Epizootiology, distribution and the impact on international trade of two penaeid shrimp viruses in the Americas

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Summary: Marine penaeid shrimp are affected by approximately twenty viruses, the majority of which were discovered as a result of their negative effects on aquaculture. In the Americas, infectious hypodermal and haematopoietic necrosis (IHHN) virus and Taura syndrome (TS) virus have had a significant negative impact on aquaculture industries and, in one instance, on a commercial fishery. Both viruses have become widely distributed as a consequence of the movement of host stocks for aquaculture. IHHN virus (IHHNV) causes catastrophic losses in cultured and wild Penaeus stylirostris. In marked contrast, P. vannamei is relatively resistant to IHHN but infection results, nonetheless, in poor culture performance. TS virus (TSV) is the ‘mirror image’ of IHHNV in its effect on P. stylirostris and P. vannamei. TSV causes catastrophic losses in P. vannamei, whereas P. stylirostris is highly resistant to TS. In the less than three years since the discovery of TSV in Ecuador in 1992, the virus has spread rapidly and caused massive production losses in most shrimp-growing countries in the Americas.


INTRODUCTION

In less than thirty years, the penaeid shrimp culture industries of the world have developed from experimental beginnings into major businesses, providing millions of jobs and billions of United States dollars each year in export revenue (74). This rapid growth has been accompanied by a recognition of the increasing importance of pathogenic agents, including significant numbers of viruses which infect penaeid shrimp.

More than twenty years have elapsed since Baculovirus penaei (BP = PvsNPV), the first known shrimp virus, was first described by Couch, in wild penaeid shrimp collected from the Gulf of Mexico coast in Florida (20, 21). By 1995, the list of penaeid shrimp viruses had grown to include nearly twenty viruses representing seven virus families (Appendix I). All but two or three of these viruses have been described in penaeid shrimp from aquaculture settings. While some of the known penaeid shrimp viruses seem to be of little economic importance, others can cause serious disease in...
penaeid shrimp hosts and provoke significant economic losses for the culture industries. Devastating epizootics due to various virus pathogens of penaeid shrimp have caused significant, and sometimes catastrophic, economic losses in commercial penaeid shrimp culture (8, 9, 18, 32, 34, 35, 72, 73, 77).

Viral pathogens have been implicated in the collapse of important shrimp aquaculture industries in Asia. Production in Taipei China fell from 100,000 t in 1987 to 30,000 t in 1988 as the result of a country-wide epizootic in which environmental degradation and viral agents were suspected to be causative factors (19, 74). The shrimp aquaculture industry in the People's Republic of China saw production collapse from 220,000 t in 1991 to 30,000 t in 1993 (18, 66, 73, 77). Likewise, recent major epizootics in Thailand, Indonesia, Japan, Taipei China and India have been accompanied by significant losses that, in some instances, reached 90% of the expected production for the year (18, 19, 22, 72, 77).

The shrimp culture industries of the Americas have also been adversely affected by serious epizootics due to viral pathogens. Infectious hypodermal and haematopoietic necrosis (IHHN) virus (13, 33, 34, 35) and Taura syndrome (TS) virus (15, 18, 24, 46) have had a significant negative impact on the developing aquaculture industries of the continents and, in the case of IHHN virus (IHHNV), on a commercial fishery as well (51, 57, 58). This paper reviews the published literature as well as recent information – as yet unpublished but available – on the effects of IHHNV and TS virus (TSV) on penaeid shrimp aquaculture and fisheries in the Americas.

INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS

Causative agent

IHHNV is the smallest of the known penaeid shrimp viruses (1, 7, 45). The IHHN virion is a non-enveloped icosahedron averaging 22 nm in diameter. It has a density of 1.40 g/ml in CsCl, contains linear ssDNA with an estimated size of 4.1 kb, and its capsid contains four polypeptides with molecular weights of 74, 47, 39 and 37.5 kD. On account of these characteristics, IHHNV has been classified as a member of the Parvoviridae family (7).

