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Surveillance and Animal Health Monitoring – Early Detection of Disease

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Abstract

Disease in aquaculture systems is the outcome of three major components, namely, the health of the animals being cultured, the condition of the culture environment and the presence of the pathogen. The early detection of signs of disease or poor health is crucial to taking measures to minimize the economic impact of disease. Routine animal health monitoring allows the generation of information necessary for immediate decision-taking. A well-established surveillance programme is key to achieving these results and it should focus on the primary enzootic pathogens in the key stages of production, as well as in the wild. It should also include exotic pathogens, as these could be introduced to the culture system through various means, including water currents, the importation of aquatic animals from infected countries or via ballast water. This paper describes the current surveillance programme for shrimp diseases being implemented in Kingdom of Saudi Arabia by the National Aquaculture Group (NAQUA), which has around 4 500 ha of culture surface. The criteria for identifying the morphological changes that indicate deviation from optimal health, its possible causes, and the mitigation measures are discussed. As the productivity of an aquaculture system is directly related to the health of the stocks, close monitoring and optimization of animal health is a key tool for profitable farming.

Keywords: aquatic animal health, disease surveillance, early disease detection, Kingdom of Saudi Arabia, shrimp diseases

Introduction

The rapid growth and development of the aquaculture business sector and international trade of aquatic animals and their products has increased the emergence of epizootics and the spread of new diseases.

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Diseases are an integral part of livestock production and are often an expression of the complex interactions between the host, the pathogen and the environment. The spread of diseases and the associated socio-economic concerns remain as one of the most relevant challenges to the industry. Prevention of diseases in aquaculture is required, as cures are hard to achieve and intervention after the onset of a disease is often difficult and costly. Well-established biosecurity practices that are strictly applied are fundamental to ensure minimal economic losses due to disease outbreaks and therefore the sustainability of the aquaculture industry. Although disease outbreaks cannot be completely averted even in the best managed ponds, early detection of disease is a key aspect of effective biosecurity.

Continuous surveillance and animal health monitoring are the major tools used to enhance biosecurity and mitigate the impact of diseases. This article describes the ongoing surveillance programme of the shrimp production at the National Aquaculture Group (NAQUA), Kingdom of Saudi Arabia, which uses approximately 4 500 ha of culture surface. This programme includes assessment criteria for morphological changes (both macroscopic and microscopic) that indicate deviation from optimal health, their possible causes, and mitigation measures applied to decrease their adverse effects and restore shrimp health. It is emphasized that productivity is always directly related to the health of the cultured animals and therefore, close monitoring and optimization of animal health are key for profitable farming.

The Surveillance Programme

The key objective of the surveillance programme is the early detection of primary enzootic pathogens, as well as exotic pathogens which are emerging or pose high risk. The health status of the shrimp population in the Kingdom of Saudi Arabia is exceptionally good. To date, the only viruses that have been detected are white-spot syndrome virus (WSSV), *Baculovirus penaei* (BP) and Taura syndrome virus (TSV). Of these, TSV was last detected in 2010, with infections limited to the southern part of the country. Other serious shrimp pathogens remain exotic to the Kingdom of Saudi Arabia (Table 1)**.**

It is important to take into account that surveillance programmes have to be designed based on the sanitary status of the zone, country or region. In addition to the commercial production, the NAQUA surveillance programme also covers the *Penaeus vannamei* Boone 1931 Specific Pathogen Free (SPF) Programme for all the pathogens of penaeid shrimp listed by the World Organisation for Animal Health (OIE) (i.e. WSSV, AHPND, IMNV, IHHNV, YHV, TSV and NHP) and other known important shrimp pathogens (EHP, CMNV, HPV, MBV and BP). As discussed below, the frequency of sampling is based on the degree of economic impact of the diseases, the type of culture system (indoor or outdoor) and the environmental conditions.

Table 1. Sanitary status of the Kingdom of Saudi Arabia with regard to major shrimp pathogens.

Enzootic pathogens	Exotic pathogens
White-spot syndrome virus (WSSV)	Infectious hypodermal and haematopoietic necrosis virus (IHHNV)
Taura syndrome virus (TSV)	Monodon baculovirus (MBV)
Baculovirus penaei (BP)	Necrotizing hepatopancreatitis (NHP)
	Hepatopancreatic parvolike virus (HPV)
	Acute hepatopancreatic necrosis disease (AHPND)
	Enterocytozoon hepatopenaei (EHP)
	Infectious myonecrosis virus (IMNV)
	Yellow head virus (YHV)
	Covert mortality nodavirus (CMNV)

Surveillance of Broodstock and Hatchery Operations

SPF monitoring programme

A major portion of the surveillance programme is focused on broodstock testing, in both the breeding and commercial broodstock development programme. To remove the IHHNV inserts from the stock, after each spawning, all brooders used for the SPF programme are tested individually for IHHNV by polymerase chain reaction (PCR) methods, followed by histopathology. The progenies from each set of brooders are reared separately to avoid mixing of populations. Later, a cold challenge at 20 °C for 48 hr will be given to the juveniles to rule out latent infection by certain pathogens, since pathogens like WSSV multiply faster and express into disease at low temperatures. All the major enzootic and exotic shrimp pathogens (see Table 1) also will be tested at 2 % prevalence by PCR or at 10 % prevalence by histology for all other serious pathogens (see Table 2) before stocking into nurseries as part of SPF status monitoring and validation.

