Stress – Various Stress Factors in Shrimp Farming

Over the last twenty years commercial aquaculture has experienced spectacular growth. A significant component of the fish and shrimp based protein that humans consume, especially in first world countries, is now provided by these activities. Many species have gone from small-scale regional production to large-scale global production. Disease has substantially impacted the profitability of many of these industries and has been instrumental in shaping the evolution of the aquaculture industry. Shrimp farming has failed to realize its potential as a direct result of disease.

Monoculture, or the rearing of a single species at a time, has few of the ecological safeguards present found in more complex natural ecosystems. It is much easier for diseases to proliferate in these environments than in the wild, where the diversity of the ecosystem provides safeguards against species threatening diseases. Stress plays a very important role in susceptibility to disease and the outcome of the disease process. Stress has been defined in many different ways, though the basic components are universally the same. The definition of Bayne, based on his work in mollusks, exemplifies an appropriate definition of stress for aquaculture.

Stress is **"a measurable alteration of a physiological steady-state that is induced by an environmental change and that renders the individual more vulnerable to further environmental change."**

Essentially anything, whether it is external or internal that disturbs the "normal" physiological balance can be considered to be stress. Stress is a normal and natural phenomenon and it is impossible for life to exist without it. In its friendly and helpful form it shapes evolutionary progress and strengthens a species ability to survive. In its evil and harmful form it weakens animals to the point where their normal physiological processes no longer can protect the host against the intense of pathogenic organisms.

Signs of stress can be exhibit openly, such as sluggishness, lack of feeding activity, slow growth, molting difficulties, hyperactivity, death, or hidden until animals become ill. The action of stressors on shrimp is varied and not widely studied. One consistent feature seems to be an elevation in blood glucose levels. Measurement of osmoregulatory capacity may also be another useful indicator of the degree of stress that animals are under. Recently it has been proposed that this may be a convenient and reliable way to monitor the overall status of stress in a population of animals. Limited field usage suggests that this may be a very important tool for determining what the relative degree of stress a population is under and thus how susceptible it might be to an infectious disease process. Should further testing bear this out, then the prospect exists that determining osmoregulatory capacity of a group of animals could become a standard component of any proactive disease management program.

Stressors

Stressors are the means by which animals become stressed. Many stressors have been identified that impact aquaculture operations. Some of these can be easily and cost effectively controlled and others cannot at any cost.

Some of the stressors impacting aquaculture

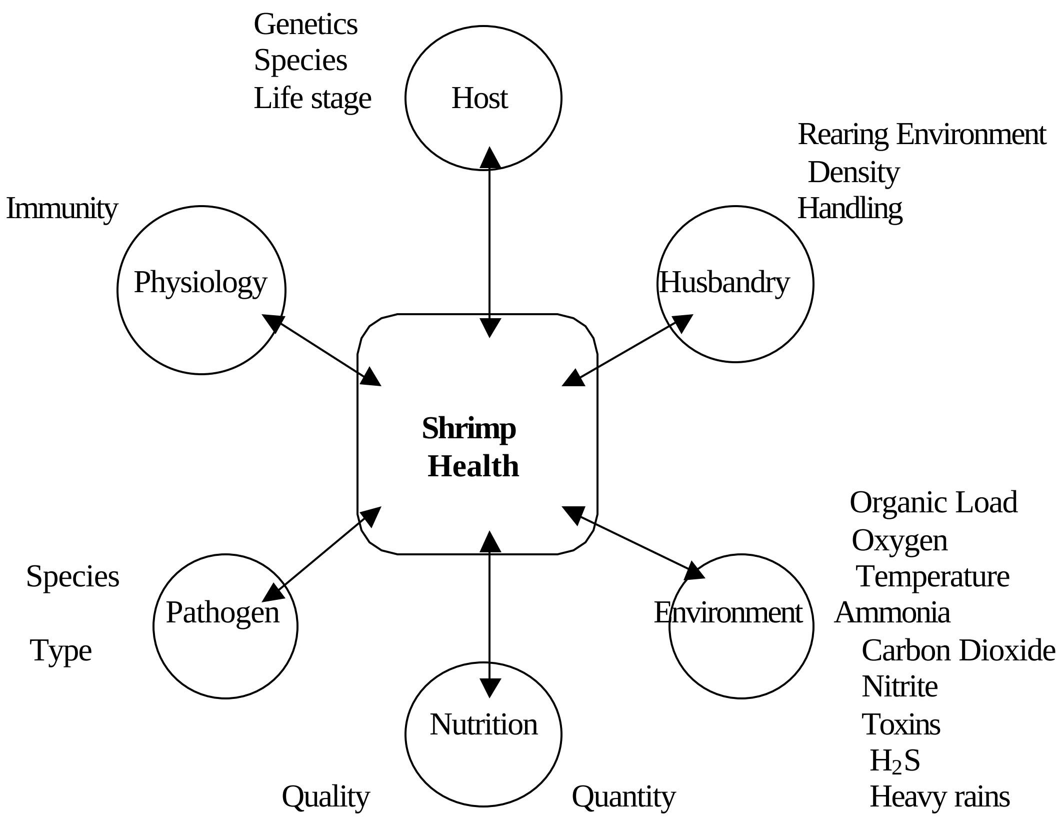
1. Rapid changes in temperature
2. Rapid changes in pH
3. Salinity fluctuations
4. High suspended solid loads
5. Density (crowding)
6. Insufficient oxygen
7. Elevated CO_2
8. Nitrites
9. Unionized ammonia
10. Heavy metals
11. Toxins (algal, bacterial, feed)
12. Pesticides
13. Nutrition (usually inadequate)
14. Molting
15. Handling
16. Parasitism
17. Low level infections
18. Disease
19. Hydrogen sulfide
20. Climatic conditions like continuous rains.