Disease

Infection by IHHNV causes serious disease in *Penaeus stylirostris*, and acute, catastrophic epizootics in semi-intensively or intensively cultured juveniles of this species (3, 13, 32, 36, 37, 43, 44). Soon after its discovery, IHHNV was recognized as a cause of infection and disease in other penaeids, such as *P. vannamei*. However, in comparison to *P. stylirostris*, *P. vannamei* was considered to be highly resistant to IHHN disease (2). Despite this resistance, ‘runt-deformity syndrome’ (RDS) in cultured *P. vannamei* was linked by epizootiological data to infection with IHHNV (16, 17, 31). Shrimp affected by RDS are characterized by variable, often greatly reduced, growth rates and by a variety of cuticular deformities affecting the rostrum (such as ‘bent rostrum’), antennae and other thoracic and abdominal areas of the exoskeleton (14, 31, 34). RDS is an economically significant disease of cultured *P. vannamei*, which has been observed in virtually every country in the Americas in
which *P. vannamei* is farmed. Populations affected by RDS may contain up to 30% runts, and consequently display a wide distribution of size classes (‘count’, or the number of shrimp per pound). Because runted shrimp have a lower market value than unaffected shrimp, RDS significantly reduces the value of *P. vannamei* crops, resulting in losses of revenue that can range from 10% to 50% of the value of similar crops which are free of IHHNV and RDS (31, 80).

**Distribution**

IHHNV has a world-wide distribution and wide host range in cultured penaeid shrimp, but its original distribution in wild penaeids remains unknown (11, 13, 32, 33, 36, 37, 38, 42). However, the occurrence of IHHNV (or a similar agent) in South-east Asia (Singapore, Malaysia, Indonesia and the Philippines) in shrimp culture facilities which used only *P. monodon* broodstock caught from the wild, and into which American penaeids had not been introduced, suggests that the region is within the natural geographic range of the virus, and that *P. monodon* may be among its natural host species (32, 40, 42).

The introduction of IHHNV into new geographical regions with imported shrimp has been well documented. Some of the accidental introductions of IHHNV (into Hawaii and Mexico, for example) have resulted in serious consequences for the shrimp culture industry in those locations (11, 13, 34, 35, 40, 43, 44, 51, 52, 57, 58). The introduction of IHHNV into the Gulf of California in Mexico, in 1987, was followed by serious epizootics of IHHN in *P. stylirostris* stocks in shrimp farms in the Mexican states of Sonora and Sinaloa in 1989 and 1990 (43, 44). Although Lightner *et al.* suggested that cross-contamination among shrimp farms was the means by which the virus was transmitted (44), a 1990 survey of wild shrimp stocks in the commercial fishery of the northern Gulf of California revealed that IHHNV infections were present at high rates of prevalence (57, 58). The broodstock used by the affected farms had been collected from IHHNV-infected wild stock. In his survey of wild penaeid stocks, Pantoja sampled and histologically examined the following species for IHHNV infection: 219 sub-adult or adult *P. stylirostris*, 74 *P. californiensis*, and 25 *P. vannamei* from thirty-nine stations representing the northern and central Gulf (57, 58). Although IHHNV-positive specimens were present in each of the three species, the species with by far the highest prevalence of infection and the greatest severity of disease was *P. stylirostris*. The prevalence of IHHNV-positive *P. stylirostris* was 50.3% in the northern region of the Gulf (67 of 133 examined), and 26% in the central region of the Gulf (19 of 73 examined). Concurrent with the high prevalence of IHHNV infections in the Gulf of California in 1990 was a 50% decline in shrimp landings at the major ports of the region from 1989 (25, 51, 68). This decline continued until 1994. However, in late 1994, Gulf fishermen reported increases in their landings of *P. stylirostris*, which suggests that the fishery was beginning to recover, perhaps due to the development of IHHNV resistance in the wild stock through natural selection.

Recent studies of wild penaeid shrimp from numerous locations on the western coasts of Mexico, Guatemala, Costa Rica, Honduras, Panama and Ecuador indicate that IHHNV is widely distributed in wild populations of Pacific American penaeids (32, 39, 40, 42, 43, 47). Historical data from the Gulf of California support the contention that IHHNV was not present in that region of Mexico before 1987, when IHHNV was first diagnosed in imported shipments of post-larval *P. vannamei* (43, 44). However, there are no such historical data for Central America and Ecuador (47).
Thus, determining whether IHHNV was introduced to the Americas, or whether it has always been here, may only be possible after serological or molecular comparisons of Indo-Pacific and American isolates of the virus.

