A review of the use of non specific immune-stimulants to reduce the impact of WSSV.

Presented By
Stephen G. Newman Ph.D.
President and CEO
Aqua-In-Tech, Inc.
6722 162nd Place SW
Lynnwood, WA 98037

The farming of shrimp has become a commercially successful global industry only during the last twenty or so years. Global production in 1998 was estimated at 737,200 metric tons (1). Unfortunately it has been stalled at or near this level for the last five years or so. Some predictions are that the commercial cultivation of shrimp may increase to as much as 1.6 million metric tons within the next decade (2). This, forecast, in light of the problems of the last five years should be regarded as overly optimistic. Viral and bacterial outbreaks have decimated the industries in Thailand (3) Taiwan, the Philippines, Indonesia, Malaysia, China, India and recently Central and South America (4). The White Spot Syndrome Virus is becoming pandemic in Central America and is Ecuador, Peru and Colombia. There is little doubt that disease is the number one problem affecting the economic viability and long-term sustainability of the industry.

From a management perspective, it is always better to prevent diseases rather than try to deal with them once they occur. This fundamental difference is reflected in the use of proactive disease management strategies versus reactive disease management strategies. All to often, the failure to take simple precautions to prevent disease problems results in disastrous and untreatable problems. Though there are many examples, the most recent example of this is WSSV. It is widely felt that this virus has been spread throughout SE Asia by the movement of infected animals in commercial trade. This scenario is being played out again with the Taura Syndrome Virus (TSV) in SE Asia. It is causing problems in Taiwan and is sure to spread to neighboring countries, all because of the lack of a proactive stance towards the disease.

In retrospect, it may turn out that the current problems with WSSV in Latin America are due to the same thing; the unrestricted movement of infected animals by commercial enterprises. Fortunately, shrimp farmers in this hemisphere have learned from the experiences of others and protocols intended to proactively manage the disease are being implemented.

Monoculture of animals is conducive to the spread of disease and shrimp farming is no exception. The management and control of disease is widely felt to be the major challenge to a sustainable industry. If the industry is to continue to grow and flourish, then the control of disease becomes paramount. Enlightened management strategies and cultural practices complemented by the use of a few essential tools will play an important role in bringing this about.

An important set of tools are those that are used for the rapid identification and detection of potential pathogens and for use in epidemiological studies designed to control the spread of disease by identifying carriers, vectors, determining how the disease is transmitted (vertical and/or horizontal), what levels of pathogen it takes to produce disease, etc. A widely used technique with WSSV is based on the polymerase chain reaction (PCR) (5).

Another set of tools are high quality diets and feed supplements that ensure that animals receive critical nutrients required for optimum growth and to cope with stressful rearing environments. Diets often contain the minimum levels of nutrients that have been found

to prevent dietary deficiency diseases under controlled laboratory conditions. Supplementation of diets with those minerals and vitamins such as Selenium and Chromium and Vitamins A, C, D and E can help.

A third group are those that can aid shrimp in defending themselves against disease outbreaks, in this case WSSV. These are compounds with non-specific immune stimulating properties. Since shrimp cannot be vaccinated, the use of these compounds will play a critical role in ensuring sustained profitability.

Prior to focusing on the use of these compounds, it is essential to consider how important the use of enlightened proactive management techniques are to ensuring the success of any program using immune stimulants. With WSSV, prevention starts with the adults. Though it is not widely believed that the virus can be vertically transmitted, published information on this is sparse (6,7) and there are anecdotal reports suggesting that it might have occurred. However, for the most part, it is felt that the cycle can be broken by the use of disinfectants and copious water washing of eggs and nauplii, much as has been done with MBV. It is important to disinfect both eggs and nauplii. The choice of the disinfectant is not likely as important as is the use of an effective disinfectant. Fortunately WSSV is very sensitive to iodine and chlorine containing compounds (8) making it easy to kill surface borne viral particles.

Disease outbreaks are not just a simple matter of pathogenic organisms being present. They are the result of complex interactions between the pathogen, the host and the

environment. Hosts must be susceptible to a pathogen and this is affected by genetics, the physiological state of the host, the presence and affect of stressors on the host and the quality of the environment that the animals are being reared in. The virulence of the pathogen and a myriad of other factors that impact viral ecology are also instrumental in determining whether a population gets ill. White Spot, as with all other infectious diseases, is impacted by interacting variables that determine the outcome of the disease process. In shrimp, genetic susceptibility, nutritional status, age and the presence of the virus at the earlier life stages all seem to be critical in determining the ability to resist the disease.

To date there is no evidence one way or another that tolerance to WSSV can be gained by genetic selection. The closed cycle of *L. vannamei* is much more conducive to exploring this than the open cycle with *P. monodon*, and within six months there should be some indications as to whether this is technically feasible (personal observations).

Certainly the nutritional status of the animals is important to their overall ability to resist any disease. This is widely documented with all animals and shrimp are no exception.
There is little doubt that the presence of the virus in stocked PLs significantly contributes to the high degree of mortality seen with the virus (9). Much more work needs to be done to better understand how to manipulate the viral ecology so as to minimize the load of virus that animals are exposed to.
Many compounds have been found to have non-specific immune stimulating properties, of which a dozen or more have been evaluated in fish and shrimp (10). The vast majority

of these have only been tested under controlled conditions in the laboratory and though a number of them do provide benefits, field tests will be the deciding factor in determining which ones find a niche in the market place. Only a few have been tested to determine if they impact viruses, specifically WSSV.