Defining what levels of stressors are normal and acceptable is not easy. A level of a stressor that is problematic under one set of environmental conditions might not be under another. Stressors that are present in combinations might be benign by themselves but pose a far greater threat because they are present together. Often times the published limits of tolerance are levels that are actually stressful. In healthy animals this may not pose a problem, but in animals that are carrying a virus such as the etiologic agent responsible for White Spot Disease (WSSV) and MBV, they can pose a serious problem. There are many observations as to what levels of specific water chemistry parameters are problematic. Most of these studies though are laboratory based and do not reflect the complexity of the pond environment. This is further complicated by the fact that there are going to be differences in species and age susceptibility.

Figure 1 shows how many of these factors interact. Many factors interact together to determine the outcome of any disease process.

Snieszko was the first to theorize that host-pathogen and environment interrelationships were applicable to fish (and by extension to shrimp) disease. This has been represented pictorially as three overlapping circles. For a disease to occur, stressful environmental conditions act as activators of the disease process. Since many potential pathogens are present as normal inhabitants of the ecosystems that shrimp are reared in, it stands to reason that stressors must play a significant role in susceptibility. In fact, observations show that as animals approach their physiological limits of tolerance to stressors, the impacts of stress increase in an accumulative fashion rather than an additive fashion. Animals are weakened in a manner that assures that even if one of the stressors is reduced or removed it has little bearing on the overall level of stress that the animal is under.

Figure 1. The Complex Interaction of Variables Affecting Shrimp Health



Host

Domestication of wild animals is a hallmark of successful agriculture and it is becoming and will be the same for aquaculture. The genetic make-up of the host has a direct impact on its ability to tolerate exposure to pathogens. It is now common knowledge that selected families of *P. vannamei* display varying degrees of susceptibility to the etiologic agents of the Taura Syndrome, IHHNV and BP. The same holds true for *Penaeus stylirostris* and IHHNV.

It is quite likely that this can also be said of stress resistance as well. Domestication implies greater uniformity and a better ability to tolerate the stresses of cultivation. Strains that are more stress tolerant are going to be less susceptible to diseases that occur as a result of this stress susceptibility. The species also impacts susceptibility. Some species may be better suited for production in certain types of environments than others are. The IHHN resistance of some strain of *P. stylirostris* is an example of this. Strains that are tolerant or resistant would be better suited for rearing in areas where other susceptible species will be affected. A good example of this is the culture of *P. stylirostris* in areas where the Taura Syndrome Virus seriously impacted the use culture of *P. vannamei*.

The life stage is also important, as there may be differences in susceptibility between different life stages.

It is also important to recognize that lower levels of pathogens may be required to produce disease in smaller animals than in larger animals. Minimizing exposure to pathogens at early stage may lend itself to greater tolerance later.