**Diagnostic methods**

Traditional methods using histology (10, 14, 33, 34, 35), and molecular methods that employ non-radioactively labelled gene probes, are the current methods of choice for diagnosing IHHNV infection (41, 45, 49). Although monoclonal antibodies have been developed for IHHNV, their use has been hampered by their cross-reactivity to non-viral substances in normal shrimp tissue (41, 59). Histological demonstration of prominent Cowdry type-A inclusion bodies (CAIs) provides a definitive diagnosis of IHHN. These pathognomonic IHHN inclusion bodies are eosinophilic with H&E (haematoxylin and eosin) stains of tissues that have been preserved with fixatives containing acetic acid, such as Davidson’s AFA and Bouin’s solution (Fig. 1a-c) (4). Such Cowdry type-A inclusion bodies are intranuclear, within chromatin-marginated, hypertrophied nuclei of cells, in tissues of ectodermal origin (epidermis, hypodermal epithelium of fore and hindgut, nerve cord and nerve ganglia) and mesodermal origin (haematopoietic organs, antennal gland, gonads, lymphoid organ and connective tissue) (13, 33, 35, 39, 42, 45).

Non-radioactive digoxigenin-labelled gene probes have been developed for IHHNV (41, 45, 49), and are now commercially available in dot blot and in situ hybridization formats (Figs 2a-d and 3). These methods provide greater diagnostic sensitivity than more traditional, histological methods. Valuable broodstock shrimp may be examined for IHHNV with gene probes by using a non-lethal biopsy (41, 45) or by testing a haemolymph sample. This haemolymph sample may be taken with a tuberculin syringe; or an appendage, such as a pleopod, may be biopsied and used as the sample for a direct dot blot test. The excised appendage could also be preserved in Davidson’s fixative and processed for routine histology (4), but reacted with the IHHNV gene probe in an in situ hybridization assay. IHHNV-infected tissues are readily apparent in the epithelial, connective tissue or in the nerve of the appendage (Fig. 2d).

**TAURA SYNDROME**

**Causative agent**

Taura syndrome virus (TSV) is perhaps the most recently identified penaeid shrimp virus. TSV has been tentatively classified as a picornavirus, based on its morphology (~ 30 nm icosahedron), cytoplasmic replication, linear ssRNA of approximately 9 kb, and the fact that it has three major polypeptides (49, 36.8 and 23 kD) and two minor polypeptides (51.5 and 52.5 kD) in its capsid (24; J.R. Bonami et al., unpublished findings).

**Disease**

In the Americas, TS emerged between 1992 and 1993 as a major epizootic disease of *P. vannamei*. It has spread rapidly in the shrimp growing regions of Latin America, and now threatens most of the shrimp farming industries of the Americas (15, 24, 29,
Infectious hypodermal and haematopoietic necrosis lesions – diagnosis by histology

Plates 1a-c show infectious hypodermal and haematopoietic necrosis (IHHN) diagnostic lesions in *Penaeus stylirostris* and *P. vannamei* by routine H&E (haematoxylin and eosin) histology. Eosinophilic, intranuclear, Cowdry type-A inclusion bodies (arrows), which are diagnostic for IHHNV infection, are shown in a section of gills (a), haematopoietic tissue (b) and nerve cord (c). H&E stain. Scale bars = 10 µm
FIG. 2

Infectious hypodermal and haematopoietic necrosis lesions – diagnosis by DNA probe

Plates 2a-d show infectious hypodermal and haematopoietic necrosis (IHHN) diagnostic lesions by DNA probe using \textit{in situ} hybridization. IHHNV-infected cells are stained darkly by the probe. Cowdry type-A intranuclear inclusions (arrows), as well as cytoplasmic areas of some infected cells, are intensely positive for the virus and are shown in gills (a & b), haematopoietic tissue (c) and nerve cord (d). Digoxigenin-labelled genomic probe BS4.5 to IHHNV. Bismarck brown counterstain. Scale bars = 20 µm (a-c), and 10 µm (d)
Dot blot hybridization assay