Table 1. Compounds with purported immune stimulating properties in shrimp and/or fish

Laminarin	Curdlan	Chitosan	Saponins
Barley Glucans	Scleroglucan	Beta 1-3 glucan	Herbal extracts
Lactoferrin	Zymosan	Dextran	Peptidoglycans
Levamisole	Schizophyllan	Lentinan	MDP
Lipopolysaccharides	Inulin	Krestin	

It is beyond the scope of this paper to discuss these.

This paper focuses on those that have been found to impact viruses in crustaceans. Most of the cell wall constituents. The group of compounds that has the greatest amount of published data on their use in shrimp are structural components of the cell walls of gram-negative bacteria. This group of bacteria includes the vibrios are the single most important group of pathogens affecting commercially reared shrimp species (11). Composed of lipids and carbohydrates, these cell wall components are often the first structures that invading bacteria present to the hosts' immune system. Classically referred to as endotoxins, lipopolysaccharides or LPS have been the subject of thousands of papers and are known to exert both specific and non-specific effects on the immune system of many animals, and potent non-specific effects in crustaceans (12, 13).

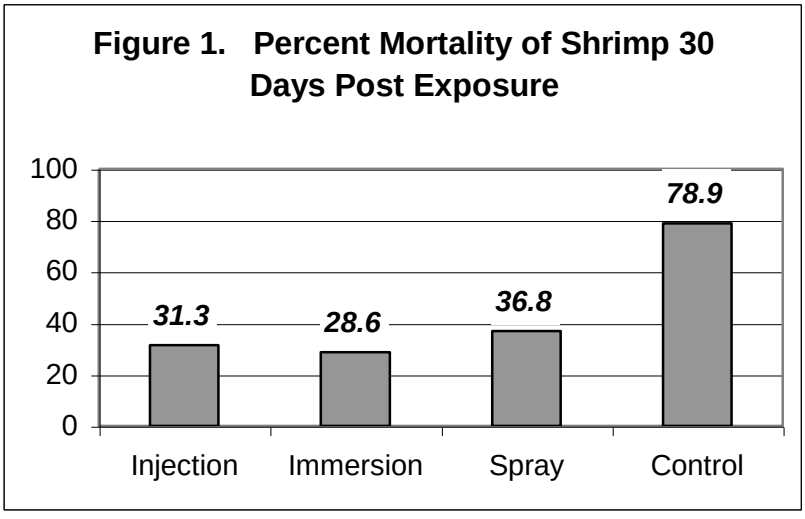
The first reported studies of the impact of LPS on shrimp date back to the late 1970's with the first published observations in the early 1980's. Crowder (14) reported on the work of Lewis and Lawrence at Texas A&M in April of 1981. Post larval *P. stylirostrus* were exposed to a dead suspension of a vibrio. Sixteen ponds were stocked, two with treated animals. Four months later the ponds were harvested. The ponds that were treated with the suspension had an 8-10% increase in production compared with the controls. One hundred treated and 100 non-treated animals were brought back to the lab and were temperature stressed in the lab. Mortality from the temperature stress was much less in the treated shrimp. Groups of 100 animals were also exposed to a pathogenic vibrio. It took almost 500,000 bacteria to kill each of the treated shrimp compared with 5000 for the non-treated shrimp. Though critical experimental details were not provided, this article marks the beginning of a series of experiments conducted with dead suspensions of vibrio bacteria over the next two decades that have demonstrated that LPS based materials exert a potent productivity increasing impact on the culture of shrimp.

In 1983, Lewis and Lawrence (15) reported additional observations on *P. setiferus*. PLs were exposed to a dead suspension of bacteria. The shrimp were stocked into 0.2-acre ponds at 12,000 animals per pond. Six weeks post stocking animals were harvested, weighed and sub groups removed for challenge. The mean weights for the three groups of treated shrimp were 7.9, 11.5 and 11.7 grams, contrasted with 4.1 and 7.2 grams for the two non-treated groups. The lethal dose of injected bacteria required to kill 50% of

the animals was more than 10,000 bacteria per animal for the treated group compared with a little more than 10 per animal for the non-treated group or almost a 1000 fold difference. The treatment resulted in substantially increased weights (presumably due to increased growth rates and decreased disease susceptibility) and considerably increased disease resistance to six weeks post-treatment. These first two studies showed that a single exposure to a suspension of dead vibrio bacteria provided a six week to a four month benefit under field conditions.

In the late 1980's, Itami et al. (16) evaluated the impact of a dead suspension of vibrio on the ability of *P. japonicus* to resist challenge with a virulent vibrio species. Groups of 8-11 adult kumura prawns were immersed in a 1% suspension for 1 hour, sprayed for 10 seconds or injected with 0.1 ml per animal. Thirty days post exposure they were challenged by injection. The results of the replicate studies are depicted in Figure 1. Statistically significant levels of protection were noted in all three of the groups, demonstrating that several routes of exposure could induce a protective effect.

Figure 1. Percent Mortality of Shrimp 30 Days Post Exposure



In 1990 Song et al. (17) reported on tests with a dead suspension of *V. vulnificus*, milled into feed at 0.1% (w/w). When fed to PL30 *P. monodon*, three times daily, for an extended period of time, an increase in the rate of growth was noted. Their analysis of protection failed. In 1991, Sung et al. (18) reported on the repetition of the immersion portion of the trial and noted a stimulatory effect on growth from a single treatment at PL13, though the animals were exposed to a 1:10 dilution of the bacterin, an impractical dilution for most hatcheries. Their analysis of protection failed as well. Challenges can fail for many reasons besides the lack of a protective effect (Newman-personal observations). These include the route of exposure to the pathogen, the virulence of the pathogen and the overall condition of the animals being challenged. Over and under challenges can mask any protective benefits.