Physiological Status of the Immune System

Vertebrates have evolved a sophisticated group of physiologic mechanisms for differentiating self from non-self. This is the immune system. In invertebrates the system is not as evolved, though it still retains a great deal of complexity. For the most part it does not appear that shrimp can respond specifically to a pathogen though there are some as yet unexplained circumstances that suggest there may be some element of specificity. Table 2 outlines some of the observed differences between vertebrate and invertebrate immune systems. The immune system of penaeids is poorly characterized contrasted with that of insects, crayfish and even the horseshoe crab.

Table 2. Differences between Vertebrate and Invertebrate Immune Systems

Vertebrates	Invertebrates
Antibodies	No antibodies
Memory	No memory
Complex assemblages of lymphocytes	Less complex
Cytokines	Cytokines
Lectins	Lectins
Clotting-Fibrin based mechanism	Clotting-not fibrin based
Variety of killing factors	Variety of killing factors
No Phenoloxidase	Phenoloxidase and Melanin

Since we evidently cannot exploit the immune system in a specific way, the Use of a variety of non-specific tools may be employed. It is clearly possible to induce a short-term increase in the ability of shrimp to tolerate exposure to pathogens and that this is a non-specific effect. Many of the compounds that have these properties work in the lab but perform less efficiently or not all in the field. Interestingly enough, a number of them have been shown to be effective in reducing susceptibility to stress and these will be briefly discussed later along with a few examples of their protective properties.

Pathogen

Pathogens generally fall into two categories. Obligate pathogens that produce disease in healthy non-compromised hosts and opportunistic pathogens that produce disease only in weakened hosts. As with fish pathogens, most of the shrimp pathogens likely fall into the opportunistic category.

Bacteria are small (1 to 10 micron-one micron is one thousandth of a millimeter) microorganisms that do not require host metabolic machinery for growth. They do however require many of the same nutrients that their hosts require and when they are present will readily complete with the host for these. Disease production by bacteria is usually associated with a toxin or some innate ability to effectively compete with the host for critical nutrients. In some cases the toxins allow the bacteria producing them to enter and weaken the host allowing other bacteria to invade and finish off the job.

The host is quite literally digested in the process of gaining the nutrients that the bacteria require to multiply. In a healthy animal a balance is maintained whereas in a stressed animal this balance is pushed in favor of the pathogen.

Viruses are even smaller microorganisms (20 to 350 nanometers-one nanometer is one thousandth of micron) that must use the metabolic machinery of the host to reproduce. They cannot reproduce independently regardless of the presence of all of the required nutrients. They produce disease primarily by damaging tissues and interfering with normal tissue function. They also weaken animals allowing secondary pathogens to establish themselves.

There are innumerable other fungal, protozoal and other types of pathogens that affect farmed shrimp. Many of them can act as stressors facilitating the entry of other pathogens (gregarines and zoothamnium for example) or are capable of eventually killing the animals by themselves, such as the microsporidians. Animals must expend energy to deal with any type of infection and this expenditure can easily increase the susceptibility of the animals to other more serious diseases.

Nutrition

This is a complex issue as most dietary nutrient requirements are determined in the laboratory under strict conditions that have nothing to do with the stressful world of the shrimp farm. What an animal requires in a laboratory tank has little to do with what they may require in the pond. Certainly if deficiency symptoms are present below a certain level a nutrient, then the diet in the field must have at least these levels. In reality though higher levels may be required depending upon the particular cultural conditions. It is known that shrimp consume an average of around 60% of the feed that they are fed during their life cycles in a pond, with the amount likely varying with respect to the size of the animal, the size of the ponds, the densities of the animals in the ponds and the feeding strategy. All of this is not likely consumed as fresh feed. Even with feed trays, animals may move feed off of the trays where it can sit on the bottom leaching critical nutrients.

There are several studies that have shown that in some pond environments feed is not actually needed. The shrimp derive all of the nutrients that they require from the pond itself. As densities increase though this is not the case. High-density cultivation requires the use of lots of feed. These feeds need to be tailored to the stresses that the shrimp have to deal with.

