Shedding some light on the confusion about SPR and SPF. Prepared by Stephen G. Newman Ph.D. President AquaInTech Inc.

It is not uncommon to hear a lot of acronyms being bandied about in shrimp farming. What do they mean and what is their impact on shrimp farming?

Perhaps the one that has had the greatest visibility and actually the greatest impact is SPF. SPF is the acronym for "Specific Pathogen Free". This is hardly an idea that is unique to shrimp farming and the concept of SPF animals comes from other agricultural sectors with the technology for application in aquaculture evolving from the ICES Code, The International Council for the Exploration of the Sea; Code of Practice to Reduce the Risks of Adverse Effects Arising from the Introduction on Non-indigenous Marine Species.(1) Lightner (2) does a great job explaining the need for SPF animals and how the original ICES code evolved to include shrimp as well as the history and rationale for SPF shrimp. There are few that would argue that this approach to shrimp farming has not been a critical element in the sustainability and rapid growth of global shrimp farming.

To generate SPF animals takes at least one complete generation and can take more depending on the starting material. Animals are housed in biosecure, indoor environments and tested repeatedly for the presence of specific pathogens that are Typically the pathogens that are listed by the United Nations OIE of interest. (another acronym that stands for the Office of International Epizootics-although they recent changed their name) as being problematic are the targeted pathogens. There is not a list of pathogens that is universal but in general, for shrimp, the list includes all of the economically important viruses, a single bacterial species and a few parasites. The list changes with time as new pathogens are identified. Today it includes the virus responsible for White Spot Disease (WSSV), the viruses that are the cause of Yellowhead (YHV) and related diseases (such as GAV-Gill associated virus), the Penaeus vannamei nodavirus (PvNV), Baculovirus penaei (BP), Baculoviral mid-gut gland necrosis (BMN), Monodon Baculovirus (MBV), the various variants of the Taura Syndrome Virus (TSV), infectious myonecrosis (IMNV), the hepatopancreatic parvovirus (HPV), and the virus responsible for Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV). (Unfortunately) This list will grow as the number of shrimp viruses that cause significant economic impact does. The sole bacterium on the list is the agent responsible for NHP or necrotizing hepatopancreatitis. The parasitic protozoans include gregarines, and spore forming protozoa microsporidians and haplosporodians.

By ensuring that animals that are not carrying these pathogens through several generations they are considered to be SPF animals. This has nothing to do with genetics or disease resistance or pathogen tolerance. It only means that that using

E mail: sgnewm@gmail.com
URLs: www.aqua-in-tech.com, www.bioremediationaquaculture.com, www.aqua-in-tech.com, <a href="mailto:www.aqua-in-tech.c

the available technologies in concert with biosecure containment to prevent infection, we are not able to find these particular organisms in these animals. Combined with history this is a powerful tool for being secure that these pathogens are not contained within the population.

Only animals that are produced and held in nuclear breeding centers and that have gone through the modified ICES protocols can be considered to be true SPF. soon as these animals leave a biosecure environment they cannot be considered SPF Some argue that if they are still negative for the presence of the pathogens on the list that they are still SPF. This is not the case. documented that the virus (and its many variants) that causes WSSV can become dormant in animals. They are PCR negative for the virus. Some have argued that geographic isolation is sufficient as the first SPF animals were produced in Hawaii, USA an area that one would consider to be geographically isolated. documented that animals from Hawaii have moved TSV. WSSV and IHHNV into areas where they did not exist before. Many companies buy SPF broodstock and then produce PLs under conditions that are not truly biosecure. They then sell the PLs as SPF. As soon as they leave a completely controlled indoor environment (as in a nuclear breeding facility) while they may technically still be SPF they should no longer be considered as such.

In the early days of SPF there was a lot of skepticism about the concept. Although a reliable and proven concept in agriculture, it was slow to catch on in shrimp farming. In areas where there was still a heavy reliance on wild seed many reported that SPF animals did not fare as well in the farm as did wild seed. Companies producing SPF animals struggled. Things changed when trials showed that SPF *Penaeus vannamei* fared much better in a multitude of environments where seed derived from wild *Penaeus monodon* broodstock were in use. It became widely accepted that SPF *P. vannamei* could be grown profitably and were less prone to a range of disease problems than wild, even though they may have been PCR screened, *P. monodon*. This began the shift away from farming *P. monodon* to *P. vannamei*. There are other reasons for this shift but the availability of true SPF animals was catalytic. It was clear that trying to produce animals that could not be absolutely established as being free of a myriad of pathogens in general was not as sure of a path towards sustainability as the use of SPF *P. vannamei*.

Some thought that SPF meant that the animals were resistant to these pathogens and that that is why they were free of them. This was perpetuated by less then ethical salesmen in some areas of the world often to sell animals that could no longer be considered SPF because of biosecurity lapses. SPF has nothing to do with the genetics of the animal. It is a term that refers to the confirmed absence of the specific pathogens mentioned earlier.