Itami and Takahashi (19) in 1991 investigated the ability of an orally delivered cell suspension to impact survival of *P. monodon*. Vibrio cells were fed at 0.05, 0.5 and 5% of the weight of a diet to zoeal stage larvae for four consecutive days. Those groups fed the material all showed higher survival and molt rates to mysis. Since no analysis was made of the presence of disease, the authors speculate that the cells may have contributed to the enhanced survival in some undefined nutritional manner. Since vibrio strains produce a variety of hydrolytic enzymes and contain a number of nutrients, it is possible that some factor did contribute to an increase in the availability of a critical or critical nutrients.

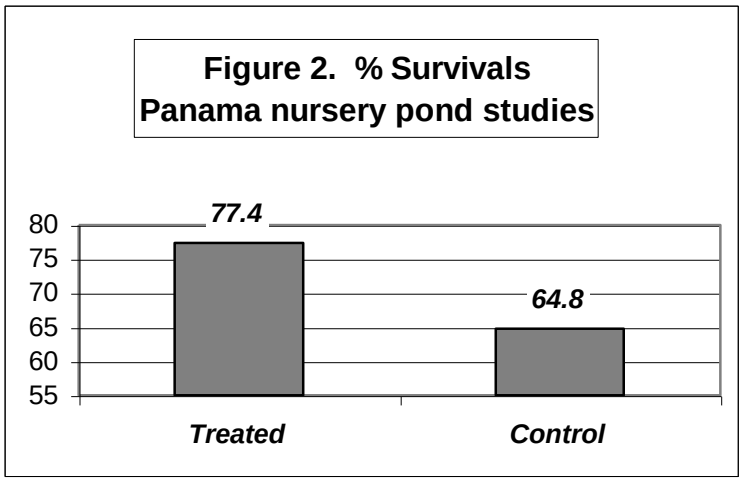
Itami et al. (20) published additional observations in 1992. Kumura prawns were exposed to three different concentrations of a dead vibrio suspension, 0.1, 0.5 and 1%, for five hours, and challenged by exposure to a virulent vibrio strain. A protective effect persisted for at least 50 days. No statistics were provided.

In a second article (21), the authors reported on their results with several cell preparations including ultrasonicated, heat-killed, cell-free and whole cell. They noted that all of the preparations protected shrimp against challenge and observed that the active component was a heat stable material located in the cells and in the culture broth-likely LPS. They were unsuccessful with a challenge of animals that had been fed material and also noted differences between bacterial strains as to the degree of protection from challenge.

The last few articles discussed dealt with lab-based studies, with the first few integrating both lab and field based. There is little doubt that exposure to LPS does have a significant beneficial impact both in the lab and the field on cultured shrimp. All of the lab challenges have been with vibrios. It is likely that since this is a non-specific effect, beneficial effects would have been noted with other pathogens, including viruses.

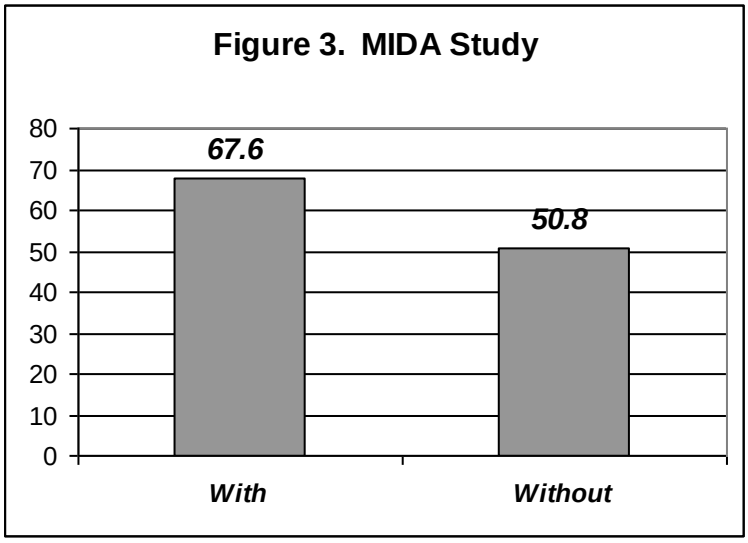
In 1992 Laramore (22) reported the results of field based studies in which post larval *P. vannamei* were exposed to a killed suspension of a vibrio species. Figure 2 shows the differential survival rates. Survival was followed through the nursery phase to harvest. The average survival in four replicates was 77.4% for treated animals and 64.8% for controls. This is a 12.6% difference or an almost 20% increase in survival. They also noted a 23% increase in yields (lbs/acre) in the treated groups. The differences noted in

survivals were no longer apparent at harvest (116 days post treatment) with survivals in both groups being in the mid 80 percentile. If the benefit of the treatment were attributed to an impact on disease, then high survivals would tend not to lend themselves towards seeing a benefit; i.e., there were no disease problems that the treatment could have protected the animals against. However the increase in yields persisted with the ponds containing the treated animals displaying a 17% greater yield, suggesting that there may have been an impact on the animals other than that of disease. Note that these observations agree with those reported by Lewis (15).