Most commercial diets contain all of the necessary ingredients to ensure that if there are deficiencies in the pond nutrient levels that the needs of the shrimp are met. However, it is not likely that they have all of the nutrients that are required to ensure a maximum ability to resist stress.

It is widely held that certain vitamins are critical to being able to withstand stress; these include Vitamin C, Vitamin E and others. Other nutrients, such as astaxanthin and highly unsaturated fatty acids have also been found to reduce stress susceptibility. Very few of the studies that demonstrate this under lab conditions show the same benefits under field conditions. Nonetheless the practice of fortifying diets during times of acute stress is consistent with the goals of proactive disease management.

Some recent attention has been paid to the nucleotides as critical nutrients. Though there are no peer-published observations of the role of these basic building blocks of DNA and RNA in aquaculture, studies in mice and other animals suggest that they can provide a benefit. Sources of nucleotides vary from enriched yeasts to bacterial suspensions to gonadal tissues. Their inclusion at elevated levels during times of stress should increase the readily available pools of nucleotides to allow DNA and RNA synthesis (and thus protein synthesis) to proceed more rapidly than under nucleotide limiting conditions. This can also be said of many of the vitamins (A, C, E, B complex), minerals (Se, Cr, trace), lipids (fatty acids), etc.

Not only is quality of feed a potential component of stress on shrimp, but so is the quantity. In the absence of natural food sources, feeding rates can become a limiting factor to growth and contribute to stress. Well-fed animals are much less likely to experiences stress associated with diseases of nutritional deficiency. Observations suggest that starved animals are much more susceptible to diseases as well. There are many philosophies regarding the best way to feed shrimp in semi-intensive systems and likely there is no one best way. The use of trays in a population of shrimp that is clinically ill or weakened might lead to worse problems then random dispersion of the feed. Though, the use of feed trays does allow a great deal of control over feeding levels, makes for improved feed conversions and thus better margins.

Husbandry

There are many routine practices that are likely quite stressful to shrimp and by things that cause weakened shrimp to die quickly. The production of shrimp involves a series of succinct yet interconnected husbandry challenges, beginning with maturation.

Maturation

P.monodon is well on its way to becoming domesticated. The cycle has been closed in most countries that produce this shrimp and efforts are underway to select the best performing animals in a wide variety of environments. This eliminates one element of potential stress; catching wild animals. Certainly netting wild animals from the ocean floor is a significant stress. By rearing shrimp from the beginning of their life cycles through the end, we can minimize and eliminate many stressors. If we do this indoors to protect the value of these animals we also have much better control over what potential pathogens we bring into our rearing facilities.

Holding adult animals for log periods of time can lead to reproductive exhaustion and result in the production of animals that are less hardy and thus less fit to tolerate stress. Improper feeding, handling and water quality control can result in wide spread mortality in maturation facilities. The animals that survive these problems may not be suitable for use as a source of nauplii or the nauplii that are produced may be more prone to problems.

Hatchery

Any type of handling can stress animals. Excessive handling certainly does. Routine washing and surface disinfection protocols are used in many state of the art hatcheries and every effort is made to ensure that animals are not stressed during this procedure.

However, failure to consider that this is a stressful process can damage nauplii and result in poor performance in the early larval stages and high mortalities. Shrimp are not reared in sterile environments and it should always be assumed that the environment contains potential pathogens.

Once the animals are stocked in larval rearing tanks, many other factors come into play. Larvae are more susceptible to deteriorated water than PLs, which in turn are more susceptible to deteriorated water quality then juveniles and adults usually are. Once animals are stressed, even though they may appear to have recovered they may still be weakened. The generous use of water exchange and monitoring of environmental parameters is essential.

Harvesting of PLs is very stressful. Large numbers of animals are collected in a short period of time and subjected to various counting strategies. Moderation of handling protocols to minimize stress or allowing animals' longer periods of time to recover form these stressors might be useful in impacting disease tolerance.

Acclimation

When shrimp are moved from hatchery tanks to the ponds they are usually subjected to stress tests to determine the suitability for transfer and then moved by a variety of methods. Though they may be able to survive stress tests, this does not mean that they are not stressed when they are moved nor does it mean that they are no stressed when they acclimated. Healthy shrimp placed into environments with low pathogen loads can tolerate stresses without ill effects. On the other hand healthy shrimp placed into poor quality environments with high pathogen loads may become ill. Recent evidence suggests that in areas where WSSV is endemic a substantial benefit can be gained by holding shrimp in raceways for variable periods of time before stocking ponds.