Figure 3 shows a dot blot hybridization assay with a DIG-labelled probe (ShrimProbe™, DiagXotics, Inc., Wilton, CT), reacting with Log dilutions of infectious hypodermal and haematopoietic necrosis virus (IHHNV)-infected and uninfected shrimp tissue samples and purified IHHNV. Labelled columns are: + C = positive control (known IHHNV-infected shrimp) tissue homogenate; − C = negative control (specific pathogen-free [SPF] shrimp) tissue; 7A, 8A, 3A and 92-7 = positive reactions for IHHNV in unknown tissue samples; 29-1 (both rows) = a Log dilution of purified IHHNV from $10^0$ to $10^{-9}$ in the left column and from $10^{-9}$ to $10^{-3}$ in the right column. The first row in columns 7A and 3A contains no sample.

46, 76). *P. vannamei* accounted for more than 90% of the farmed shrimp production in the Americas (about 132,000 t), or about 15% to 20% of the world production of farmed shrimp in 1993 and 1994 (62, 63). *P. vannamei* is the principal penaeid shrimp species used in aquaculture in the Americas (66, 77). For this reason, TS may pose the most serious biological threat yet to the penaeid shrimp aquaculture industries of the Americas, and perhaps to its commercial shrimp fisheries as well.

Taura syndrome was first discovered in commercial penaeid shrimp farms near the mouth of the Taura River in the Gulf of Guayaquil, Ecuador, in mid-1992 (29, 46, 71, 76). Shortly after the syndrome appeared, both toxic and infectious aetiologies were suggested (29, 46, 71, 76), but the disease was eventually shown to be caused by a previously unrecognized infectious agent which is now called Taura syndrome virus or TSV (15, 24). Studies found that Taura syndrome could be induced in healthy, juvenile *P. vannamei* by exposure to the virus via the injection of cell-free homogenates, prepared from the carcasses of TSV-infected *P. vannamei* (from Ecuador and Hawaii), or by directly feeding those same carcasses to the indicator shrimp (15, 24). Identical results were obtained with TSV-positive shrimp from both Hawaii and Ecuador (15, 24).

Taura syndrome is now known to have occurred in shrimp farms throughout Ecuador, as well as in single or multiple farm sites in Peru, on both coasts of Colombia, in western Honduras, El Salvador, Guatemala, Brazil and in the United States of America (USA). Less than two years after its initial discovery in Ecuador,
TS had made its way into the USA, occurring at isolated sites in Florida and Hawaii, where it may have been contained (15, 46).

Taura syndrome virus has been documented in Mexico. In early 1995, the disease was detected in wild, adult *P. vannamei* collected from the offshore fishery of the southern Mexican state of Chiapas, near the Mexican border with Guatemala, and near to where TSV had first appeared in Guatemalan shrimp farms in 1994. Within weeks, TSV had appeared in post-larvae produced from the imported broodstock, or in the broodstock itself, in at least two farms in north-western Mexico. In addition to documenting the introduction of TSV into north-western Mexican shrimp farms with wild broodstock, the findings also suggest that TSV may be vertically transmitted, as well as through its known methods of horizontal transmission (D.V. Lightner, unpublished findings).

**Effect on the shrimp aquaculture industry**

The impact of TSV on penaeid shrimp aquaculture in the Americas has been severe. In Ecuador, where TSV first emerged in 1992 as a significant disease of cultured *P. vannamei*, the infection caused losses of between 15% and 30% of Ecuadorean production in 1993 and 1994 (62, 64, 65, 66, 76). In 1991, before TSV became a problem, the shrimp production of Ecuador was 100,000 t. At current shrimp wholesale prices (~ US$13.00/kg for 31/35 count tails [67]), a 30% reduction from that figure represents a loss of nearly US$400,000,000 in revenue per year. Taura syndrome has had an equally devastating effect on the shrimp farms of Honduras. The US Embassy in Honduras reported that shrimp farm production in 1994 was 2,300 t. In 1993, before TSV became established, production had reached 7,200 t (68). Published reports examining the impact of TSV on the aquaculture industries of other affected countries (e.g. Colombia, Peru, Guatemala and Brazil) are not yet available. However, the information which has accompanied diagnostic case submissions to the University of Arizona Aquaculture Pathology Laboratory indicates that losses are occurring in a similar pattern to those experienced in Ecuador and Honduras (D.V. Lightner, unpublished findings).

