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Developing a specific pathogen-free shrimp: the case for IHHN virus

The IHHNV or the infectious hypodermal and hematopoietic necrosis virus has determined to some extent the direction of shrimp farming in the United States. The wide-spread use of *Penaeus vannamei* rather than the faster growing *P. stylirostris* is largely because *P. vannamei* is more resistant to IHHNV.

Juvenile *P. stylirostris* with acute IHHN disease exhibit reduced feeding, mottling of the cuticle, unusual swimming behavior, and ultimately, 80-95% mortality. The infection in *P. vannamei* induces no clear signs of disease and no dramatic mortality but can cause disease syndromes in grow-out farms. In 1989, an extensive epizootic of IHHNV in *P. vannamei* occurred throughout the western hemisphere. There were reports of runting, deformities, and decreased production in farms. There was also concern over the possible release of the exotic virus into coastal waters.

The Gulf Coast Research Laboratory Consortium (GCRLC) in the U.S. which was formed in 1984 developed a strategy to control the spread of IHHNV. They wanted to relocate IHHNV-free postlarvae to an isolation facility, grow them to broodstock, and produce the next generation of IHHNV-free postlarvae for stocking in farms.

IHHNV in wild penaeids

IHHNV is distributed in wild penaeids of the Pacific Coast of the Americas. The highest density is in the Gulf of California, where all samples of wild adult *P. stylirostris* were IHHNV-positive. The Gulf of Panama is next. The lowest IHHNV density seem to be north central America. No IHHNV-positive samples were found from Guatemala or northern Costa Rica; however, the virus was detected in samples from Nicaragua and El Salvador during quarantine. Wild penaeids in Ecuador may have IHHNV, but survey has not been extensive. Southern Mexico has not been surveyed.

The geographic distribution of *P. stylirostris* is as extensive as that of *P. vannamei*, and presumably that of IHHNV in the latter species.

Acquiring specific-pathogen free stock

Selecting specific-pathogen free stock from the wild rests on the assumption that not all animals from a contaminated wild population carry the pathogen. If the prevalence of a pathogen in the wild is known, securing animals free of the pathogen is a statistical sampling problem -- that is, what is the optimum sample size to be certain no infected animals are present in a given sample? The American Fisheries Society "blue book" provides a table that recommends how many fish should be examined. For example, if a parasite is present in 10% of fish and the lot being evaluated contains 4,000 fish, 27 should be examined in order to be at least 95% confident that the pathogen will be detected, if present.

In principle, obtaining specific pathogen-free stock from contaminated farms is the same as obtaining them from the wild. However, the likelihood of finding specific pathogen-free individual is reduced because the animals in culture are at higher densities than in nature. Prevalence of infection can be 100%.

Quarantine and detection of viruses

The assumed specific pathogen-free stock are quarantined. Quarantine provides the time necessary for shrimp to develop signs of infection. It is usually stressful and can also provide the stimuli to amplify the presence of pathogens which can then be detected by examining a few samples.

If IHHNV is not detected by histology after 60 days in quarantine, a bioassay diagnosis for IHHNV is performed. This is done by feeding an IHHNV-infected *P. vannamei* to the more susceptible *P. stylirostris*. After 9-30 days, infected *P. stylirostris* will die and show intranuclear inclusions characteristic of IHHNV infection.

During quarantine, water that is routinely settled, filtered and disinfected with chlorine is

used. Routine sanitary work procedures include restricted access, the use of foot baths at the entrances to all doors, segregation of equipment by tank, regular cleaning and disinfecting of equipment and rooms, disinfection of shrimp waste and debris, and clean feed preparation areas. Effluent water is disinfected with chlorine prior to discharge into municipal sewers that terminate at landfills.

Founder populations

If collected from 10 sites, about 240 breeders (120 males and 120 females) are needed to maintain, if not increase, genetic diversity in founder populations. This number is based on the resources available, the biology of the shrimp, and the experiences in animal breeding programs.

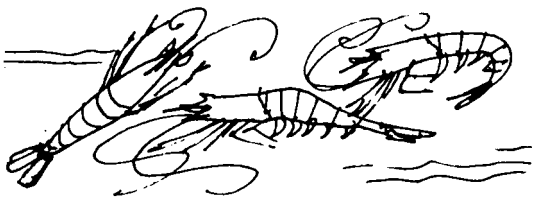
Other than IHHNV, several pathogens and potential pathogens are excluded from founder populations. These are:

- hepatopancreatic parvo-like virus, *Baculovirus penaei* and other baculoviruses. The reo-like viruses can not be excluded because no diagnostic method exists.
- rickettsia-like bacteria. Other bacteria are secondary invaders and exclusion is fruitless.
- microsporans like *Ameson* sp., *Agmasoma* sp., *Pleistophora* sp., and *Thelohania* sp.
- intermediate hosts of gregarine protozoans and of helminths

Fungi like *Fusarium solani* and fouling protozoan ciliates like *Zoothamnium* sp., *Acineta* sp., and *Hyalophysa* sp. are not excluded. They may only indicate stress. Crustaceans like the bopyrid isopods can be eliminated by removing infected *P. vannamei.* Their intermediate hosts are not found in quarantine tanks.

Some private fish farms in the U.S. are test-farming the specific pathogen-free fry produced from SPF broodstock provided by GCRLC.

Reference: JM Lotz. 1992. *Developing a specific pathogen-free (SPF) animal populations for aquaculture: a case study fo IHHN virus of penaeid shrimp.* p. 269-283. In: W Fulks and KL Main (eds). **Diseases of cultured penaeid shrimp in Asia and the United States.** The Oceanic Institute, Hawaii.



Farming high-health shrimps

Pond trials

Harlingen Shrimp Farms in Texas, U.S.A. has obtained yields ranging from 2.5 to 4.5 metric tons per hectare-crop. To achieve more consistent yields, the farm entered into a cooperative research agreement with the Gulf Coast Research Laboratory Consortium (GCRLC) in September 1990. The GCRLC supplied the farm with specific pathogen-free (SPF) broodstock to produce postlarvae for commercial-scale comparisons with selected farm stocks, named Texas broodstock source (TBS), which were IHHNV positive. The SPF broodstock were maintained in isolation from the farm stocks housed in the same facility. Regular inspection of the postlarvae indicated that the offspring were also SPF. The ponds stocked with postlarvae produced from SPF broodstock outperformed the TBS postlarvae in terms of survival, overall yield, and decreased size variation.

Reference: F Jaenike, K Gregg and L Hamper. 1992. *Shrimp production in Texas using specific pathogen-free stocks.* In: W Fulkfs and KL Main (eds).

In late 1982, Amorient Aquafarm in Hawaii initiated work on *Penaeus vannamei* at their maturation and hatchery site in Kahuku. From 1983 to 1989, no known viruses and other obligate pathogens were detected in the shrimps. In early 1989, however, infectious hypodermal and hematopoietic necrosis virus (IHHNV) was discovered in *P. vannamei.* The effect on shrimp production was dramatic, very slow growth rate that is characteristic of runt-deformity syndrome (RDS). In the IHHNV-infected RDS groups, the coefficient of variation in size (CV) increased from