also been no reports of YHV or WSSV transfer from Thailand to Australia, although the volume of imported shrimp (22,000 metric tonnes 1994-1998) was less than 1/10 of that to the USA and Japan.

Actual risks associated with the import of frozen shrimp for human consumption will depend upon the subsequent

treatment of these products. Laboratory infection tests^{30,56} demonstrate that transmission by this route is theoretically possible but the likelihood of disease through this route needs to be assessed based on the actual risks and probabilities involved as shown in Fig. 1. It can be seen that there is little risk associated with standard importing and marketing practices and end-use (cooking at home, for example). Indeed, given normal household and restaurant practice, it is difficult to imagine a route for viral transfer that would present any significant level of probable risk. Other risks arising from improper disposal of processing waste or product diverted for bait have already been considered.

As an example of risk calculation, shrimp exports from Thailand to Australia for 1997 and 1998 are shown in Table 3. In both years the majority of shrimp were imported as cooked product which carries no risk of transfer of WSSV or any other virus. Of the frozen raw shrimp, most is peeled or head-off product which has a limited risk. The highest potential risk, from frozen raw head-on product represented 1.1% and 6.9% of total imports in 1997 and 1998 respectively. The risk associated with these imports is represented in Fig. 2. Of the approximately 7% of shrimp which were imported as frozen raw head-on in 1998, most would probably have

been subsequently cooked as either head-on or after de-heading. The main risk would be with any infected, uncooked raw waste of which an unknown proportion may be disposed of in such a way as to bring it into contact with potential hosts during the time within which the virus remains infective.

In summary, there has been no known disease transfer from household or restaurant shrimp to aquaculture ponds or wild populations and we believe there is no reason to restrict trade in these products. This is in agreement with the AQIS prawn risk study for Australia (Draft prawn risk analysis, Australian Quarantine Inspection Service, Canberra, 2000).

Shrimp head meal and pelleted shrimp feed

Shrimp heads discharged from processing plants are often used for the production of shrimp head meal, a common ingredient in pelleted shrimp feeds. Even though these animals might have been infected with WSSV/YHV, the time-temperature regime used during head meal processing and feed manufacture would be sufficient to inactivate the virus. For example, some studies on viral inactivation have been carried out and some of these have been published⁵⁸⁻⁶². A review of the head meal processing conditions and feed processing conditions revealed that

Figure 2. Risk of WSSV/YHV transfer through Thai shrimp exports to Australia, 1998

the temperatures involved excluded the possibility of viral transfer by the feed route, and bioassay studies with WSSV in headmeal have confirmed this⁶⁰⁻⁶²). As a result, we are completely confident that these viruses cannot be transferred via the feed and it is accepted that shrimp headmeal inclusion in shrimp feed presents no reasonable risk of disease transfer. No experience from here or elsewhere has given any indication that this conclusion is invalid for IHHNV or TSV either.

Ballast water and fouling organisms

Increased travel and trade are providing many more opportunities for the introduction of new diseases. Cargo ships contain huge ballast tanks that are routinely filled and emptied to maintain stability with a vessel holding up to 3,500 m³ of water. Together with the water, infected/carrier animals can be collected and discharged. Indeed, discharged ballast water and sediment has led to unplanned and unwanted introductions of non-native plants, animals and pathogens⁶³). Fouling organisms on hulls and ropes have also been introduced. These routes of introduction are particularly worrisome in the case of WSSV for which over 50 crustacean carriers have been found¹⁰), some of which are very small and planktonic.

The Smithsonian Institute's invasion site (<http://invasions.si.edu/nmic.htm>) shows that known invaders at Chesapeake Bay amount to 228 species with fish 21%, mollusks 11% and crustaceans 9%. The Australian Center for Research on Introduced Pests (CRIMP) gives data from Port Phillip Bay, Victoria⁶⁴) with an estimate of 300-400 exotic marine species already established in the bay and an estimate of 2-3 new exotic species being established each year. Just for one harbor, that would account for 14 to 21 newly established species, including perhaps 1 to 2 crustaceans (10%) since the beginning of the WSSV epizootic in China. This does not account for those species that remain for a while but do not become established. The estimated numbers for released organisms are staggering. One can get some idea of the magnitude by using a spreadsheet for calculation of the theoretical of introductions from ballast water developed by Gollasch⁶⁵). This spreadsheet is based on results of the first European ship sampling study on ballast water and tank sediments undertaken on nearly 200 vessels in German ports from 1992 to 1996 and relates the potential scale of introductions by the volume of traffic and type of shipping using the port. We used the spreadsheet to calculate the estimated number of living zooplankton specimens from foreign sources entering the water at Lamchabang port in Thailand in the year 2000. The result was approximately 100 to 1,900 specimens per sec-

ond or 3.6 billion to 61 billion per year. This is only one port. Although there are several caveats in the use of this spreadsheet for calculation of ballast water introductions in different situations and in calculating the potential risk of disease introduction by this route, we believe it provides important information related to the possible scale of the problem associated with this previously little considered route of transmission. We believe that the risk of disease transfer by this route, although considerably lower than that from the import of live animals for aquaculture, may be greater than that from frozen products for human consumption due to the volume of ballast water and potential scale of introductions involved. The increasing size and speed of bulk carriers on international shipping routes makes survival of organisms in ballast water even more likely. Further spread of introduced organisms via coastal currents and domestic shipping may occur.