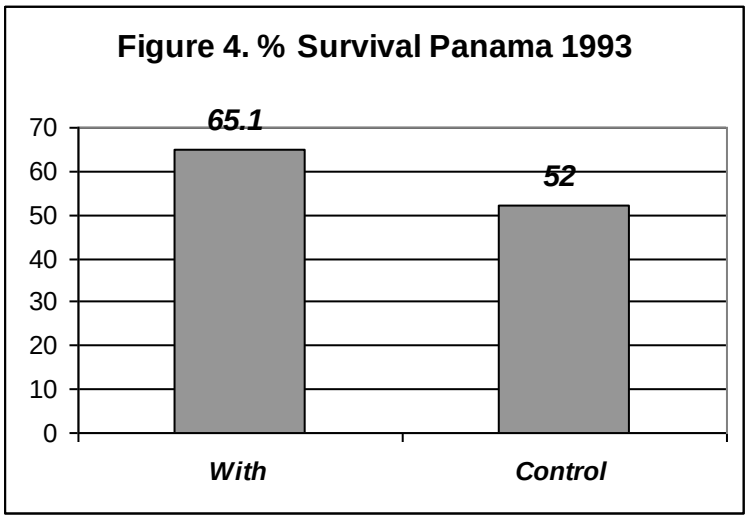
Dr. Laramore also reported on the results of testing performed in cooperation with the Ministry of Agricultural Development (MIDA) (22), Panama in which PLs exposed to his LPS preparation were directly stocked. These results are depicted in Figure 3. There was a significant difference between the treated and non-treated groups with the difference in average survival being almost 17%. These studies demonstrated that exposing shrimp to dead suspensions of bacteria under field conditions resulted in a benefit in terms of increased survivals and/or yields that persisted to harvest. In all of the field studies

reviewed, the impact of these treatments on the actual incidence of disease can only be speculated upon as no diagnostic work ups were done to determine if any of the observed differences could be related to disease incidence. Nonetheless the increases in survival and yields were statistically significant. The consistent increase in yields could be attributable to a general overall increased resistance to disease or to other unknown factors. The observation that when survivals were high, increases in survival did not occur and that when survivals were lower there was a significant increase in survival is similar to those noted in subsequent experiments reported by Newman et al. (10).



In the technical literature of International Aquaculture Biotechnologies Ltd., the results of both an immersion treatment and an oral evaluation of a commercially available suspension of vibrio were described in 1993 (Figure 4). *P. vannamei* PL's were immersed in a 1:1000 dilution of the suspension for 90 minutes and stocked. Nine groups were treated and six were not. They were harvested 110 days later. Approximately 1.5

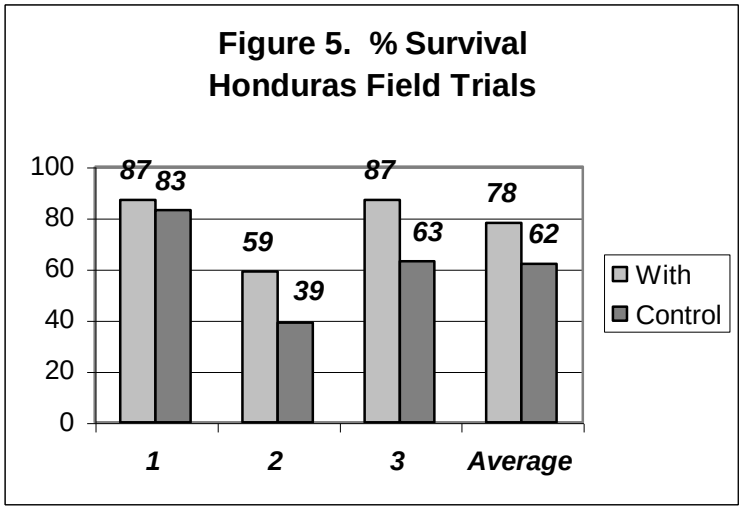
million animals were in each group. In the treated group, survivals averaged 65.1% compared with 51.8% in untreated controls. At this time oral evaluations were also done (unpublished observations). Though the results were disparate, there was an indication that three oral treatments (one in the nursery for three days) spaced 30 days apart for 6 days impacted the presence of vibriosis.

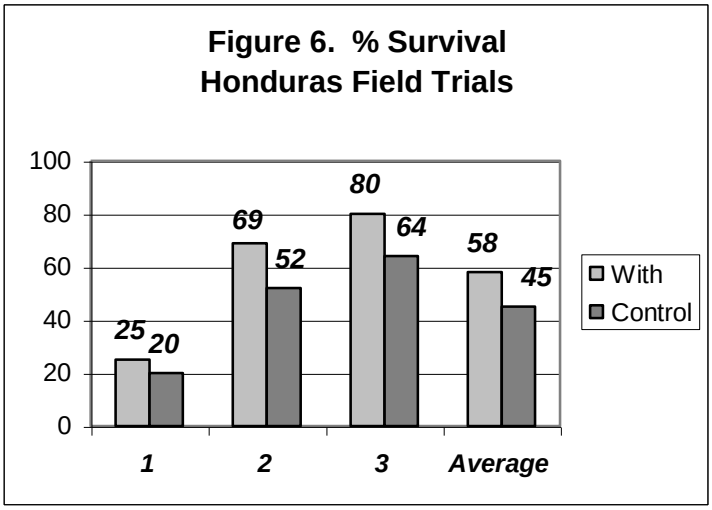


Newman et al. (10) reported similar observations to those of Laramore using a commercial product. The results are depicted in figures 5 and 6.

