Metadata of the chapter that will be visualized online

Chapter Title	Aquaculture Disease Diagnosis and Health Management	
Copyright Year	2015	
Copyright Holder	Springer India	

Corresponding Author	Family Name	**Raja**
	Particle	
	Given Name	**R. Ananda**
	Suffix	
	Organization	Central Institute of Brackishwater Aquaculture
	Address	75. Santhome High Road, R. A. Puram, Chennai 600 028, Tamil Nadu, India
	Email	anandarajars@gmail.com
Author	Family Name	**Jitendran**
	Particle	
	Given Name	**K. P.**
	Suffix	
	Organization	Central Institute of Brackishwater Aquaculture
	Address	75. Santhome High Road, R. A. Puram, Chennai 600 028, Tamil Nadu, India

Abstract

Aquaculture is growing by leaps and bounds and is one of the world's fastest-growing industries in food production. Unlike other terrestrial farm animals and plants, aquatic animals require more attention in order to monitor their health. They live in a complex and dynamic environment and are not readily visible except under tank-holding conditions. Similarly, feed consumption and mortalities are also equally well hidden under water (Bondad-Reantaso et al. 2001). So the problems faced by the aquatic animals are also species and system specific. The complexity of the aquatic ecosystem makes it difficult to understand the difference between health, suboptimal performance, and disease. The range of diseases found in aquaculture is one among the major problems faced by aquaculturists all over the world. Diseases in aquaculture are caused by the outcome of a series of linked events involving the interactions between the host, the environment, and the presence of a pathogen (Snieszko 1974). Environment includes not only the water and its components (such as oxygen, pH, temperature, toxins, and wastes) but also the kind of management practices (e.g., handling, drug treatments, transport procedures, etc.). There are three factors such as stocking density, innate susceptibility, and immunity which are particularly important in affecting host's susceptibility to diseases. The intensive shrimp aquaculture has parallely brought disease problems leading to great economic loss. Diseases may be caused by a single or combinations of multifarious factors. Generally, diseases are broadly classified in to infectious and noninfectious. The former is caused either by virus, bacteria, fungi, parasites, or rickettsia, while the latter is due to environmental stresses,

Author's Proof

genetic factors, and nutritional deficiencies. The most important steps to reduce or prevent losses due to diseases in aquaculture are monitoring as regularly as possible and appropriate action at the first sign(s) of suspicious behavior, lesions, or mortalities. These fundamental approaches should be followed in many aquatic animal production sectors as in animal husbandry and agricultural production. Some farmers hesitate to reveal the disease problems due to their ignorance that it may result in failure in the competitive market price. It should be made understood that hiding or denying health problems can be as destructive to aquatic animals as it is elsewhere.

Author's Proof

Aquaculture Disease Diagnosis and Health Management

R. Ananda Raja and K. P. Jitendran

Introduction

Aquaculture is growing by leaps and bounds and is one of the world's fastest-growing industries in food production. Unlike other terrestrial farm animals and plants, aquatic animals require more attention in order to monitor their health. They live in a complex and dynamic environment and are not readily visible except under tank-holding conditions. Similarly, feed consumption and mortalities are also equally well hidden under water (Bondad-Reantaso et al. 2001). So the problems faced by the aquatic animals are also species and system specific. The complexity of the aquatic ecosystem makes it difficult to understand the difference between health, suboptimal performance, and disease. The range of diseases found in aquaculture is one among the major problems faced by aquaculturists all over the world. Diseases in aquaculture are caused by the outcome of a series of linked events involving the interactions between the host, the environment, and the presence of a pathogen (Snieszko 1974). Environment includes not only the water and its components (such as oxygen, pH, temperature, toxins, and wastes) but also the kind of management practices (e.g., handling, drug treatments, transport procedures, etc.). There are three factors such as stocking density, innate susceptibility, and immunity which are particularly important in affecting host's susceptibility to diseases. The intensive shrimp aquaculture has parallely brought disease problems leading to great economic loss. Diseases may be caused by a single or combinations of multifarious factors. Generally, diseases are broadly classified in to infectious and noninfectious. The former is caused either by virus, bacteria, fungi, parasites, or rickettsia, while the latter is due to environmental stresses, genetic factors, and nutritional deficiencies. The most important steps to reduce or prevent losses due to diseases in aquaculture are monitoring as regularly as possible and appropriate action at the first sign(s) of suspicious behavior, lesions, or mortalities. These fundamental approaches should be followed in many aquatic animal production sectors as in animal husbandry and agricultural production. Some farmers hesitate to reveal the disease problems due to their ignorance that it may result in failure in the competitive market price. It should be made understood that hiding or denying health problems can be as destructive to aquatic animals as it is elsewhere.