Table 2. Specific pathogen free (SPF) monitoring programme.

Process	Target sample	Target pathogen	Percentage of stocks tested	Diagnostic method
Broodstock SPF programme	Broodstock after spawning	IHHNV	100%	PCR
		All	100%	Histology
	Juveniles (cold challenge)	WSSV, IHHNV, BP, NHP, MBV, HPV,AHPND, EHP, TSV, IMNV, YHV, CMNV	Once per batch (2% prevalence)	PCR
		All	Once per batch (10% prevalence)	Histology

Surveillance of commercial broodstock

Broodstock for commercial seed production are selected individually for quality before they are moved to maturation. Any mortality during maturation will be tested for WSSV by PCR and all animals with clinical signs will be analyzed by histology. One of the important sources of potential pathogen entry into the system is through live or fresh feeds. Hence *Artemia* biomass used as maturation feed is cooked to make sure pathogens are eradicated prior to feeding. All batches of commercial as well as breeding programme broodstock are checked fortnightly by animal health monitoring, examination of wet mounts and histology.

Table 3. Surveillance of broodstocks.

Process	Target sample	Target pathogen	Frequency	Diagnostic method
Broodstock production	Routine	All	Fortnightly	Animal health monitoring, wet mount, histology
	Clinical signs	All	N/A	Histology
Broodstock for maturation	100%	All	Individual selection	Animal health monitoring (morphological)
Broodstock at maturation	Standard mortalities	WSSV	100% mortalities	PCR
	Clinical signs	All	N/A	Histology

Surveillance of hatchery

Bacterial infections are one of the causes of mass mortalities at early stages. So *Artemia* and algae that are used to feed larvae must be free of green colonies on thiosulfate-citrate-bile salts-sucrose (TCBS) agar. PCR analysis for WSSV, IHHNV, AHPND and EHP is also done for *Artemia* because these pathogens can be introduced to the system by contaminated *Artemia*. All the larval tanks are checked on a daily basis by animal health monitoring and wet mount as shown in Table 4.

Table 4. Surveillance of hatchery.

Process	Target sample	Target pathogen	Frequency	Diagnostic method
Larval production	Routine	All	Daily	Animal health monitoring & wet mount
	Artemia	WSSV, IHHNV, AHPND, EHP	Every batch	PCR
	Algae and *Artemia*	Vibrio (green colonies – TCBS)	Weekly	Microbiology

Surveillance of nurseries and grow-out ponds

Considering that shrimp will lose their SPF status once they are exposed to the external environment of the nurseries, samples from each nursery are also cold challenged at 20 °C for 48 h prior PCR testing for WSSV and then transferred to grow-out ponds to make sure that they are free of WSSV. Samples from all nurseries and grow-out ponds also undergo animal health monitoring and wet-mount examination, as described in Table 5. During winter, due to the risk of WSSV, the nurseries and ponds are monitored fortnightly by animal health monitoring and examination of wet mounts. During the summer, the procedure is performed once a month.

Table 5. Surveillance of nursery and grow out.

Process	Culture period	Target sample	Target pathogen	Frequency	Diagnostic method
Nursery and grow-out ponds	Summer	Cold challenge (only nursery)	WSSV	Before transfer to grow out ponds	PCR
		Routine	All	Monthly	Animal health monitoring & wet mount
		Clinical signs	WSSV	N/A[1]	Rapid field test/PCR
			All	N/A	Animal health monitoring & wet mount
			AHPND	Based on hepatopancreas wet-mount results	PCR
			All	N/A	Histology
	Winter	Routine (only nursery)	All	Fortnightly	Animal health monitoring & wet mount
		Clinical signs	WSSV	N/A	Rapid field test/PCR
			All	N/A	Animal health monitoring & wet mount
			AHPND	Based on hepatopancreas wet-mount results	PCR
			All	N/A	Histology

[1]N/A = not applicable.

Surveillance of wild animals

Wild crustaceans (e.g. crabs, shrimp, zooplankton) can be carriers of shrimp pathogens. These are collected and tested for WSSV and in the case of wild shrimp, also for TSV. For this purpose, samples from various locations (including feeder canals) and from local fishermen who are engaged in fishing in the same area are collected. The information thus generated is useful in making decisions regarding stocking season, type of stocking, stocking density and even emergency harvest. The details of testing are given in Table 6.

Table 6. Surveillance of wild animals.

Location	Target sample	Target pathogen	Frequency	Diagnostic method
Main feeder canals, subfeeder canals & intakes	Crabs, shrimp & zooplankton	WSSV (crabs, shrimp & zooplankton); TSV (shrimp)	Monthly (summer) Fortnightly (winter)	PCR
Local fishing boats (wild catch)	Shrimp	WSSV, TSV, AHPND, EHP	Monthly	PCR
	Crabs	WSSV		
	Filter feeders	TSV, AHPND		

Surveillance of seafood markets

Because of the transboundary nature of many pathogens (many diseases are spread globally through the importation of infected animals), and the fact that the Kingdom of Saudi Arabia is importing seafood from other countries having much lower sanitary status, samples are collected from local fish markets and analyzed for both enzootic and exotic pathogens. The details of testing are given in Table 7.