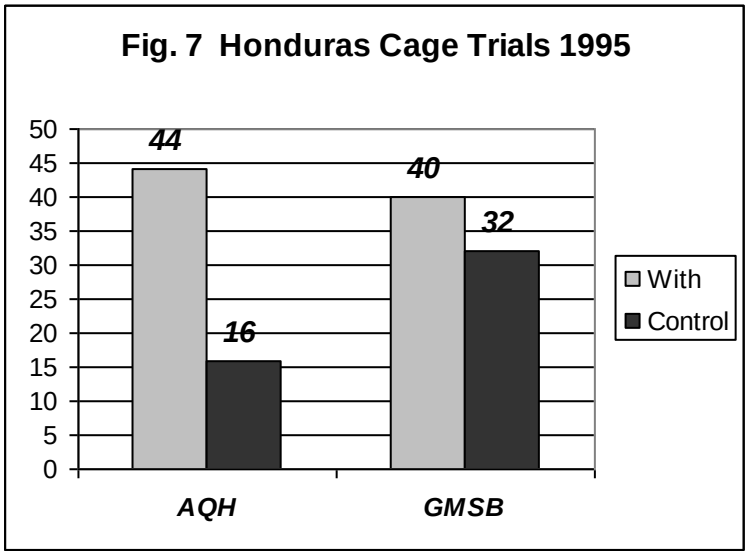
The experiments depicted in figure 5 took place in 2-hectare nursery ponds stocked at high densities, 200-300 PL/m2. These observations were made at about 50 days post stocking after an immersion treatment of three hours at a 1:1000 dilution. The average difference in survival noted in the treated groups was 14.7% with an increase of 21%. Figure 6 depicts the results of another study in nursery ponds. These results were from nurseries located on a dead end reservoir and were made 28 days post stocking. Discounting the group with the low survival (though there was still an increase in

survival) there was an average difference of 18.5% and an increase in survival of 42%. Note that in those experiments when the survival of untreated animals was at its highest, Figure 5, experiments 1 and 3, in one of the groups the differences in survival between treated and non-treated groups was relatively small. This could be accounted for by the lack of a problem that the product could have had a benefit against. In experiment 2, this was not the case. It was evident from these experiments that a single immersion exposure could enhance survival for at least 60 days post exposure in the field.



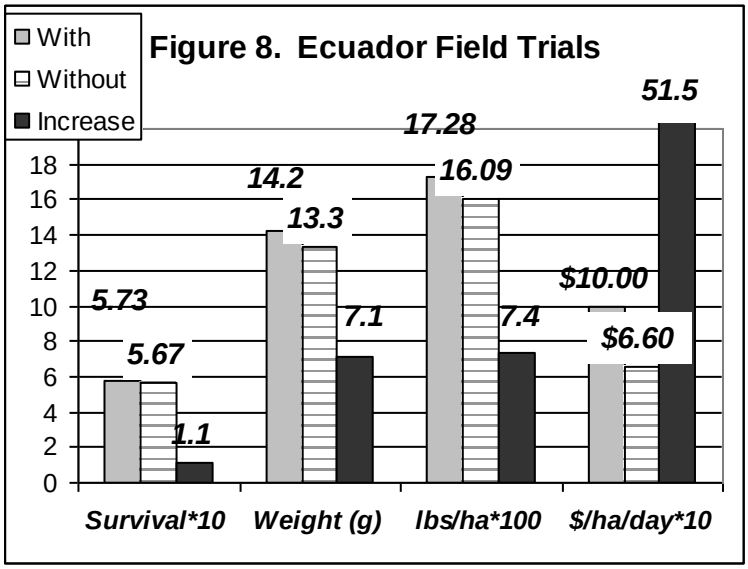


These experiments were repeated using cages in grow out ponds. The results are depicted in Figure 7. Two sites were examined, with differences in survival at one site of 28% and 8% at the other. At Aquacultivos de Honduras (AQH), the cages were placed into a single pond at 40 animals per cage with 4 experimental cages and 4 controls. At Granjas Marina San Bernardo (GMSB), a single cage was placed into a single pond. There were three control and three experimental cages each containing 60 animals. Survivals were determined at 56 days post treatment. As is apparent a single immersion exposure to this material resulted in a substantial benefit 56 days later.



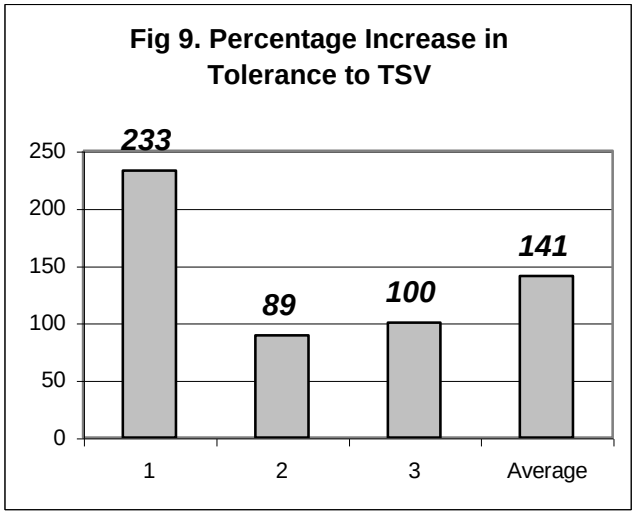
In late 1994 and early 1995, at a farm in the outskirts of Guayaquil, Ecuador a much larger field experiment (Figure 8) was performed in which animals were followed to harvest. Wild larval *P. vannamei* were treated at a 1:1000 dilution for 3 hours with a commercial LPS preparation prior to being directly stocked. Nine groups with controls were tested for a total of more than 20 million animals. The average pond size was 10 hectares with survivals being about the same, in the 50-60 percentile ranges. The treated