Grow Out

In general, stressors are controlled by mechanical means, such as aeration, by chemical means, such as the addition of quick lime to lessen algal blooms, to the use of diets fortified with critical nutrients and stress reducing and immune enhancing substances. Environmental stress plays a significant role in mortality and disease susceptibility in grow out ponds.

Environmental Stress

The major cause of mortality in farmed fish is husbandry techniques and environmental disturbances. Environmental affects are considered to be accumulative, not additive. This means that the stress from multiple environmental factors is intensified many fold. This is the number one area that farmers have a fair amount of control over that is all too often rarely exerted. There are ranges of tolerance for all of the aspects of the environment that are critical.

A classic example is that of dissolved oxygen. Animals may be able to tolerate relatively low levels of dissolved oxygen, but there is a level below which they are stressed. Shrimp slow down their rate of respiration when dissolved oxygen levels and temperatures are lower.

In a recent study in aquariums the threshold DO level was 5 ppm. This is several parts higher than what is usually considered to be an oxygen stress. The actual level that will cause stress will depend on the oxygen needs of the individual, its metabolic rate, and the degree of gill parasitism (this will interfere with oxygen transfer). Very little is known about the metabolic requirements of shrimp under field conditions. Molting has been shown to be highly stressful, increasing oxygen demand and disease susceptibility. We should not be aiming at maintaining oxygen levels at or slightly above the minimum accepted level. Low oxygen levels impair metabolic activity and can negatively impact many normal physiological functions including molting and immune function. Many environmental factors will affect the immune response leaving animals more susceptible to disease. Reduced immune function with substantially increased disease susceptibility is often the consequence.

Some Suggested Solutions:

Prevention and minimization of the number of factors that cause and contribute to stress is an important component of achieving success. This should begins with the selection of sites that have access to high quality, clean water. Filtering of water through 150 to 200 micron filters is required to minimize and lessen the impact of water borne vectors of some diseases such as WSSV. These filters need to be maintained and checked regularly for integrity. Minimizing water exchange in areas where there are potentially serious vector problems is a viable solution as long as this is offset by measures that ensure that here will be no significant deterioration of water quality. Perhaps the most important single aspect is oxygen. Aeration should always be available and ponds should never be allowed to drop below 4-ppm oxygen.

Production of genetic stocks of shrimp that can tolerate some of the environmental extremes better than others is necessary. This extends as well to stress tolerance in general and disease tolerance specifically.

The use of specific dietary additives in the feed can go a long way towards mitigating some of the negative influence when they do occur. Cell wall fractions or whole cell walls from yeast and bacteria have been found to impact the ability of shrimp to tolerate stress, in addition to providing the potential of an immune stimulating effect.

Glucans

These are chains of glucose molecules linked together. The manner in which the chains are linked and cross-linked impact their ability to be effective. Furthermore they are readily digested by crustaceans and need to be effectively protected from digestive enzymes. The primary source is bakers or brewers yeast particularly Saccharomyces cerevisiae. The tolerance of glucan-treated shrimps was enhanced to stresses including catching, transport and ammonia. Glucan have been shown to activate polyphenoloxidase in the haemolymph, which is need to synthesize bactericidal melanin and this will give resistance to shrimp against Vibrio sp. The growth and survival rates of treated and untreated shrimps were significantly different.

Peptidoglycan (PG)

PG is a cell wall component of many bacteria. The whole cells of *Brevibacterium lactofermentum*, when fed to shrimp, conferred a higher tolerance to dissolved oxygen and salinity stresses. PG included at 100g/ton of feed gave the best results in terms of stress resistance. It gives observed a substantial protective benefit against the Yellow Head Virus and also protected shrimp against WSSV.

Lipopolysaccharides (LPS)

LPS is also a component of the cell walls of bacteria. LPS has been shown in many studies to impact disease resistance against vibriosis and WSSV.

The key to the management of stress in farmed shrimp is avoidance through the use of enlightened animal husbandry techniques, optimization of animal genetic tolerances, appropriate nutritional and feeding strategies and the selective use of biologically active compounds to promote heightened immunity during times of stress.