Given this risk, it is perhaps not surprising that WSSV first caused widespread outbreaks in Central America, including Panama, in early 1999. In April 1999, we assisted in PCR testing of freshly captured broodstock shrimp at the Smithsonian Institute in Panama. The results showed 9/60 positive for WSSV (unpublished), suggesting that the virus had been established in the local marine environment for a considerable period before the outbreaks. One possibility is that it was introduced there by Asian vessels approaching the Panama canal although this has been disputed on the basis that ballast discharge is not permitted in the canal zone. On the other hand, this does not discount the possibility of disease transmission through organisms fouling the hulls of vessels.

The International Maritime Organisation (IMO) has established some voluntary guidelines on ballast water handling although some of the suggested solutions are impractical from an operational viewpoint, being either prohibitively expensive or physically impossible. One option, the use of chemicals, would require approval by government agencies and could pose a threat to the ship crew and environment. However, judicious use of short-lived insecticides might be effective in eliminating arthropod carriers of WSSV and other diseases, reducing the probability of their transmission.

The IMO regards mid-ocean ballast water exchange (MOE) where the depth exceeds 2,000 m as the most effective method of ballast treatment. The idea is that species picked up in harbours are unlikely to survive when pumped out into the open ocean and that organ-

isms taken from open oceans would be generally less likely to survive when released in coastal waters. However, this method has some limitations as the amount of ballast water that can be exchanged varies with ship design and sea conditions and ships moving along the coast would not be able to follow these guidelines. In addition, a recent study by Botnen⁶⁶⁾ indicates that organisms including fish and crustaceans survive in ballast water tanks for long distances and times and that MOE changes the fauna assemblage in the ballast water, but that some organisms either remain in the tanks during water exchange or are brought back in along with the new water. Thus, for various reasons, even if MOE is implemented, ballast water would still be a possible means to spread diseases.

Whatever method is adopted, introduction and acceptance of ballast water exchange methods can only buy time until better technologies and ship designs are available to reduce the risk of exotic species transfers. The Marine Environment Protection Committee is presently working on an annex to MARPOL (the IMO convention for the prevention of pollution from ships) to make the application of the guidelines mandatory. We believe that there is a real risk of introducing WSSV and other virus diseases through the discharge of ballast water in coastal areas but the degree of this risk is difficult to estimate since it depends upon a number of variable factors. Estimations of risk associated with ballast water would have to be done on a case-by-case basis. However, mandatory ballast water exchange rules established for vessels moving between specific regions may help to limit the problem.

CONCLUSIONS

The highest risk of introducing pathogens is through the import of live animals (broodstock and fry) from infected areas. Intentional movement of live animals should be restricted and carried out following ICES regulations. This should also be applied to SPF stocks and in particular to SPR stocks of shrimp, since we already know these are likely to be infected with the viruses they resist. In addition, local initiatives can be taken such as the Asia Regional Guidelines for Responsible Movement of Live Aquatic Animals which are being formulated through a project of the Network of Aquaculture Centers in Asia Pacific (NACA), the United Nations Food and Agriculture Organisation (FAO) and the Office Internationale des Epizooties (OIE). These guidelines will form a basis for responsible and safe movement of live aquatic animals.

The degree of risk of introducing pathogens through frozen food depends more on the possibility of viable viral particles contacting a new host, than on the viral load of the carcasses themselves. Therefore, frozen products used for bait and feeding live stocks, would be classified as high risk as they would be released into the environment or fed directly to susceptible hosts. Products imported for the processing industry, would represent a risk only when the waste was not properly disposed of and where there existed the possibility of mechanical transport of a sufficient dose of viable pathogen to susceptible hosts within the time during which the pathogen remained infective. In our opinion, the possibility of this happening would represent a low risk. For similar reasons, we believe that frozen product imported for direct human consumption represents an even lower risk. Processed shrimp and shrimp head meal would pose no threat as temperature and drying procedures would inactivate pathogens. The release of ballast waters into coastal areas and the presence of fouling organisms on ship hulls poses a threat difficult to assess, and needs further investigation, but to us seems to present a much bigger threat for disease transfer than that from imported shrimp for human consumption due to the volumes of water and number of organisms involved. Although ballast water exchange as recommended by the IMO, is the most practical method so far suggested to reduce the disease transfer risk, we believe that the risks associated with ballast water and fouling organisms deserve further study.

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