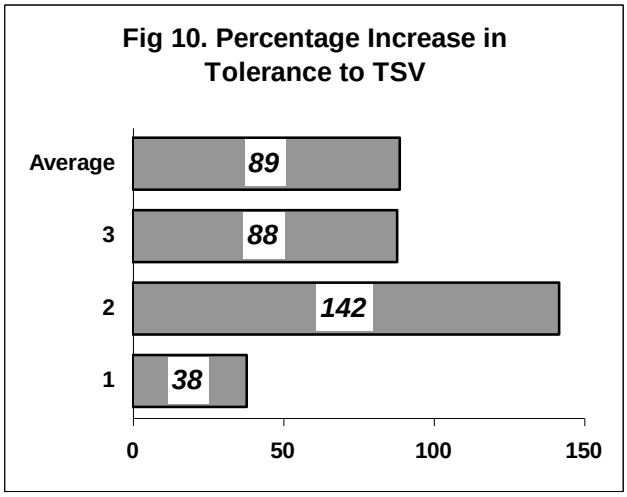
animals weighed, on an average, almost 1 gram more at harvest. In six of the nine groups there was a substantial increase in the average profit per hectare per day. One was the same as the control and in two others the controls fared better. One of the best measures of success is increased profit. There was a substantial cost benefit from using the product and a solid indication that a variety of factors in the pond could be affected. These experiments were repeated at the same farm and several others in 1996, 1997 and 1998 with similar results.



In 1997 and 1998, International Aquaculture Biotechnologies Ltd., in conjunction with a large farm in Honduras evaluated the ability of an LPS based material to protect shrimp against the Taura Virus as determined by LC50s. The results are depicted in Figures 9 and 10. Groups of *P. vannamei* PLs were exposed to the LPS suspension and subsequently exposed to a waterborne suspension of virulent TSV. Each data point represents a replicate test. The data in figure 9 is based on the animals being exposed to

five different levels of tissue (corresponding to five different levels of virus) 24 hours after being bathed in the LPS suspension. The data in figure 10 was generated after the animals were held for 6 days post exposure. Both studies showed that the animals could tolerate a greater level of exposure to the virus after they had been exposed to the LPS. This effect lasted for at least six days and was substantial. Twenty-four hours post exposure to the LPS the PLs could withstand an average of 141% more tissue (virus) than could the controls (figure 9). Six days post exposure to the LPS the PLs could withstand an average of 89% more tissue (virus) than could the controls (figure 10).





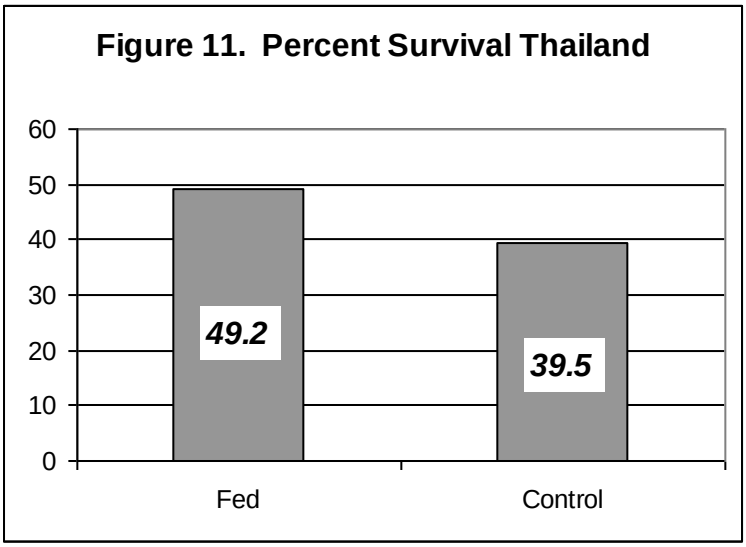
Several additional observations showing a similar benefit from exposure to the LPS preparation have also been made (10). This is likely a non-specific effect and a similar result should be seen with any virus.

Horne et al. (23) published the results of an extensive evaluation on the use of dead suspensions of vibrio for the control of vibriosis in *P. monodon*. Their observations were consistent with those reported by previous authors. Their testing was extensive and they reported significant protection by injection, immersion or by the oral route. When animals were immersed in a 1:100 diluted suspension of their test material for 6 hours, held in the laboratory for 60 days and challenged by injection, they reported a 38% percent survival compared with 21% for controls. They also noted that oral administration of their material, when tested 14 days post administration, conferred a protective benefit (20% survival in controls and 30-70% in fed groups). When animals were held in the field and challenged at 50 days post treatment they also noted a difference between controls and treated groups. They concluded that a single immersion

treatment conferred a benefit that lasted 4-6 weeks and that repeat oral treatments at 4-6 week intervals were required to maintain the protective effect. They also concluded that LPS based treatments provided much higher levels of protection than did beta glucans in a lab based challenge.

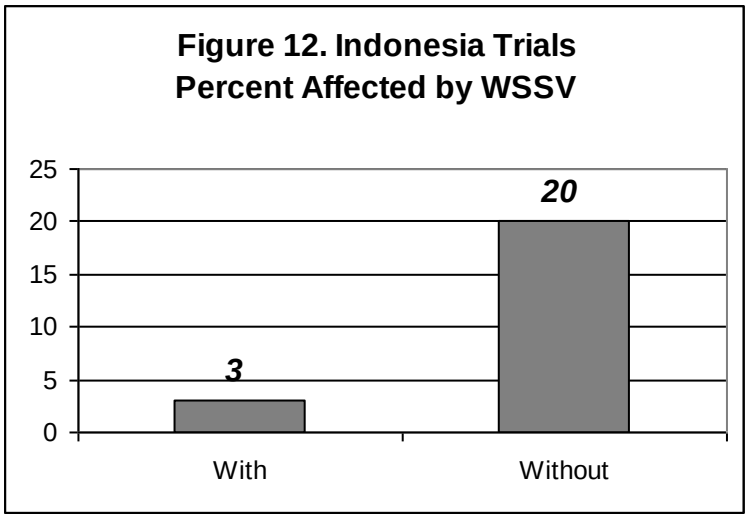
Almost all of the data presented so far has focused exclusively on exposing animals to a single level of LPS early in their life cycle. The reports from field and lab observations suggest that if animals are not under serious stress or being overwhelmed by a pathogen that the short term (6 weeks or so) benefit from this single exposure will be sufficient for a benefit through to harvest. It is however important to take a look at the best way to extend this benefit and the following field trials were performed with this in mind.