Table 7. Surveillance of seafood markets.

Location	Target sample	Target pathogen	Frequency	Diagnostic method
Local fish markets	Shrimp from different countries	WSSV, TSV, IHHNV, NHP, AHPND, EHP, MBV, HPV	Monthly	PCR

Animal Health Monitoring Programme

The Animal Health Monitoring Programme helps to determine if there is any building up of problems that could eventually lead to a disease outbreak (whether it is caused by primary or secondary pathogens). A primary pathogen is an agent that can cause disease by itself (such as viruses), while a secondary or opportunistic pathogen requires a primary cause to initiate the onset of disease (e.g. a primary pathogen or a change in culture conditions). An effective animal health monitoring programme takes into account first-level diagnosis, real-time physicochemical parameters of water and soil, and animal performance data obtained from the field. The complete information will help in obtaining a proper diagnosis. In order not to lose the opportunity for early detection of disease, it is important to prioritize tanks, nurseries or ponds with abnormalities or poor performance.

Importance of Correct Sampling

The observation of animals is time consuming. It should be taken into account that the number of animals that will be sampled is very small when compared to the population size; and that the information generated from these few animals will be used to determine the health status of the whole population. Hence, it is crucial to conduct targeted sampling.

Weak animals can be obtained from the pond sides (especially during an outbreak of white-spot disease (WSD), see Fig. 1b), from check trays (Fig. 1a) or from outlet screens after a thorough flushing (Fig. 1c, d). Assumed prevalences of 2 % and 5 % are most commonly used for surveillance of presumed exotic pathogens with a 95 % confidence limit. More details of prevalence calculation are given in the Table 8.

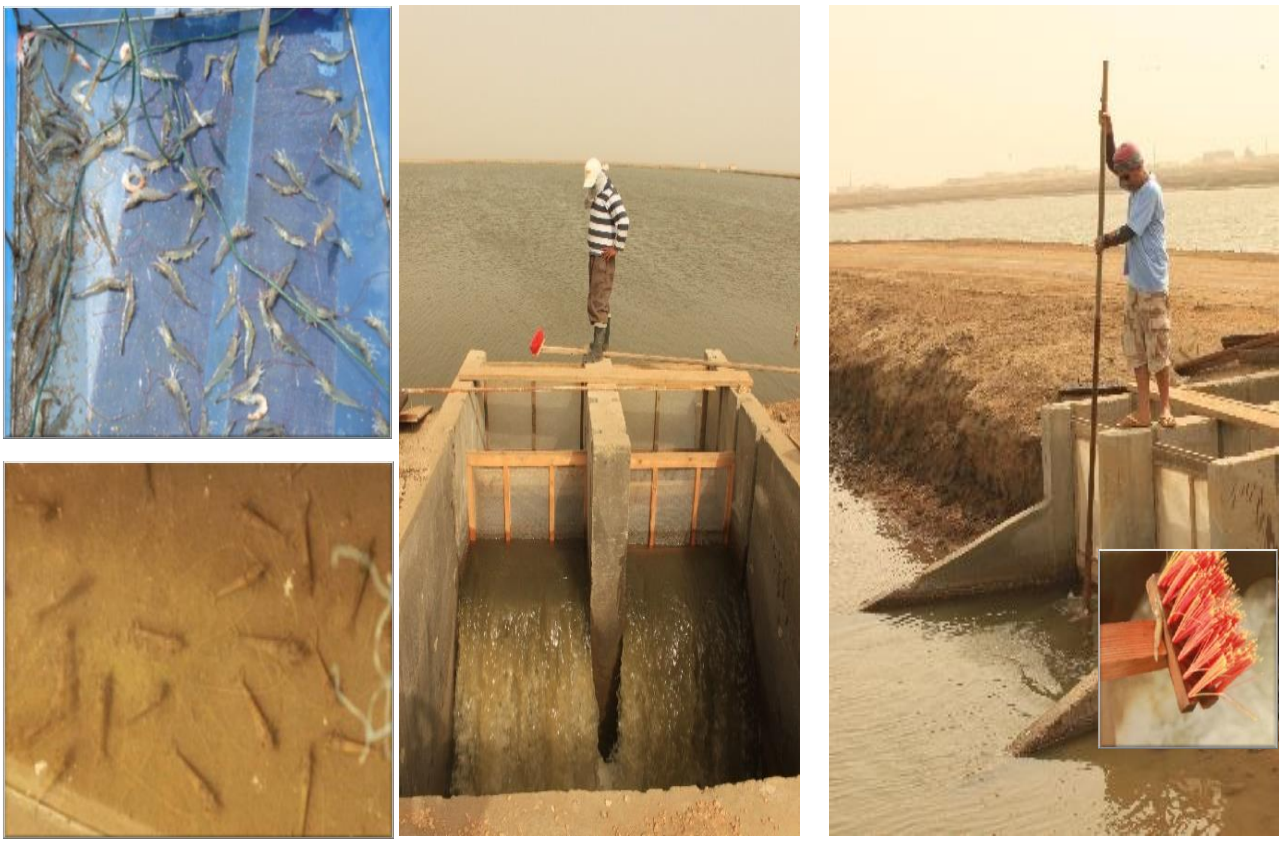

Fig. 1. (a) Weak animals in check tray; (b) weak animals near pond side; (c, d) collection of weak animals from outlet screen after thorough flushing.