In Hawaii, the outbreak of TSV at two adjacent farms on Oahu, in mid-1994, meant that a viral aetiology could be experimentally demonstrated for the disease (15, 24). On one of the affected farms, the outbreak caused > 95% cumulative losses in stocks of juvenile *P. vannamei*, which were stocked at > 1,000 shrimp/m² in super-intensive raceway culture (52), within ten to fifteen days of the first observed mortalities (15). One of the Oahu farms was forced to close, and the second farm changed its operation to grow only TSV-resistant *P. stylirostris* (15).

In Florida, the disease was diagnosed in wild, adult *P. vannamei* that had been imported from Central America. These shrimp had been collected from the Gulf of Fonseca off the Pacific coast of Honduras and El Salvador. The affected, adult *P. vannamei* showed high mortalities and diagnostic lesions of the disease (D.V. Lightner, unpublished findings). It is sobering to consider this occurrence of TSV in wild broodstock from Honduras and El Salvador, together with the discovery of the disease in wild post-larvae collected during mid-1993, off Puna Island near the mouth of the Gulf of Guayaquil in Ecuador, and in wild adults from Chiapas, Mexico. All three examples illustrate the potential for this disease to become established in wild stocks, in regions where its potential effect on commercial penaeid shrimp fisheries cannot be known.
Possible vectors

Complicating the situation further is the very recent discovery that an aquatic insect may be involved in the epizootiology of TS (24). Increasing populations of the salinity-tolerant water boatman, *Trichocorixa reticulata* (Corixidae), were initially noted at a farm site in Ecuador which was in the midst of a severe epizootic of TS (27). Bioassays demonstrated that TSV was present in a sample of the insects. Taura syndrome was induced in specific pathogen-free (SPF) juvenile *P. vannamei* by the injection of crude, cell-free homogenates of insects collected from TSV-positive shrimp ponds (K.W. Hasson and D.V. Lightner, unpublished findings).

*T. reticulata* is common in estuarine ecosystems in the coastal areas of the Americas. It is distributed from California and Hawaii southwards to Peru on the Pacific side of the Americas, and from Florida to Texas and southwards to Brazil on the Atlantic coast of the Americas (27, 50). The insect has been reported as being abundant in TS-affected ponds in Honduras (J.A. Brock, personal communication) and Colombia (R. Bador, personal communication). Perhaps this insect is a factor in the rapid spread of TS in the Americas.

Diagnosis

The current diagnostic methods for TSV include the demonstration by histopathology in acutely affected shrimp which show gross signs of the disease, and bioassay. The latter demonstrates the presence of the virus in asymptomatic carrier shrimp (or other appropriate samples), using SPF juvenile *P. vannamei*, which serve as the indicator for the presence of the virus. Shrimp with acute natural or induced TSV infections display a distinctive histopathology. This histopathology consists of multifocal areas of necrosis of the cuticular epithelium and subcutis (of the general cuticle, gills, appendages, foregut and hindgut), which are characterized by the presence of cytoplasmic inclusion bodies. These inclusion bodies range from several to very many; they are variable in size, eosinophilic to basophilic, and they give TSV lesions a characteristic ‘peppered’ or ‘buckshot’ appearance (Fig. 4a-d), which is considered to be pathognomonic for the disease (15, 46).

A cDNA probe has recently been developed for TSV. A non-radioactive digoxigenin-labelled probe has been used successfully as a diagnostic reagent in dot blot assays with haemolymph, and by in situ hybridization assays with fixed tissue. Both techniques have distinguished TSV-infected samples from uninfected control samples (J. Mari, J.R. Bonami and D.V. Lightner, manuscript in preparation). Pathognomonic TS lesions show a very strong reaction with cDNA probes by in situ hybridization assays (Fig. 5a and b).