Importance of Diagnostics in Aquaculture

Diagnostics play an important role in aquatic animal health management and disease control. Confirmatory diagnosis of a disease is often

R.A. Raja (✉) • K.P. Jitendran
Central Institute of Brackishwater Aquaculture,
75. Santhome High Road, R. A. Puram, Chennai 600 028,
Tamil Nadu, India
e-mail: anandarajars@gmail.com

S. Perumal et al. (eds.), *Advances in Marine and Brackishwater Aquaculture*,
DOI 10.1007/978-81-322-2271-2_23, © Springer India 2015

Author's Proof

considered as complicated and costly which may be true in some newly emerging diseases, for instance, early mortality syndrome (EMS) outbreak in shrimp aquaculture and its confirmatory etiological diagnosis, but not in all the cases with already standardized and validated diagnostics. Incorrect diagnosis can lead to ineffective or inappropriate control measures which may be even more costly. Disease diagnostics should be made available throughout the entire life cycle of the host till it reaches table for consumption. There are multifarious recent diagnostics available in aquaculture for disease diagnosis at different levels. Some diagnostics are used to screen healthy animals to ensure that they are free from any infection at asymptomatic levels with specific pathogens. This kind of screening is mostly done on aquatic animals which are transferred live or as products from one area or country to another. Such screening reduces the risk of carrying infectious agents including opportunistic pathogens which might proliferate during shipping, handling, or change of environment (Bondad-Reantaso et al. 2001). Further, it reduces the risk of resistant or tolerant animals transferring a significant pathogen to a susceptible population. Diagnostic tests may be applied to diagnose clinically diseased individuals and screen specific disease surveillance and as a confirmatory and calibration tests to validate the other diagnostics and procedures adopted. Valid laboratory results are essential for diagnosis, surveillance, and trade.

Disease Diagnosis in Aquaculture

It is a dynamic field; what found new yesterday becomes dated today, and latest today would become obsolete tomorrow. Disease diagnosis can be basically divided into two types such as *presumptive diagnosis* where a preliminary diagnosis based on gross observations and circumstantial evidence is done and *confirmatory diagnosis* in which the etiological agent is confirmed with a high degree of diagnostic confidence.

Gross and Clinical Signs

Gross observations can be easily made at the farm or pond side. In most cases, such observations are insufficient for a definite diagnosis. But such information is essential for preliminary understanding of the "case description" or "case history." Accurate and detailed gross observations can also help in effectively reducing the losses or spread of the diseases by means of destruction or isolation of affected stocks and treatments or alterations to husbandry practices. Clinical signs such as behavioral change which includes changes in feeding behavior, weight loss, lethargy, erratic swimming movement or unusual aggregations, parasitism, cuticle softening, discoloration, hemorrhagic lesions, ulcers, predator activity, and unusual mortalities are considered to be the first signs of stress or disease problem in an aquaculture system. Environmental parameters such as temperature, dissolved oxygen, pH, etc., play a significant role in aquaculture both directly (within the ranges of physiological tolerances) and indirectly (enhancing susceptibility to infections or their expression).