Observation of Culture Conditions

Some observations of culture conditions that could indicate the health status of the pond population are given below.

Table 8. Estimation of sample size needed to detect different levels of prevalence in a population (modified from Amos 1985).

Population size	Prevalence (%)						
	0.5%	**1%**	**2%**	**3%**	**4%**	**5%**	**10%**
50	46	46	46	37	37	29	20
100	93	93	76	61	50	43	23
250	192	156	110	75	62	49	25
500	314	223	127	88	67	54	26
1 000	448	256	136	92	69	55	27
2 500	512	279	142	95	71	56	27
5 000	562	288	145	96	71	57	27
10 000	579	292	146	96	72	29	27
100 000	594	296	147	97	72	57	27
1000 000	596	297	147	97	72	57	27
>1 000 000	600	300	150	100	75	60	30

Bird activity

Birds are the best indicators of abnormalities in a pond, and will be the first sign a farmer can notice when entering a farm. Birds are attracted to the animals that move to the pond surface, either due to a disease outbreak, mortalities or even dissolved oxygen depletion. Weak animals gathered at the pond peripheries also attract birds. Figure 2a-c shows bird activity in culture ponds.

Fig. 2. (a-c): Bird activity in culture ponds due to oxygen depletion.

Water discolouration

One of the basic and important aspects of successful shrimp farming is managing water transparency and productivity in a stable manner. A sudden increase or decrease in algal or microbial population can result in drastic changes in water quality. Excess feed will provide nutrients for algal and microbial communities to develop exponentially. This is particularly common in the second phase of production. If the phytoplankton population is not managed properly, it could cause "die-off" and eventually lead to high transparency and an anaerobic pond bottom. This dead and decaying organic matter could provide nutrients for pathogenic bacteria. The acceptable range of transparency for semi-intensive ponds is 45–60 cm on Secchi disk. Figure 3a-d shows water transparency and discolouration in shrimp ponds.

Fig. 3. (a) Normal pond water; (b) *Dunaliella salina* bloom; (c) filamentous algae; (d) dinoflagellate bloom.

Presence of extraneous populations

Crabs and fish must be excluded from shrimp culture ponds, as they can introduce primary pathogens like WSSV, increase the chance of spread of muscle microsporidians, increase food conversion ratio (FCR) and even prey on postlarvae (PL). Carnivorous fish (Fig. 4a,b) play a significant role in reducing the survival of PL when they are stocked into a pond. The results of a small trial that was conducted to assess the impact of fish in the ponds at the time of stocking is given in Table 9. A similar trial was conducted with juveniles; however, no significant difference in survival was observed.

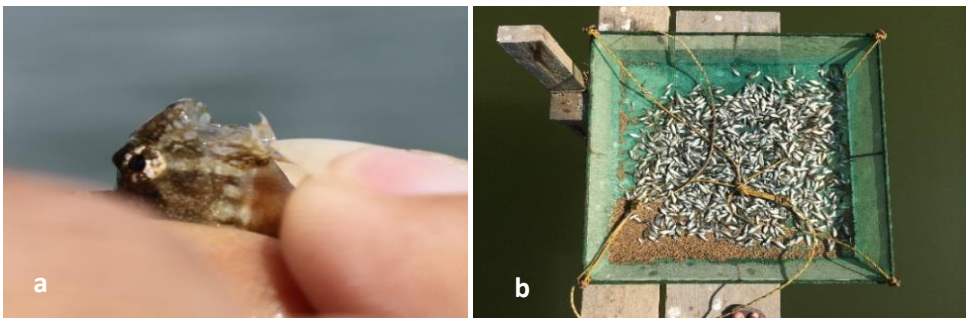

Fig. 4. (a) Fish used for the trial; (b) fish found in check tray.

Table 9. Results of a trial conducted to determine the effect of carnivorous fish on the survival of *Penaeus vannamei* during the early stages (trial duration – 24 hr).

	Stocking	Survival
Happa 1	200 postlarvae + 5 fish with feeding	24%
Happa 2	200 postlarvae + 5 fish without feeding	10%
Happa 3	200 postlarvae + no fish with feeding	93%

Morphological and physiological observations

Behaviour

Behaviour of the animals is the first observation you are should make when conducting shrimp health monitoring. Animals with clinical signs of disease and weak animals at the shore are a clear indication of abnormal behaviour.

Body colouration

Discolouration of the carapace and pleopods also often indicates the occurrence of disease or stress. Reddish discolouration can be a sign of WSD. Discolouration of the shrimp body is shown in Fig. 5a-c.