**ECONOMIC FACTORS CONTRIBUTING TO PATHOGEN INTRODUCTIONS**

Industry dependence on wild stock

One factor which has played a significant role in the spread of IHHNV and TSV within the Americas, as well as in most of the devastating disease epizootics that have affected the shrimp culture industries of Asia, is the near-total dependence of these industries on wild shrimp for ‘seed stock’ (61, 79). Nearly all of the seed stock
Plates 4a-d show Taura syndrome (TS) diagnostic lesions in *Penaeus vannamei* by routine H&E (haematoxylin and eosin) histology. Pathognomonic lesions for TS are focal to multifocal and diffuse. The lesions present a characteristic 'peppered' or 'riddled-with-buckshot' appearance, which is due to the presence of multiple, pale to darkly staining, often spherical inclusions of varying diameters (arrows). The structures in these cells are pyknotic or karyorrhectic nuclei and areas of cytoplasmic virogenetic stroma. Such diagnostic TS lesions are shown (in low and high magnification) as multifocal lesions in the cuticular epithelium (a & b), gills (c) and stomach hypodermal epithelium (d). Scale bars = 50 µm (a), and 20 µm (b-d)

required to stock the world's 1.15 million hectares of ponds is derived either from post-larvae gathered directly from the wild, or produced from spawners which were also caught in the wild (61, 66). To provide for the needs of the rapidly growing shrimp farming industry, international commerce in wild, penaeid shrimp seedstock (nauplii, post-larvae and broodstock) has become a characteristic of shrimp
FIG. 5

Taura syndrome lesions – diagnosis by genomic probe

Plates 5 a & b show Taura syndrome (TS) diagnostic lesions by a genomic probe using in situ hybridization. TS virus (TSV)-infected cells are stained dark by the probe. Cytoplasmic inclusions containing TSV nucleic acid are intensely positive in the cuticular epithelium of the sections of appendages shown, while pyknotic and karyorrhectic nuclear fragments, which give the lesion a characteristic ‘riddled-with-buckshot’ appearance (arrows), do not react with the probe. Digoxigenin-labelled cDNA probe to TSV. Bismarck brown counterstain. Scale bars = 50 µm (a), and 20 µm (b)

aquaculture. This practice, however, has been implicated in the transfer and introduction of a number of important viral pathogens of penaeid shrimp from one geographical region to another (37, 40, 43, 44). International commerce in TSV-infected penaeid seedstock and broodstock is the most plausible explanation for the rapid dissemination of TS in the Americas.

Importation of frozen product

The importation of frozen shrimp from regions with epizootic disease in their aquaculture industries also poses a threat to the aquaculture industry of the importing country. This is especially true for pathogens, such as TSV and IHHNV, which remain infectious after one or more freeze-thaw cycles. Introducing undetected pathogens with frozen shrimp products remains a lesser threat than introducing pathogens with live, virus-infected shrimp. However, there is a significant possibility that pathogens of concern to aquaculture (or human health) may be imported with frozen shrimp and inadvertently released into a domestic fishery or aquaculture industry by waste streams from shrimp processing plants or retail outlets, where imported shrimp are thawed and sold or reprocessed and repackaged for subsequent marketing. Another possible route for the introduction of such pathogens as IHHNV and TSV could be the use of cheap, imported, frozen shrimp as fishing bait near shrimp farm seawater intakes. This possibility has only recently begun to be addressed (5). Because emergency harvests
are a common practice in the industry when serious epizootics strike shrimp farms, and because many shrimp pathogens remain highly infectious in frozen shrimp tissue, this mechanism may explain the way in which some otherwise unexplainable shrimp pathogen introductions have occurred.

SOLUTIONS TO THE DILEMMA

Domestication and development of specific pathogen-free and specific pathogen-resistant shrimp

*International Council for the Exploration of the Sea guidelines*

Several sets of guidelines have been developed to help governments and private organizations to import non-indigenous, aquatic animal stocks for fisheries or aquaculture in a responsible manner, which is designed to reduce the risk of accidental introduction of pests and pathogens. In 1979, the International Council for the Exploration of the Sea (ICES) adopted a code of practice to reduce the risks of adverse effects arising from the introduction of non-indigenous marine species (69, 70). These guidelines provide a series of steps for evaluating the pathogen/disease status of the stock being imported. According to the guidelines, the evaluation of the stock should begin at its source, before importation. Monitoring of disease status continues after importation, throughout the life-long quarantine of the stock, during the development of broodstock, and the production of an F₁ generation. Only the F₁ stock is developed for fishery or aquaculture use, and its pathogen/disease status is also monitored.

