Management and Prevention of Stress in Aquaculture
With a focus on farmed shrimp

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Introduction:

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. Concomitant with this rapid growth there also has been the increased occurrence of problems that accompany all agricultural endeavors. 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 (1), 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 benevolent form it shapes evolutionary progress and strengthens a species ability to survive. In its malevolent form it weakens animals to the point where their normal physiological processes no longer can protect the host against the onslaught of pathogenic organisms. There are many examples in finfish culture where specific stressors are associated with disease outbreaks (2). The analogies between fish and shrimp farming make a compelling argument that shrimp farming is affected in a similar manner.

Signs of stress can be overt, 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 (3). Measurement of osmoregulatory capacity may also be another useful indicator of the degree of stress that animals are under (4). 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 (5). 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 (1,5). Some of these can be easily and cost effectively controlled and others cannot at any cost. Table 1 lists some of these.

Table 1: Some of the stressors impacting aquaculture

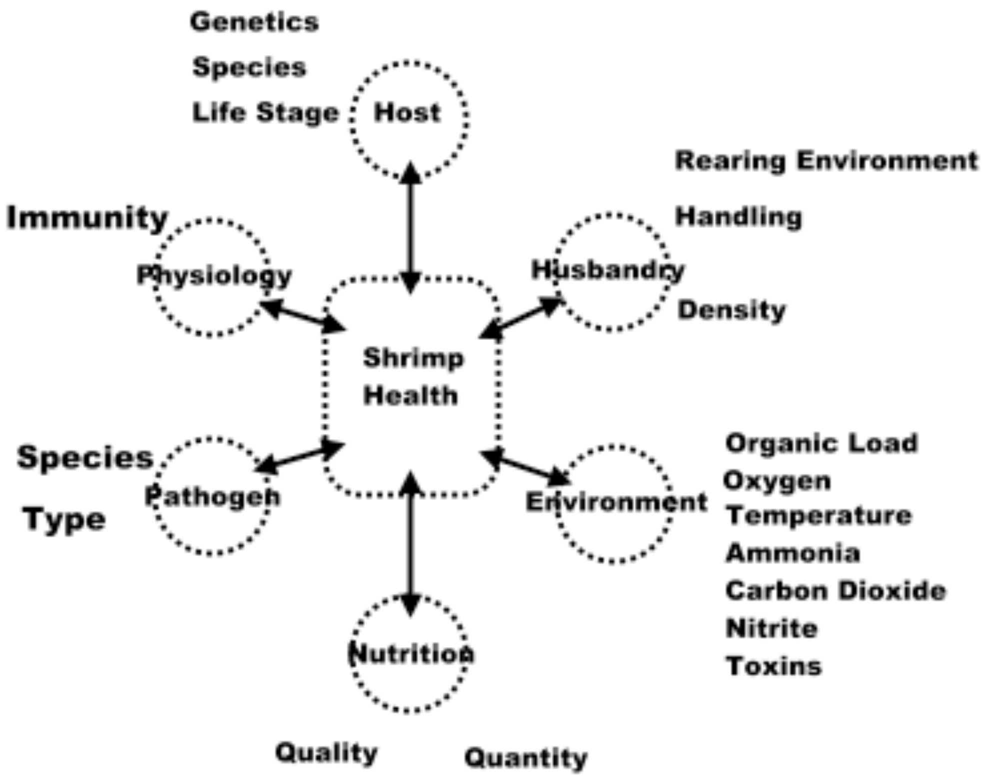
Unionized Ammonia	Nitrites	Insufficient Oxygen
Elevated CO_2	Salinity	Nutrition (usually inadequate)
Density (crowding)	Rapid changes in pH	Rapid changes in temperature
Molting	Handling	Pesticides
Heavy Metals	Toxins (algal, bacterial)	High suspended solid loads
Low level infections	Parasitism	Disease

Defining what levels of stressors are normal and acceptable is not easy. Many papers have addressed this and it is beyond the scope of this presentation to even begin to tackle this issue. 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), 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 (after Plumb (1)). Many factors interact together to determine the outcome of any disease process.

Snieszko (6) was the first to theorize that host-pathogen and environment interrelationships were applicable to fish (and by extension to shrimp) disease. This has

Figure 1. The Complex Interaction of Variables Affecting Shrimp Health



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 fish or 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.

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 (7). It is now common knowledge that selected families of *L. vannamei* display varying degrees of susceptibility to the etiologic agents of the Taura Syndrome, IHHNV and BP (8). The same holds true for *Penaeus stylirostris* and IHHNV (7). I believe that we will find it holds true as well for most if not all pathogen mediated diseases.