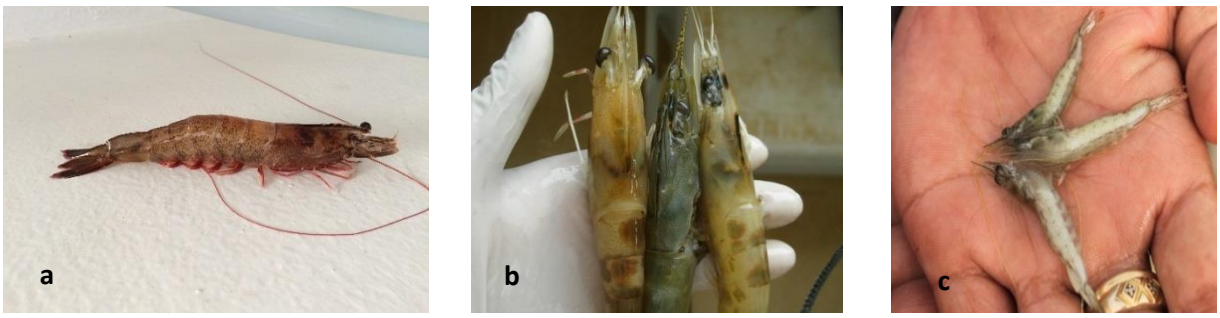

Fig. 5. Discolouration of the shrimp body. (a) reddish discolouration; (b) whitish discolouration; (c) yellowish discoloration.

Presence of loose shell

Loose shell is another crucial indication of abnormality. It may be a clinical sign of enteric bacterial infection. Inflammation of the hepatopancreatic tubules results in malfunctioning and eventually leads to impairment of nutrient absorption and lipid storage. This will result in weight loss and subsequently gap formation between the shell and muscle. It is important to remember that loose shell can also be due to malnourishment. Loose shell caused by enteric bacterial infections is shown in 6a,b.

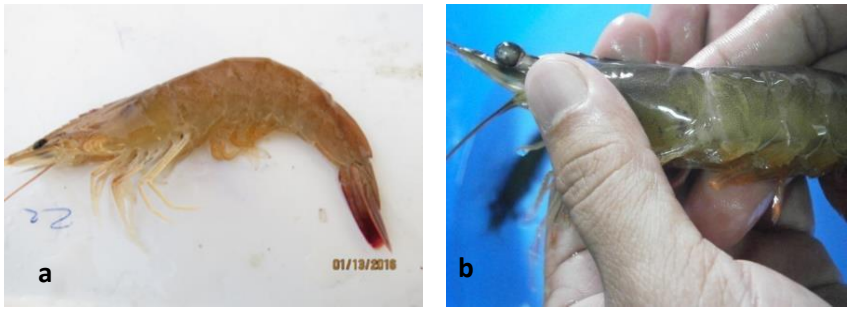

Fig. 6. (a, b) Loose shell due to enteric bacterial infection.

White spots on carapace

Even though the presence of white spots on the shell has no diagnostic value, as they are not pathognomonic, it can raise an alarm to increase vigilance for WSSV in the pond (Fig. 7a,b). In the Kingdom of Saudi Arabia, WSSV is the major enzootic disease affecting the shrimp industry. However, the presence of white spots on the shell could be non-specific.

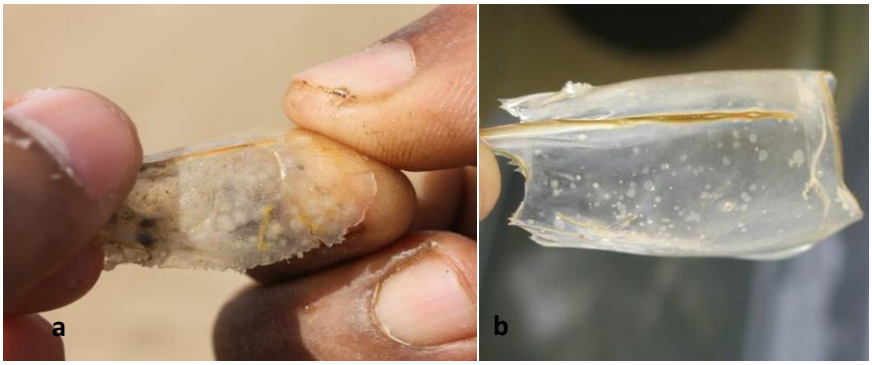

Fig. 7. (a,b) White spots on the carapace of shrimp.

Gill colour

It is important to check gill colour frequently; if they are becoming brown (Fig. 8a,b) or black (Fig. 8c) it will be either a sign of poor bottom condition, algal bloom or even bacterial infection. These can be easily distinguished by placing the affected animals in an aquarium or bucket with clear water with sufficient oxygen.

If the animals are able to clear the discolouration by themselves, it can be concluded that pond bottom deterioration, heavy algal bloom or algal die off are possible causes. However, if the animals do not clear the colour, it means that the dark gill colouration is caused by melanization and thus could be related to bacterial infection. In both cases, a thorough water exchange can help to restore the normal condition of the shrimp.