The ICES guidelines have been used as a model for importations of penaeid shrimp stocks and, when combined with appropriate pathogen-detection methods, some noteworthy successes have been achieved. Marine Culture Enterprises was an operation that had been plagued by IHHNV in its imported stocks at its research and development facility on Oahu, Hawaii (36, 37). After adopting the ICES guidelines, it was able to restock its facility and develop captive, domesticated, SPF breeding stocks of Mexican *P. stylirostris*. The organization used these SPF stocks for its commercial development in 1984, and was free of IHHNV for four years. However, in mid-1987, Marine Culture Enterprises was struck by a massive IHHNV epizootic which caused catastrophic losses in its stocks of highly susceptible *P. stylirostris*. The source of the contamination could not be confirmed but it was likely to have come either from neighbouring facilities, or from other facilities on Oahu, which were culturing imported IHHNV-positive stocks that had not undergone the type of quarantine steps advocated by ICES (43, 44, 52). The IHHNV outbreak at Marine Culture Enterprises resulted in a ~ US$10 million loss. The facility was ultimately sold and converted to farm the more IHHNV-resistant *P. vannamei* (52).

*Development of specific pathogen-free shrimp*

The United States Marine Shrimp Farming Consortium (USMSFC) is a group of non-profit, research institutions that have been actively involved in shrimp culture technology since 1984. The USMSFC has made the development of breeding stocks of SPF shrimp its highest priority. The organization has learned from the experience of Marine Culture Enterprises, and believes that the threat of introducing unrecognised pathogens with imported, live, wild seedstock can be reduced, in time, by developing
domesticated breeding lines of the commercially important penaeid shrimp species. Thus, the USMSFC has adopted the ICES guidelines in its efforts to develop fully domesticated, SPF stocks for the fledgling US shrimp culture industry (79, 80). The list of specific, excludable pathogens has been published previously (48, 79), and the most recently revised list is given in Appendix II.

While the production of farmed shrimp was in sharp decline in many parts of the world, due to massive epizootics of infectious disease (18, 19, 54, 61, 73, 74, 77), the US industry grew from 1,200 t in 1991 to 2,500 t in 1993 (60). Crop projections for 1995 indicate that US production will be more than 4,800 t (A. Lawrence, personal communication).

Although many countries have tried to domesticate wild shrimp stocks for aquaculture, virtually nowhere else in the world has the shrimp culture industry been successful in domesticating and maintaining breeding lines of shrimp. Nearly all attempts to domesticate wild penaeid shrimp have ended in failure, and the only fully domesticated lines of penaeid shrimp currently in existence have been developed in the USA by members of the USMSFC (79) and in French Polynesia (75). Experience has shown us that previously unrecognized pathogens are commonly present at low prevalence rates in wild populations, and that these pathogens emerge to cause serious disease or poor culture performance when the rearing and domestication of captive wild populations are attempted (35, 48, 79). Predominant among such pathogens are certain viruses, which were unrecognized in wild shrimp stocks, but which caused serious problems under aquaculture conditions, and prevented the establishment of breeding populations in the USA, as well as elsewhere in the world (13, 35, 45). Thus, while the industry learned to breed shrimp in captivity, it was unable to domesticate the stocks needed because of the adverse effects of pathogens (mostly viruses), which were present in most of the stocks in demand, and which were not detectable with available diagnostic methods. The absence of reliable and sensitive methods to detect and exclude shrimp that were infected with these pathogens hampered the development of a sustainable, domestic, shrimp farming industry until such tools were developed early in the 1990s. The expansion of the US shrimp culture industry since 1991, with the use of SPF shrimp stocks, indicates that this technology can be successfully applied.