E mail: sgnewm@gmail.com
URLs: www.aqua-in-tech.com, www.aqua-in-tech.com, www.aqua-

Shrimp have very sophisticated immune systems although they are much different than that of the vertebrates. Estimates from the fossil record suggest that shrimp have been in their current form for many tens of millions of years. implies that they are able to successfully resist disease. As shrimp farming grew and expanded and different paradigms developed, shrimp were increasingly being cultured under high stress conditions in environments where disease could significantly impact productivity. Stress precipitated disease outbreaks were often associated with what are termed as non-obligate pathogens. These are pathogens that do not produce disease under most circumstances in healthy animals. Concomitantly there appeared pathogens that were obligate. While few would argue that the role of stress was still a component of susceptibility these obligate pathogens wreaked havoc. Interestingly many of the viral pathogens seemed to become less of a problem over time, even though some have high mutation rates and there are many different variants that may cause disease. At this time there is some interesting research that may explain why this happens. These shrimp were described as being resistant to these pathogens and the term SPR or specific pathogen resistant was coined

The ability to withstand exposure to these pathogens could be observed in the field and then confirmed by lab observations or the other way around. Lab challenge studies, by their very nature, do not replicate real world conditions. stress free environments using genetically uniform animals that are exposed to the pathogen over a short period of time (in most cases). The relative levels of survivals are determined at the end of the experiment and the conclusions In the real world shrimp are stressed and exposed to pathogens (usually more than one) on a more or less constant basis. Lab studies eliminate many of the variables and say nothing about whether exposure to different levels of the pathogen would elicit the same response. This is best accomplished by doing comparative LC50's. This entails exposing animals to a range of levels of the pathogens of interest and then comparing survivals at different levels of exposure. This approach is a powerful tool whereby one can effectively determine if a group of animals is SPR or SPT (specific pathogen tolerant). It does not work in every instance though. While there are some families that appear indeed to be SPR against certain types of pathogens, the vast majority that are being sold as SPR are SPT.

If one cannot kill a shrimp by any route of exposure at any level of exposure then the animal is truly resistant. This is a bit simplified as one can overwhelm an animals ability to defend itself in a manner that is unrealistic and not likely ever to happen in the real world--such as injecting large numbers of bacteria that are not able at any level of waterborne or oral exposure to kill animals. The lipopolysaccharide overload can kill them. The message though is that true resistance implies that no level of the pathogen in the environment can kill the animal. With a virus this

E mail: sgnewm@gmail.com
URLs: www.aqua-in-tech.com, www.aqua-in-tech.com, www.aqua-

would mean that no level of exposure including by injection could infect and kill the animal. Animals that are touted as being SPR for TSV can indeed be infected with the virus, as can those that are being touted as being SPR for WSSV.

The use of the term SPR is being used in a manner, which connotes an absolute in the minds of many farmers, much as the term SPF was when these animals first appeared. Even if animals can tolerate high levels of exposure to a pathogen under controlled conditions, this says nothing about what happens when stress and other pathogens are involved. A good example of this is WSSV. Anecdotally, when WSSV first hit Panama, many larger farms were seeing horrific mortalities from what looked to be the virus. Shrimp were dying within days of the apparent exposure often deeply red colored in huge numbers. Mortality rates could easily reach into the high 90 percentiles. There was a small farm, with four small ponds in the middle of this area. The animals were WSSV positive by PCR, yet they were unaffected and grew well. The owner did an excellent job of managing the stress that these animals were under and they never developed the disease. This was a clue about what was going on. Many savvy farmers understand now that WSSV infected shrimp in well managed and stress free environments do not die from WSSV. There is a very strong correlation between the overall stress that the shrimp are under and the presence of both obligate and opportunistic pathogens. Shrimp can carry the virus without becoming visibly ill from it. That does not mean that they are SPR (or even SPT).

It appears that shrimp have a sophisticated mechanism to cope with viral infections and that selection pressures will very strongly select against strains that are highly virulent. Shrimp are highly evolved and have the ability to adapt and likely continue to evolve. The extent to which those that sell and market SPF animals have exploited this process is not clear as most of the focus is on growth, where it should be. No doubt there have been some incremental gains against some pathogens and there are efforts underway on the part of many to further select for animals that are truly resistant and not just tolerant. Rapidly growing animals produced in largely stress free conditions with excellent feed conversions that are not carrying pathogens in with them have been a formula for profitability and sustainability for many. Be wary of salesmen and others telling you that they are selling SPR shrimp. While they may actually be SPT, one cannot infer that stressed animals will not be susceptible at some level to the disease. They are tools and not solutions.

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- 2. Lightner, D.V. 2011. Status of shrimp diseases and advances in shrimp health management, pp.121-134. In Bondad-Reantaso, M.G., Jones, J.B., Corsin, F. and Aoki, T. (eds.). Diseases in Asian Aquaculture VII. Fish Health Section, Asian Fisheries Society, Selangor, Malaysia

E mail: sgnewm@gmail.com
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