Fig. 8. (a,b): Brown gills; (c): black gills

Tail muscle colouration

Tail discolouration can occur for various reasons; for example, when the animals are infected with muscle Microsporidia such as *Ameson* sp. the tail becomes whitish and opaque (Fig. 9a); this disease is known as cotton shrimp disease. In some cases, there will be reddish discolouration of the abdominal segments (Fig. 9b,c). This could be due to IMNV or to a systemic bacterial infection. Whitish muscle can also be caused by muscle cramp.

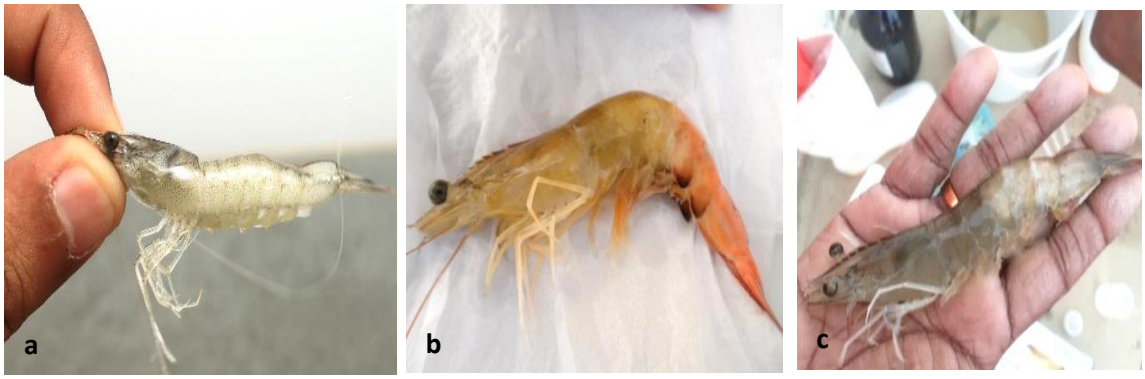

Fig. 9. (a): Muscle microsporidian infection; (b,c): systemic bacterial infection.

Cuticular melanization

Cuticular melanization is another striking character occurring mostly due to bacterial infection if the bacterial flora of the culture water is dominated by pathogenic bacteria (e.g. *Vibrio parahaemolyticus*). A thorough water exchange will help to replace the bacterial flora and remove moults, as the chitin component of shells acts as a substrate for *V. parahaemolyticus*. Melanization caused by infection by *V. parahaemolyticus* in the cuticle of *P. vannamei* can be seen in Fig. 10a-c.

Fig. 10. (a-c): Cuticular melanization/erosion caused by *Vibrio parahaemolyticus.*

Uropod reddishness/tail rot

Reddish and melanized uropods (Fig. 11a,b) are often the result of poor pond bottom quality and bacterial infections. This condition is common in poorly managed broodstock ponds and also in recirculated aquaculture systems (RAS).

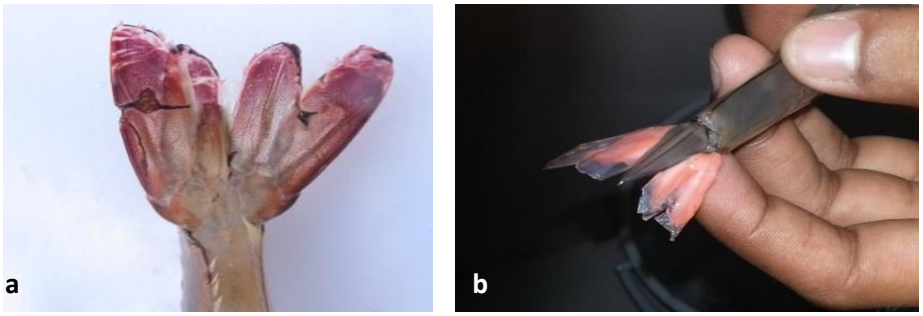

Fig. 11. (a) Uropod reddishness; (b) necrosis (tail rot).

Haemolymph clotting test

In shrimp, haemolymph will normally clot within 1–1.5 min after extraction. However, if the animals have viral infections (like WSSV) or bacterial infections (like vibriosis), the clotting time will be extended. Care should be taken to conduct this analysis immediately after sampling, because stress can also cause extended clotting time. Extraction of haemolymph and the haemolymph clotting test are shown in Fig. 12a,b.

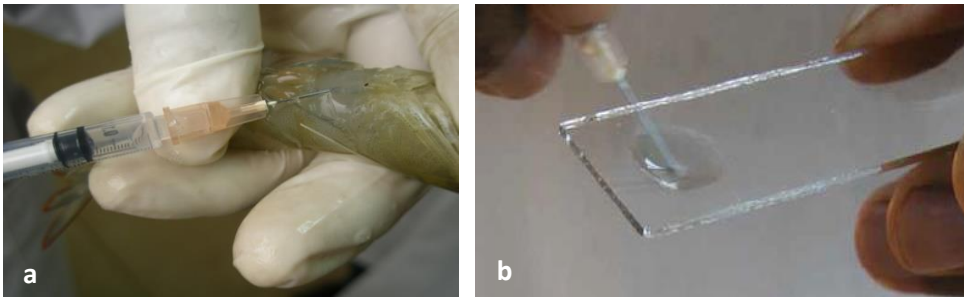

Fig. 12. (a): Extraction of haemolymph; (b): performing haemolymph clotting test.