Development of specific pathogen-resistant shrimp

The alternative approach to developing SPF domesticated shrimp stock is to select and breed survivors of ‘specific pathogen-infected’ stocks to develop ‘specific pathogen-resistant’ (SPR) stock. Following this method, French researchers successfully developed a stock of IHHNV-resistant P. stylirostris in French Polynesia (75). This stock has been successfully used to develop the shrimp culture industries of Tahiti and New Caledonia (75). Recently, IHHNV-resistant P. stylirostris was introduced into an area of south-western Mexico, where IHHNV is enzootic. It is hoped that this stock can be developed as an alternative to the slower-growing P. vannamei, which currently makes up > 90% of the shrimp farmed in Mexico (66). In view of the recent accidental introduction and spread of TSV in Mexico, this SPR stock, which is resistant to disease when infected by IHHNV and TSV, may provide an alternative to the highly TSV-susceptible stocks of P. vannamei and the highly IHHNV-susceptible stocks of P. stylirostris which have been the only viable options for shrimp farming in Mexico until now.
The successful application of the ICES guidelines and the SPF concept depend on the ability to exclude certain pathogens. In situations where specific pathogens may not be able to be excluded, the development and use of SPR stocks may be the only alternative. The fact that IHHNV and TSV have become widely distributed in the Americas indicates that either government or industry-supported pathogen exclusion measures must be implemented and enforced to achieve the use of SPF shrimp stocks, or, alternatively, that SPR stocks should be developed and used.

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ÉPIZOOTIOLOGIE ET DISTRIBUTION DE DEUX VIRUS DES CREVETTES PÉNÉIDÉS DANS LES AMÉRIQUES ET LEUR IMPACT SUR LE COMMERCE INTERNATIONAL. – D.V. Lightner.


EPIZOOTIOLOGÍA Y DISEMINACIÓN DE DOS VIRUS DE LOS CAMARONES PENEIDOS EN LAS AMÉRICAS Y SU IMPACTO SOBRE EL COMERCIO INTERNACIONAL. – D.V. Lightner.

Resumen: Se conocen aproximadamente veinte virus que afectan a los camarones peneidos, la mayor parte de los cuales han sido descubiertos a raíz de sus efectos negativos sobre la acuicultura. En las Américas, el virus de la necrosis hipodérmica y hematopoyética infecciosa (NHHI) y el virus del síndrome de Taura (ST) han tenido un impacto sumamente devastador sobre la industria acuícola y, en un caso, sobre una empresa pesquera. Ambos virus se han propagado extensamente debido al movimiento de reproductores infectados utilizados para la acuicultura. El virus de la NHHI afecta a los camarones Penaeus stylirostris tanto salvajes como de vivero, y es responsable de pérdidas catastróficas. P. vannamei, por contraste, es relativamente resistente al virus de la NHHI, aunque la infección se traduce por resultados mediocres en la producción. El virus del ST tiene los efectos inversos sobre ambas especies: ocasiona fuertes pérdidas en P. vannamei, mientras que P. stylirostris se ha demostrado muy resistente a este virus. Desde su descubrimiento en 1992 en Ecuador, el virus del ST se ha extendido con rapidez y ha provocado enormes pérdidas de producción en la mayoría de los países americanos en los que se practica la cría del camarón.

# Appendix I

## Viruses of penaeid shrimp

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Appendix II

A working list of excludable and certifiable pathogens of American and Asian penaeids

Pathogen type and specifically listed pathogens

Viruses

Infectious hypodermal and haematopoietic necrosis virus (IHHNV) - a systemic parovirus
Hepatopancreatic parovirus (HPV) - enteric paroviruses of HP
Lymphoidal parvo-like virus (LPV) - a systemic IHHNV-like parovirus
Baculovirus penaei-type (BPV) - an occluded enteric baculovirus
Penaeus monodon-type (MBV) - an occluded enteric baculovirus
Baculoviral midgut gland necrosis types (BMN) - a non-occluded enteric baculovirus
Yellowhead virus of P. monodon (YHV) - a systemic non-occluded cytoplasmic rhabdo-like virus
White spot syndrome viruses (WSSV) - (non-occluded systemic baculoviruses)
Taura syndrome virus (TSV) - a picornavirus

Protozoa

Microsporidians
Haplosporidians
Gregarines

Metazoan parasites

Larval nematodes
Larval trematodes
Larval cestodes

For additional information on these pathogens and the most appropriate diagnostic methods, see references 10, 13, 14, 22, 23, 30, 33, 34, 35, 45, 69, 70.
REFERENCES