In Thailand, early in the WSSV epidemic, an experiment was performed in which juvenile *P. monodon* were exposed to LPS prior to stocking and then fed LPS top dressed in the feed for 7 day periods at thirty day intervals for 90 days. The results are in figure 11. The 9.7% difference represents a 25% increase in survival.



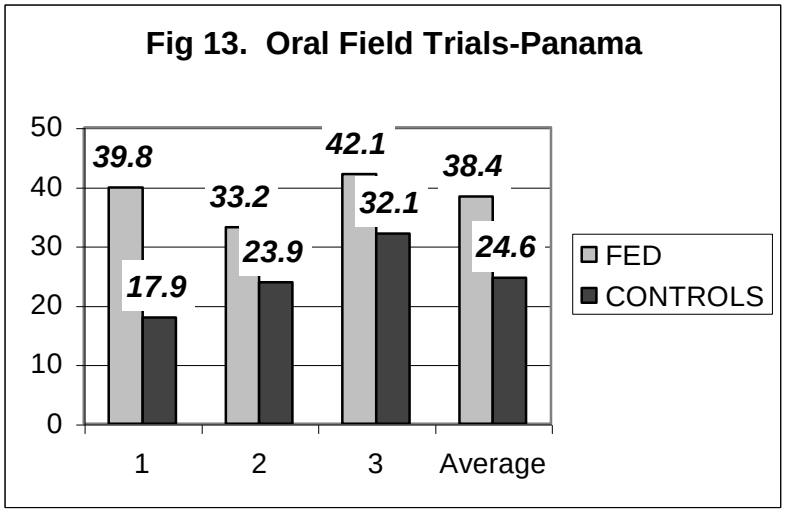
The data shows that there was a clear-cut benefit to the use of this approach. It is noteworthy that though there may have been WSSV problems in some of these ponds the overall survival was much higher than some farms have been reporting in Central America in the face of the current outbreak.

Subsequent to this a large trial was conducted in Indonesia in which WSSV was a serious problem. Success of this approach was measured on the basis of a difference in the number of ponds that were affected with the virus (Figure 12).



Out of 65 ponds treated with the LPS, only 3 developed WSSV contrasted with 2 out of 10 control ponds. As with the trial in Thailand though, the viral loads were clearly not as high as they have been in outbreaks in Central America. Despite this, this data does provide evidence that the virus can be impacted by this particular approach and offers hope that with continued fine-tuning it may be possible to substantially increase survivals in affected ponds.

At the same time that these trials were being run in SE Asia trials were being run in Central America. At a large farm in Panama, three 10-acre ponds were fed a combination of LPS and a commercially available glucan starting at 28 days post stocking for one week on and one week off for most of the cycle. Fig 13 shows the results in percent survivals. The ponds were matched as to the source of the PL's, location on the farm and the age and cycle of the ponds. The fed animals showed a 55% increase in survival over those not fed. The animals were larger in the fed ponds, by 9.3%, and there was a 15.4% difference in yield. Based on current pricing of these materials, they would have realized a 12-fold benefit on their investment.



This farm had been experiencing some serious problems with a variety of pathogens and the data suggests very strongly that there was a benefit from the oral treatment.

In 1998 these experiments were repeated in Ecuador on a larger scale, four ten hectare ponds were fed and five were used as controls. Survivals were very high in both groups and the differences noted were small, with one very important exception. All of the control ponds were medicated repeatedly during the cycle while none of the ponds fed the LPS/glucan combination were. These experiments are being repeated at this time and early indications are that the effect is reproducible.

Devaraja et al. (24) reported that a combination of an LPS preparation and a yeast glucan provided a greater degree of apparent immune stimulation than did either alone. They observed a substantial elevation in an indicator of immune function in groups of shrimp fed a combination of both compounds contrasted with those fed either one alone. This

supports the field observations that the combination provides a strong benefit in terms of increased survival.

CONCLUSIONS

We are in the early stages of trying to optimize the use of non-specific immune stimulants in shrimp. There are many possible compounds and combinations that can be evaluated. LPS based materials have proven that they can provide a benefit under many conditions in both the field and under controlled laboratory conditions. More than twenty years worth of tests have proven this. Further studies should address the effectiveness of combinations of LPS and other materials in conjunction with compounds such as fucoidans (25) or other materials with anti-viral properties.

References

1. Rosenberry, Bob. World Shrimp Farming 1998.

2. Csavas, Imre. The status and outlook of world aquaculture with special reference to Asia. Aquaculture towards the 21st Century. Proceedings of Infofish-Aquatech'94, International Conference on Aquaculture, Colombo, Sri Lanka. p. 1-13. August 1994.

3. Lin, C. Kwei and Nash, Gary L., compilers. Mass mortality caused by systemic bacilliform virus in cultured penaeid shrimp, *P. monodon*, in Thailand. p. 178-181. In Asian Shrimp News. Collected Volume. 1989-1995. 1996.

4. Hasson, K.W., Lightner, D.V., Poulos, B.T., Redman, R.M., White, B.L., Brock, J.A. and Bonami, J.R. Taura syndrome in *Penaeus vannamei*: demonstration of a viral etiology. Diseases of Aquatic Organisms 23:115-126. 1995.

5. Lo, C.F. and Kou, G.H. Virus-Associated White Spot Syndrome of Shrimp in Taiwan: A Review. Fish Pathology 33(4): 365-371. 1998.

