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Disease Management in Shrimp Culture Ponds - Part 3

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In previous articles we reviewed the importance of preventing the entry of disease into the pond system, and minimizing the effects of disease by reducing stress to the shrimp, which may boost their disease resistance. In this article we discuss the importance of on-farm sampling programs to monitor the health and performance of the shrimp, and detect the onset of disease. We also present a number of health conditions that can be quantified in the field from pond samples.

A thorough sampling program can be time consuming, labor-intensive, and often costly to sustain. Consequently, many producers attempt to economize by minimizing their pond sampling routine. This is unfortunate, because a good sampling program can produce substantial savings by identifying disease problems before they become unmanageable or catastrophic. We recommend that whenever possible shrimp farms implement the following sampling/monitoring programs.

1. Perform a visual inspection of the pond on a daily basis.
2. Monitor feed consumption on a daily basis
3. Sample for growth once a week.
4. Sample to estimate the number of shrimp once a week.
5. Monitor shrimp health and the appearance of disease using animals collected in the weekly growth and population samples

Visual Inspection of the ponds. There is no substitute for a visual inspection of the ponds. Each morning, pond personnel should walk the length of at least one dike of each pond in production, looking for signs of oxygen problems, dead or moribund shrimp, birds circling overhead or wading birds (e.g. herons, egrets), uneaten feed, phytoplankton crashes, theft, or any other unusual observations. Shrimp swimming erratically at the surface, or birds feeding on shrimp are symptomatic of either low dissolved oxygen, water quality problems, or disease. If oxygen levels in affected ponds are normal, samples of moribund shrimp should be collected for disease examination. Whenever possible, dead or moribund shrimp should be removed from the ponds to minimize horizontal disease transmission due to cannibalism.

Monitoring Feed Consumption. One of the first indicators of a serious disease problem is an abrupt decline in feed consumption, which can be provoked by either population loss or appetite suppression due to disease or water quality problems (e.g. low dissolved oxygen). (Synchronous molting by the majority of the population of shrimp in a pond can also result in a sudden decline in feed consumption, but this is not a common phenomenon in most shrimp species.) In either case it is essential that the daily feed ration be promptly reduced in a timely manner to prevent excess feed from contaminating the pond bottom. One of the most effective means of detecting sudden reductions in feed consumption is the use of feed trays (Figure 1), a topic that has been addressed in previous articles in this magazine (Jul-Aug 97, Nov-Dec 97 and Jan-Feb 98). Several virulent shrimp diseases, such as WSSV and NHP, evoke abrupt and drastic reductions in feed consumption during the acute phase of the disease, usually due to loss of appetite in affected animals. At the onset of a disease event in a pond, personnel inspecting the feed trays will frequently observe dead shrimp in the trays. (Most likely the dead shrimp did not expire in the trays, but instead were transported there in the process of being cannibalized by other shrimp.) Growers not using feed trays will be unable to react promptly to the sudden reduction in feed consumption triggered by these diseases.

A secondary benefit of using feed trays to gauge feed consumption is that the resulting information can be used to estimate shrimp populations. Using a standard percentage body weight feed curve, and the average weight of the shrimp in the pond, the number of animals in the pond can be estimated according to the following formula:

$$TP = \frac{[FI \div \%BW] \times 1000}{AW}$$

where, TP = total shrimp population
FI = daily feed intake (kg) for the pond
%BW = the percentage of body weight to be fed according to the feed curve AW = average weight (grams) of the shrimp in the pond

Sampling for Growth and Survival

Sampling for population estimates (survival), average weight (growth) and health monitoring can all be performed simultaneously from the same samples collected from a pond. Thirty days after a pond is stocked with PL's, sampling with a cast net should begin at a frequency of once per week. (If a pond is stocked with juveniles from a nursery, sampling should start a week after stocking.) Initiating sampling on day-30 of culture establishes a base line for comparison of initial growth rates between ponds. If more than one stocking was required to fully stock a pond, an average stocking date can be calculated based on the number of days between stockings and the number of PL's in each stocking.