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 (7).

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 *L. vannamei.*

The life stage is also important, as there may be differences in susceptibility between different life stages (9). 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 early on 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 pathogenic insult though there are some as yet unexplained circumstances that suggest there may be some element of specificity (10). 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 (11), the use of a variety of non-specific tools may be employed. These have been reviewed elsewhere (12,13). 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 (1).

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 fish and shrimp (1,14). Many of them can act as stressors facilitating the entry of other pathogens (gregarines 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 stringent conditions that have nothing to do with the stressful world of the shrimp farm. What an animal requires in a benign 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 (15), Vitamin E and others. Other nutrients, such as astaxanthin (16), and highly unsaturated fatty acids (17) 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 (18). 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. Healthy shrimp may seem to be unfazed 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

L. vannamei 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. (19). 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 (20), 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 benign 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. The exact reasons for this benefit are unclear but they likely range from simply losing the shrimp in the raceway to when the cost of losing an animal is less, to allowing for a greater degree of nutritional and immune system fortification to a general overall reduction in stress at the younger life stages.

Grow Out

There are as a many disparate philosophies regarding all of the aspects of grow out, as there are farmers. 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 (1). 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 published ranges of tolerance for all of the aspects of the environment that are critical. These are usually based on controlled lab studies and have little meaning in the field.

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 (21). 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 (21). 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 (4) and disease susceptibility (22). 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 (23,24). Many environmental factors will affect the immune response leaving animals more susceptible to disease (24,25). Reduced immune function with substantially increased disease susceptibility is often the consequence.

Another important gas, the role of which in shrimp ponds is likely under appreciated is carbon dioxide. This gas it produced by algae when they respire. When algal biomass uses the oxygen in the pond they produce carbon dioxide. Much of this is buffered by seawater, but elevated levels can be problematic. (26, 27)

The list of environmental stressors is very large, a review of which is beyond the scope of this article. Some of these are reviewed elsewhere (24,25). Some of these are expected and others may be surprising. In particular, the role of algal toxins in stress and disease susceptibility is poorly appreciated (28).

Some Suggested Solutions:

Prevention and minimization of the myriad of factors that cause and contribute to stress is an important component of achieving success. This should begins with the selection of sites that are free of agricultural run-off influences and 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 (Newman-personal observations). 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.

Selection and use 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.

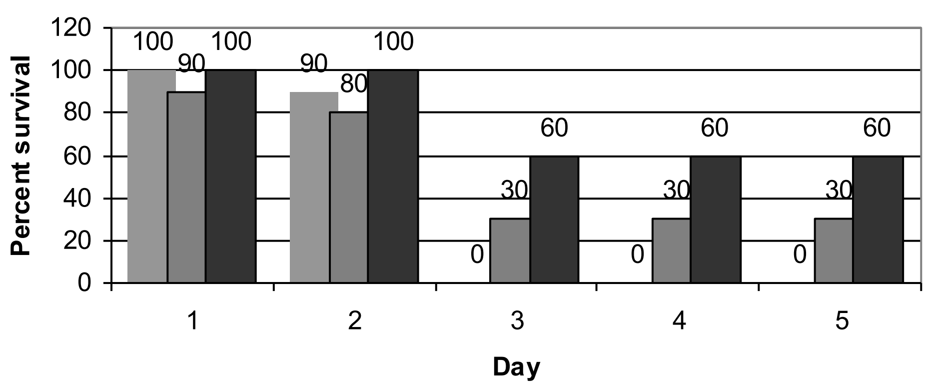
Glucans

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. Many glucans are ineffective or poorly so (29). Furthermore they are readily digested by crustaceans (30) and need to be effectively protected from digestive enzymes. Though the primary source is bakers or brewers yeast, almost all of the benefits ascribed to them are from another source (31) or from more highly purified material that are not sold in the market place because of their cost (32). Song et al. (31) noted that the tolerance of glucan-treated shrimps was slightly enhanced to stresses including catching, transport and ammonia. The growth and survival rates of treated and untreated shrimps were not significantly different in their experiments.

Peptidoglycan (PG)

PG is a cell wall component of many bacteria. Studies conducted in Thailand (33) demonstrated that whole cells of *Brevibacterium lactofermentum*, when fed to shrimp, conferred a higher tolerance to dissolved oxygen and salinity stresses (Figure 2).