6. Chu-Fang Lo, Ching-Hui Ho, Chau-Huei Chen, et al. Detection and tissue tropism of white spot syndrome baculovirus (WSBV) in captured brooders of *Penaeus monodon*. Diseases of Aquatic Organisms 30:53-72. 1997.

7. Venegas, C.A., Nonaka, L., Mushiake, K., Shimizu, K., Nishizawa, T., and Muroga, K. Pathogenicity of Penaeid Rod-shaped DNA Virus (PRDV) to Kuruma Prawn in Different Developmental Stages. Fish Pathology 34(1):19-23. 1999.

8. Maeda, M., Kasornchandra, J., Itami, T., Suzuki, N., Hennig, O., Kondo, M., Albaladejo, J.D. and Takahashi, Y. Effect of Various Treatments of White Spot Syndrome Virus (WSSV) from *Penaeus japonicus* (Japan) and *P. monodon* (Thailand). Fish Pathology 33(4):381-387. 1998.

9. Chanratchakool, P., and Limsuwan C. Application of PCR and Formalin Treatment to Prevent White Spot Disease in Shrimp. Aquatic Animal Health Research Institute, Dept of Fisheries, Bangkok, Thailand. 1998.

10. Newman, S.G. and Deupree, R.H. The Impact of Biotechnology on Aquaculture. Aquaculture towards the 21st Century. Proceedings of Infofish-Aquatech'94, International Conference on Aquaculture, Colombo, Sri Lanka p. 147-158. August 1994.

11. Lightner, D.V. Diseases of cultured penaeid shrimp. In "CRC Handbook of Mariculture. Crustacean Aquaculture " McVey, J.P. (Ed.) pp. 289-320. CRC Press. Boca Raton, Florida. 1983.

12. Sung, H.H., Yang, Y.L, and Song, Y.L. Enhancement of microbiocidal activity in the tiger shrimp *Penaeus monodon* via immunostimulation. Journal of Crustacean Biology. 16(2): 278-284. 1996.

13. Söderhäll, K., and Häll, L. Lipopolysaccharide induced activation of prophenoloxidase activating system in crayfish haemocyte lysate. Biochem. Biophys. Acta. 797:99-104. 1984.

14. Crowder, B. Viral disease control study develops shrimp culture immunization techniques. Aquaculture Magazine. May-June: 12-15. 1982.

15. Lewis, D.H. and Lawrence, A.L. In Proceedings of the First International Conference on Warm Water Aquaculture-Crustacea. G.L. Rogers, R. Day and A. Lim (eds.) Brigham Young University Hawaii Campus. p 304-307. 1983.

16. Itami, T. , Takahashi, Y. and Nakumura, Y. Efficacy of vaccination against vibriosis in cultured kuruma prawns *Penaeus japonicus*. Journal of Aquatic Animal Health. 1:238-242. 1989.

17. Song, Y.L. and Sung, H.H. Enhancement of growth in tiger shrimp *(Penaeus monodon)* by bacterin prepared from *Vibrio vulnificus*. Bulletin of the European Association of Fish Pathologists. 10(4):98. 1990.

18. Sung, H.H., Song, Y.L. and Kou, G.H. Potential uses of bacterin to prevent shrimp vibriosis. Fish & Shellfish Immunology. 1(311-312). 1991.

19. Itami, T., Takahashi, Y., Yoneoka, K. and Yan, Y. Survival of larval giant tiger prawns *Penaeus monodon* after addition of killed vibrio cells to a microencapsulated diet. Journal of Aquatic Animal Health. 3:151-152. 1991.

20. Itami, T., Yan, Y. and Takahashi, Y. Studies on vaccination against vibriosis in cultured kuruma prawn *Penaeus japonicas* - I. The Journal of Shimonoseki University of Fisheries. 40(2):83-87. 1992.

21. Itami, T., Yan, Y, and Takahashi, Y. Studies on vaccination against vibriosis in cultured kuruma prawn *Penaeus japonicas* - II. The Journal of Shimonoseki University of Fisheries. 40(3):139-144. 1992.

22. Laramore, R. Shrimp Culture Technologies, Inc.: Research to improve shrimp genetics and health. In Diseases of Cultured Penaeid Shrimp in Asia and the United States. Fulks, W. and Main, K.L. (Eds.) Oceanic Institute. Honolulu, Hawaii. p.305-310. 1992.

23. Horne, M.T., Poy, M., Pranthanpipat, P. Control of vibriosis in black tiger shrimp, *Penaeus monodon*, by vaccination. In Diseases in Asian Aquaculture II. M. Shariff, J.R. Arthur and R.P. Subasinghe (eds.), p. 459-467. 1995.

24. Devaraja, T.N., Otta, S.K., Shubha, G., Karunasagar, I., Tauro, P. and Karunasagar, I. Immunostimulation of Shrimp Through Oral Administration of Vibrio Bacterin and Yeast Glucan. In Flegel TW (ed) Advances in Shrimp Biotechnology. National Center for Genetic Engineering and Biotechnology. Bangkok. p. 167-170. 1998.

25. Takahasi, Y., Uehara, K., Watanabe, R., Okumura, T., Yamashita, T., Omura, H., Yomo, T., Kawano,T., Kanemitsu, A., Narasaka, H., Suzuki, N., and Itami, T. Efficacy of Oral Administration of Fucoidan, a Sulfated Polysaccharide, in Controlling White Spot Syndrome in Kumura Shrimp in Japan. In Flegel TW (ed) Advances in Shrimp Biotechnology. National Center for Genetic Engineering and Biotechnology. Bangkok. p. 171-173. 1998.