For direct stocked ponds, a small mesh cast net (e.g. 1/8") can be used until the animals attain a gram or more, at which point a standard 3/8" mesh cast net can be employed (Figure 2). The sampling program should be organized so that each pond is sampled each week on the same day of the week. In addition, each pond should be sampled early in the morning before daylight can affect the shrimp's escape response to the cast net. Fixed sampling stations are recommended, at a minimum density of three per hectare (1-2 per acre) in large ponds and five per hectare in small ponds. Distribution of shrimp within a pond is not uniform. *L. vannamei* tend to migrate in schools, and congregate in the deeper areas of the pond. For this reason sampling stations need to be scattered throughout the pond in proportion to the shallow and deep areas of the pond. For example, if a large semi-intensive pond with 24 sampling stations has 30 percent of its bottom area in canals and a harvest basin, we would install seven stations in the deep areas and 17 in the shallow areas.

A population sampling program designed to estimate survival generally requires many more samples than that required to determine the average weight of shrimp in the pond. An effective combined sampling program involves sampling for population at 3-5 sampling stations per ha., and at each station, randomly collecting 5-10 shrimp for average weight determination. (A random sample of shrimp can be collected from a single cast net sample by grabbing the antennae of a bunch of shrimp and lifting them up and out of the sample.) These random sub-samples of shrimp are collected from each cast net sample until 100-150 animals from each pond have been accumulated. After the first 100-150 shrimp are collected for weighing, the remainder of the shrimp captured are counted as rapidly as possible for the population estimate, and returned to the pond. It is not recommended that all the animals for weighing be collected from the first few stations sampled in a pond, owing to the fact that certain areas of the pond favor small or large shrimp due to differing behavioral preferences.

Growth sample. In addition to calculating the average weight of shrimp in the sample, which provides data necessary for calculating growth rates, it is also informative to individually weigh each shrimp in the sample. Plotting the frequency of individual weights creates a size distribution curve that can yield important information on intra-population growth trends. For example, a healthy population of shrimp typically exhibits a tall, compact size curve symmetrically centered over the mean weight. A flattened size distribution with a wide range in sizes, or a skewed curve may be symptomatic of under- feeding or feed competition. (A pond stocked over a large period of time, or with different size animals will also generate a similar size distribution curve.) By comparing size distribution curves in the same pond from week to week, and studying the evolution of the curves over the course of the production cycle, valuable insight may be gathered regarding the health and growth performance of the whole population of shrimp in the pond. For example, a bimodal size distribution with two or more distinct groups of animals in a pond with significantly different average weights may be indicative of Runt Deformity Syndrome (RDS) caused by the IHHN virus, especially if the smaller population of shrimp grows at a slower rate than the larger animals. If growth of the smaller group parallels the larger animals, it may be an indication that wild post-larvae have penetrated the pond.

Disease monitoring.

When collecting the random sub-sample of 100-150 animals from the population samples for subsequent weight determination, symptoms of disease or compromised health in the same animals can be noted merely through a simple visual inspection of the sampled shrimp by trained personnel. The animals in the sample can be examined in the field for clinical symptoms of disease (Figure 3), or transported to a wet lab on the farm, where additional health indices can be evaluated. As each shrimp from the growth sample is being weighed, symptoms of disease or compromised health can be detected and quantified from a simple visual inspection by trained technicians. A week to week comparison of health indices in a pond will determine if the incidence of a particular health problem is worsening or improving.

Listed below are a number of health indices and symptoms of disease from the random sample of shrimp that can be detected by a trained person in the field or wet lab without the use of a microscope or other sophisticated equipment. In a continuation of this article, we will discuss similar disease indices that can be quantified from the same random sample of shrimp, but only through the use of microscopes, bacterial cultures, histopathology, or more sophisticated diagnostic techniques.