Fig 2. Effect of PG on Salinity Stress tolerance



PG included at 100g/MT of feed gave the best results in terms of stress resistance. They also observed a substantial protective benefit against the Yellow Head Virus (Figure 3). Itami et al. (34) noted that PG from another source (whole cell material) protected shrimp against WSSV (Figure 4). Three different feeding regimes were used. Animals were fed 0.2 mg of PG per kg/body weight/day. The feeding regime used in number one (one in the table below) was 7 days with PG followed by 7 without. This was repeated for 95 days until the experiments were terminated. The second group (two in the table below) was fed the diet daily and the third group (three in the table below) 2 days on and five days off. The groups were small, twenty shrimp each, and they were challenged by a continuous waterborne exposure to the virus initiated at the onset of the experiment. There have been several other unpublished observations that PG when incorporated into feed can impact stress resistance and disease tolerance. A recent study in Belize, though only on one pond, suggests that feeding PG might have an impact on the incidence of RDS.

Figure 3. Brevibacterium (PG) YHV

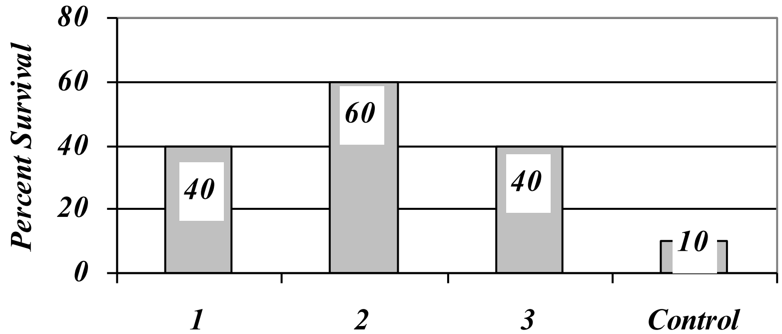
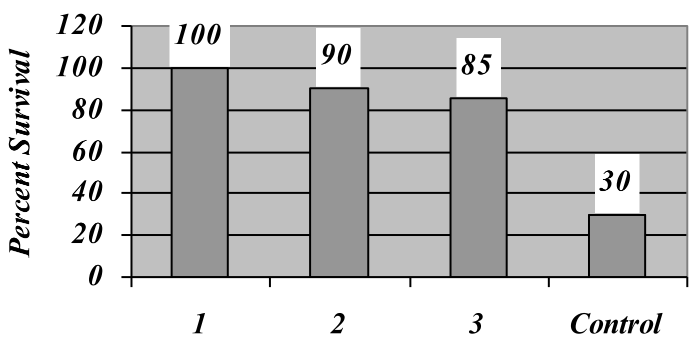


Figure 4. WSSV trials

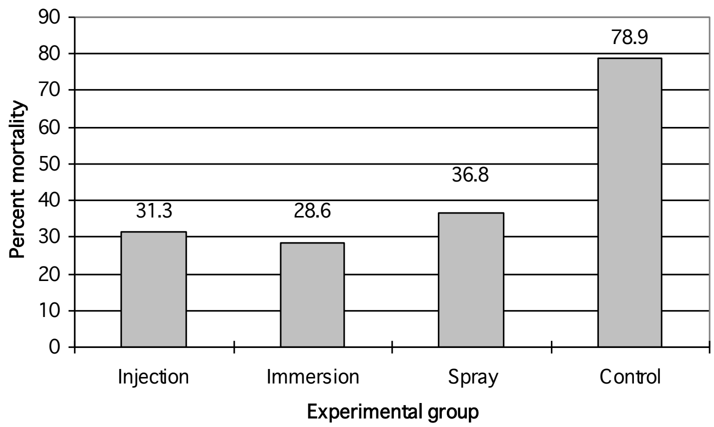


<u>Lipopolysaccharides (LPS)</u>

LPS is also a component of the cell walls of bacteria. Many studies have been conducted with LPS based materials and shrimp. The earliest of these data date to the early 1980s in which it was noted that shrimp exposed to a suspension of dead bacteria were substantially more tolerant to a water temperature stress than non exposed animals. (35 In a subsequent study it was noted that shrimp exposed to a similar suspension also were substantially larger than controls (36). This was also observed in 1990 (37). There are no studies that ascribe an exact mechanism for this though it has been suggested that there may be some critical nutrients present in the bacterial suspension.

LPS has been shown in many studies to impact disease resistance, ranging from vibriosis (figure 5) (38) to TSV (13) and WSSV (39). These are the subject of many articles and have been reviewed elsewhere (13).

Figure 5. Comparative survival of kumura prawns exposed to virulent vibrio species 30 days post exposure to a dead vibrio suspension (after Itami et al.)



The key to the management of stress in farmed fish and 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.

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