Microscopic Observations or Wet Mount

Gill wet mount

Gill wet mount is done to determine the presence of parasites, debris or melanization in the gills. These results will give an idea about the pond conditions and also the health status of the animals. Gill melanization is one of the clinical signs of systemic vibriosis; in some cases it can also be caused by toxicity. Photomicrographs of gill wet mount showing gill melanization (Fig. 13a) and various infections (Fig. 13b,c) are given below.

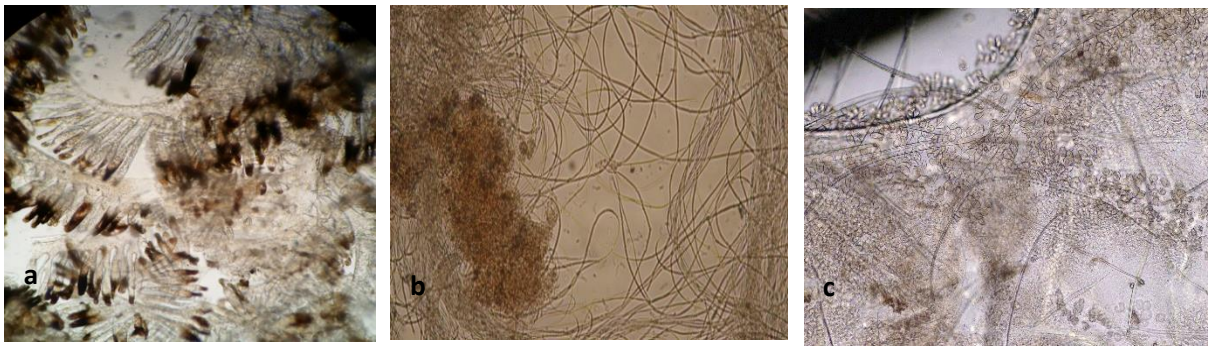

Fig. 13. (a) Gill melanization, probably due to bacterial infection; (b) filamentous bacteria (*Leucothrix mucor*) infection in gills; (c) *Zoothamnium* sp. infection in gills.

Hepatopancreatic tubular constrictions

It is always important to observe changes in the tubules of the hepatopancreas, as this organ is exposed to water and feed quality. Hepatopancreatic tubular constrictions are the first sign of toxic effects due to acute hepatopancreatic necrosis disease (AHPND), vibrios, blue-green algal toxins, etc. Usually, if the percentage of hepatopancreatic tubular constrictions is high, it is not advisable to stock the larvae, as this can have a significant impact on production and also on health. Fig. 14a,b shows constrictions in the hepatopancreatic tubules of *P. vannamei* collected from grow-out ponds.

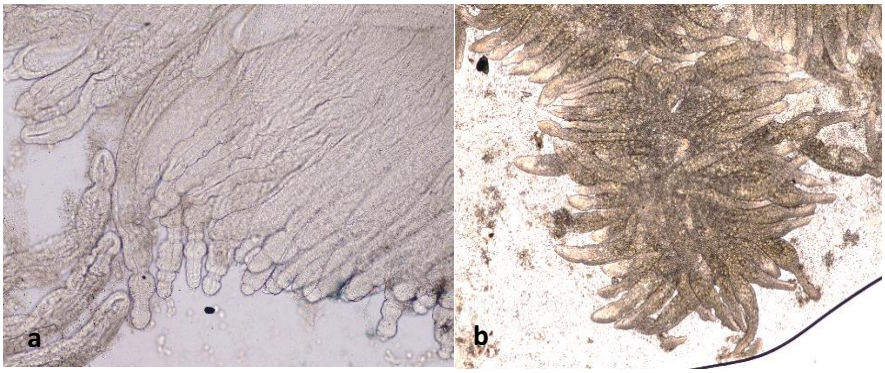

Fig. 14. (a,b) Hepatopancreatic tubular constrictions.

Hepatopancreatic tubular melanization

Tubular melanization of the hepatopancreas could be due to bacterial infection or the result of chronic toxicity. In some cases, if hepatopancreatic tubular constriction is not managed properly, melanization can occur, resulting in the malfunctioning of the hepatopancreatic tubules and therefore, poor absorption. Animals with more hepatopancreatic tubular melanization will also have loose shells. Figure 15a-c presents photomicrographs of melanized hepatopancreatic tubules of animals with enteric bacterial infection.

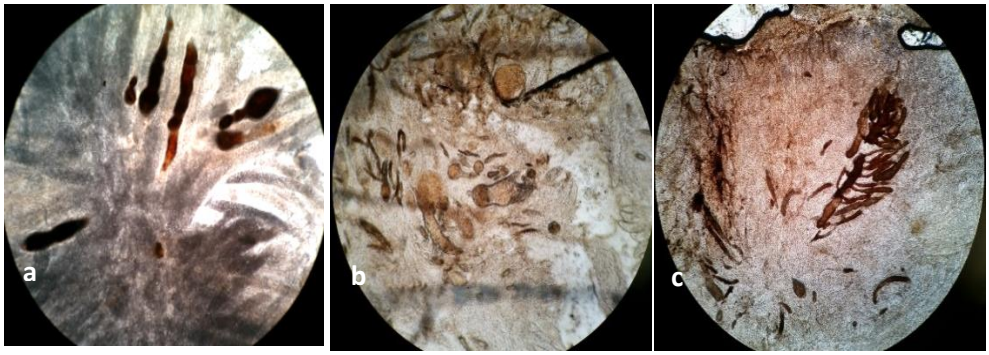

Fig. 15. (a,b,c) Hepatopancreatic tubular melanization in *Penaeus vannamei*.