Gut content. In general, 80% or more of the shrimp randomly sampled from a healthy, well nourished, recently fed pond should display the intestinal tract (mid-gut) running the length of the tail to be full of food. A numerical grade can be used to quantify the gut fullness index:

GUT FULLNESS	ACCEPTABLE LEVEL (%)	GRADE
Full	> 80	2
Semi-full	10 – 20	1
Empty	< 10	0

Each shrimp from the growth sample is examined and assigned a numerical gut fullness index based on the ranking system described above. (This examination is best conducted when the shrimp are still alive, or before the normally opaque transparency of the tail muscle begins to fade.) If the overall average for the sample is 1.6 or less, it may indicate under-feeding or disease (e.g. WSSV, NHP, TSV). If the daily feed ration for that pond is at least 80% of the amount stipulated by a commercial (% body weight) feed curve, and you are feeding at least 3x/day, then a follow-up examination for disease should be carried out.

In addition to quantifying gut fullness and using it to detect under-feeding or predict the onset of disease, the color of the shrimp's gut contents can also be very informative:

GUT CONTENT COLOR	PROBABLE FOOD ITEM	PROBABLE CAUSE(S)
Black, dark brown	Benthic detritus, sediment	Under-feeding; inadequate feeding frequency
Light or golden brown	Manufactured feed	Normal
Red, pinkish	Cannibalized body parts from dead shrimp	Disease event in pond
Green	Benthic algae	Under-feeding
Pale, whitish	None (disease condition)	Gregarines, or some other disease condition (*)

(*) In heterotrophic culture systems in which a grain or carbohydrate food source is provided, shrimp may be encountered with white guts, which reflects the color of the feed.

In addition to gut fullness, and contents, the appearance of the gut can also provide insight into the shrimp's health. Advanced cases of haemocytic enteritis (HE) can alter the appearance of the intestinal tract in the first segments of the tail. HE is manifested as inflammation or damage to the gut epithelium caused by toxins secreted by certain species of bacteria or algae. The anterior section of the mid-gut of shrimp severely affected by HE will often appear swollen and distended. Further inspection of the gut contents or a histopathological examination of the gut would be required to confirm a diagnosis of HE.

Gill fouling and discoloration. The presence (or absence) of epicommensals such as filamentous bacteria, protozoans, or algae on the gills of the shrimp is regarded as a general indicator of shrimp health. Biofouled or damaged gill lamella can affect the respiratory and osmoregulatory capacity of the shrimp, and can precipitate asphyxia in severely affected animals, or generate sub-lethal effects at dissolved oxygen concentrations that would normally not be deleterious to healthy shrimp. The most conspicuous symptom of compromised gill function is a change in the coloration of the gill tissue. Thus, in the sampled shrimp the percentage of shrimp with darkened or discolored gills should be recorded. Black or brown colored gills can be caused by 1) biofouling by epicommensals, 2) melanized bacterial lesions, 3) a melanization reaction to toxins in the water, 4) iron precipitation, or 5) silt or detritus. Discoloration due to detritus or silt accumulation in the gill lamella can be easily diagnosed by forcefully shaking the shrimp in clean water, which will generally clean the gills of any unattached debris. A confirmed diagnosis of the other causes of darkened gills can only be confirmed via microscopic examination of the gill lamella, a topic that will be addressed in a continuation of this article. If it is confirmed that the discolored gills are colonized by fouling organisms such as ciliate protozoans or filamentous bacteria, this is generally symptomatic of excessive organic matter in the water, or slow growth (i.e. reduced molting frequency). An acceptable level of incidence of shrimp with black or brown gills in a random sample from a pond would be 5%.

Melanized cuticular lesions. Melanized cuticular lesions (MCL) can be caused by: 1) *Vibrio* sp., 2) TSV, and 3) physical injury. Although MCL's caused by *Vibrio* sp. may be symptomatic of poor water quality, they are generally not considered to be life threatening to the shrimp unless the necrotic lesions have penetrated through the exoskeleton into tissue. Animals in the transitional or chronic phases of TSV will often display classic TSV-related MCL's. Regardless of the etiology, MCL's will affect the commercial value at harvest of the afflicted shrimp (unless they are marketed in the peeled form). An acceptable level of incidence of shrimp with melanized cuticular lesions in a random sample would be 5-10% during the production cycle, and less than 2% prior to harvest.

Abnormal coloration of appendages and chromatophores. Color changes in the appendages and chromatophores of the shrimp usually are symptomatic of viral or bacterial diseases. Shrimp infected with TSV or WSSV often display reddish uropods and expanded chromatophores. A pale pinkish coloration of the muscle is also an early symptom of WSSV. Reddish pleopods or pereiopods are often associated with TSV or vibriosis. Considering the seriousness of the diseases that are typically associated with abnormal coloration of appendages and chromatophores, there is no acceptable level of incidence for these symptoms.

Occasionally, the pleopods and pereiopods of the shrimp will take on a dark brown, black or rusty coloration. This is usually caused by adherence of detritus or other substances to the setae of the appendages, and is associated with poor pond bottom conditions. An acceptable prevalence for this condition would be 15%, as long as the harvest of the pond is not imminent, in which case a much lower incidence should be tolerated so as to not substantially diminish the market value of the harvested product.

Soft shelled shrimp. In addition to normal softness immediately following post-molt stages, soft shell condition is also associated with WSSV, TSV, NHP, and in rare instances by mineral imbalances. An acceptable level of incidence of soft shelled shrimp would be 5% during the production cycle, and less than 2% at harvest.

Cramped tail. Occasionally, a percentage of shrimp collected with a cast net will exhibit cramped tail syndrome (CTS). Although the complete etiology of CTS has not been definitively ascertained, it is thought to be a stress reaction in susceptible shrimp, caused by one or more of the following conditions: 1) high temperature stress, 2) vibriosis, 3) mineral imbalances, 4) toxins in the water. Normal, healthy shrimp should not cramp even when handled. Even though it is believed that CTS occurs only after handling, it is symptomatic of reduced tolerance to stress, and indicates a fundamental deterioration in the overall health of the shrimp. An acceptable level of CTS would be 5%, or less.

Opaque, whitish musculature. Like CTS, this is also considered to be a stress related reaction to being handled. Normal, healthy animals should be able to resist being handled without experiencing opaque or whitish transformation of the musculature. An acceptable level of incidence of white or opaque muscle condition would be 2%.

Edema. Edema, in this case, is characterized by an inflammation of tissue in appendages or extremities of the shrimp, and frequently *Vibrio* is the culprit. For example, in the tail it may feel like a small blister at the extreme end of the uropod, or a swelling of the opercular membrane that covers the gill chamber. An acceptable incidence of edema in sampled shrimp would be 5%.

Deformities. Anatomical imperfections such as twisted, stunted antennae, truncated or skewed rostrums missing rostral teeth, convoluted intestinal tracts, asymmetrical tail segments, swollen cephalothorax, and rough cuticle are often symptomatic of the IHHN virus. These abnormalities can also be caused by genetically originated malfunctions in the shrimp's molting processes. An acceptable level of incidence of physical deformities would be 5%.

White spots. The presence of white spots on the cuticle can be caused by several disease conditions, the most notorious being the chronic phase of the White Spot Syndrome Virus (WSSV). Often WSSV-related white spots are only visible in *L. vannamei* if the cephalothoracic cuticle is removed, dried, and held up to the light. Tentative confirmation of white spots associated with WSSV can be made by immersing the cuticle in dilute acid; the calcified spots caused by WSSV will generally be removed by acidification. Cuticular white spots can also have other etiologies. For example, certain bacteria and fungi can also provoke white spots on the shrimp's cuticle. It has also been suggested that mineral imbalances in the water can induce the formation of calcified white spots on the exoskeleton. Excluding white spots associated with WSSV, for which there should ideally be zero tolerance, an acceptable level of non-WSSV related white spots would be 10%.

Haemolymph coagulation time. It is thought that the presence of elevated numbers of bacteria in the shrimp's haemolymph will prolong the normal coagulation time of the haemolymph upon exposure to air. Although there exists some controversy over the actual physiological mechanism of this phenomenon, it has been employed as a practical method for determining if shrimp are afflicted with vibriosis. Using a small gauge (insulin) syringe, a few drops of haemolymph are extracted and placed on an inclined glass slide, and “teased” with the tip of the syringe, counting the seconds before the haemolymph coagulates. In most cases, the haemolymph of healthy shrimp will coagulate in as little as 10 seconds, and usually within 30 seconds. A longer coagulation time suggests health problems in the shrimp.