Lipid vacuolization in the hepatopancreas

Observations of lipid storage levels in the hepatopancreas may help in optimization of feeding. It is determined by checking for the presence of lipid vacuoles in the hepatopancreas (Fig. 16a-c).

- Correct feeding: more 80 % of the animals with medium to low lipids 1 h before and more than 80 % with high lipids after 1 h of feeding
- Under-feeding: less than 80 % of animals with high lipids after 1 h of feeding
- Over-feeding : more than 60 % of animals with high lipids before 1 h of feeding

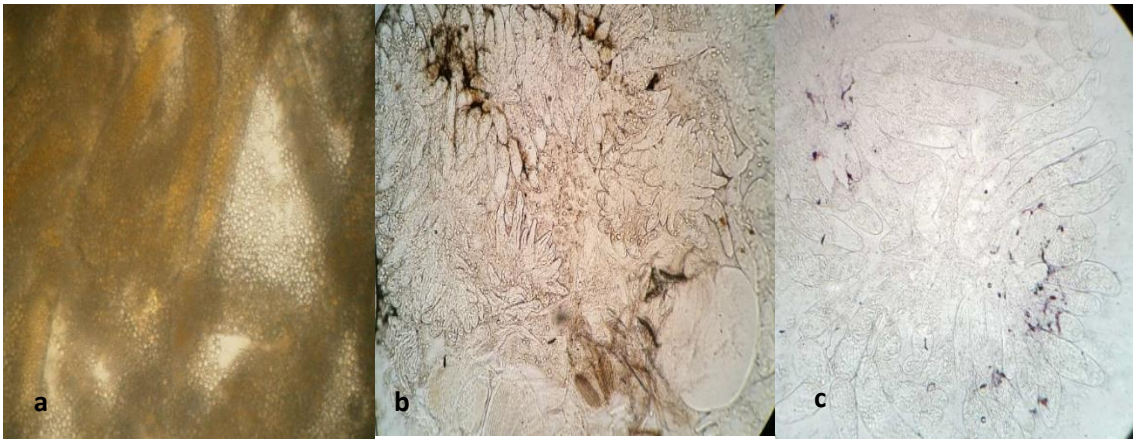

Fig. 16. (a): High level of lipids storage in hepatopancreas; (b, c) low level of lipids.

Gut content analysis

Wet-mount examination of gut contents is done to detect the presence of diseases like *Baculovirus penaei* (BP) (Fig. 17a), monodon baculovirus (MBV) (Fig. 17b) and intestinal gregarines (Fig. 17c). All these pathogens can affect the growth of cultured shrimp.

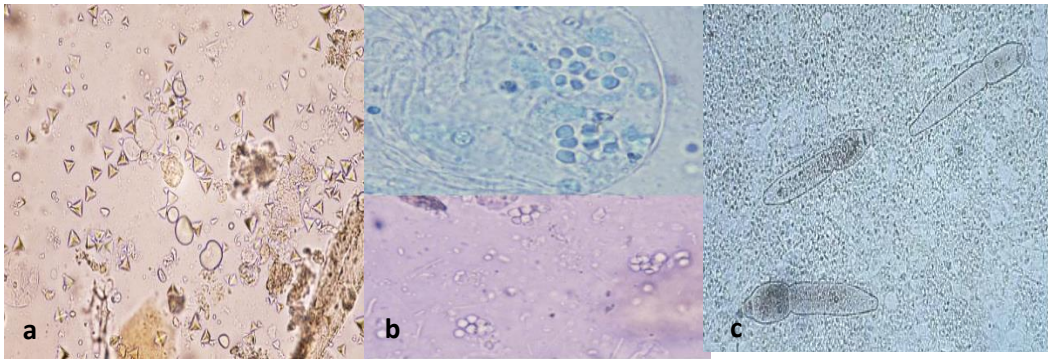

Fig. 17. (a) *Baculovirus penaei* (BP); (b): Monodon baculovirus (MBV); (c) gregarine in midgut (courtesy Dr D.V. Lightner).

Factors Influencing Frequency of Monitoring

Health monitoring frequency should be flexible to adapt to the season and sanitary status of the biosecurity zone. It has to be increased when there is a disease detection or outbreak at the farm, at neighbouring farms or any other farm within the same biosecurity zone. During winter and after a heavy rain, the farms and nurseries need to be monitored more critically, as low temperature can trigger diseases like WSSV. The objective of performing an animal health monitoring and surveillance programme is to achieve early detection of disease, thus minimizing the economic losses. If the clinical signs or field observations are ignored, that can cause failure and will be very expensive.

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