Clinical Biochemistry

Clinical chemistry in shrimp–fish pathology is in its infancy state. But routine application of clinical biochemistry will help in arriving at confirmatory diagnosis in future and also identification of any blood-borne parasites. Hematological, immunological, and clinical biochemical values such as bleeding time, coagulation time, total hemocyte count (THC), differential hemocyte count (DHC), bacterial clearance activity, phagocytosis, prophenoloxidase activity, serum acid phosphatase, serum alkaline phosphatase, total serum protein, glucose, cholesterol, total protein, total albumin, alanine transaminase (ALT), aspartate transaminase (AST), triglycerides, and lactate dehydrogenase (LDH) will also give some specific clue in making confirmatory diagnosis.

Uncorrected Proof

Author's Proof

Environmental Parameters

Often environmental parameters are not included in routine diagnostic procedures done in aquaculture. But it is essential to assess the water and soil quality parameters such as salinity, temperature, pH, dissolved oxygen (DO), ammonia nitrogen (NH_3–N), nitrite nitrogen (NO_2–N), nitrate nitrogen (NO_3–N), phosphate phosphorus (PO_4–P), and microbial load since they play vital role in deciding any disease outbreak in aquaculture system. Sometimes any one of these environmental factors alone can lead to high mortality, but mere presence of certain pathogenic organism in the host and pond ecosystem can mislead our confirmatory diagnosis.

Necropsy Examination

Necropsy examination is performed to inform farmer, clinical staff, researcher, academicians, or legal authorities about the cause of death. It is essential for getting new information and guidance for the future. Post mortem examinations can provide information about illness and health that would not be discovered in any other way and help to understand why the animal died. The rare pathological conditions can be preserved, and retention of whole animal/organ/tissue would benefit to future needs. Much of what we know about illness today came from such examinations. They help to:

- Identify the cause of death.
- Confirm the nature of the illness and/or the extent of the disease.
- Identify other conditions that may not have been diagnosed.
- Identify complications or side effects of treatments and drugs.

It is also possible that the information gained may benefit future generations in the family, or other animals suffer similar problems. Before proceeding to post mortem examination, one should ascertain when the fish first showed signs and the treatment given (Noga 2010; Roberts 2012).

Isolation and Identification of Pathogen

The organ of choice for isolating systemic bacterial pathogens in fish is kidney which can be approached either dorsally or ventrally, and in shrimp it is hepatopancreas being a vital organ. Fish–shrimp pathogens should be cultured at room temperature (22–25 °C), not at 37 °C, as is routinely done in many microbiology laboratories since some of the fish pathogens grow poorly or not at all at 37 °C (Bondad-Reantaso et al. 2001). For example, *Vibrio salmonicida* grows at 17 °C. Samples from marine and brackish water source to be cultured on a medium that has high salt content at least 1.5 % (Bruno 1996). Special media like thiosulfate–citrate–bile sucrose agar (TCBS) can also be used. Live specimens should be used for culture whenever possible. Identification of an obligate pathogen (*Aeromonas salmonicida*) (Drinan 1985) in a dead fish is a stronger diagnosis than the isolation of an opportunist (*A. hydrophila*). The other pathogens like virus, fungi, parasite, etc. should be isolated as per the standard protocol for each species of the organisms. It is very important to understand that mere isolation and identification of pathogen from any host do not warranty that the disease and mortality are due to its presence in the system. The specific cause of death should only be ascertained when the Koch's postulate is proven.

Bioassay

It is a quantitative procedure that uses susceptible organisms to detect toxic substances or pathogens. Bioassay is done with samples collected from suspected or asymptomatic carriers and tested using a highly susceptible host (life stage or species) as the indicator for the presence of the pathogen. In this assay Koch's postulate is well proven.

Microscopy

Bright-field microscopy is the simplest of all the light microscopy techniques where the sample is

Author's Proof

illuminated with white light from below and observed from above. The technique is very easy and simple to do with minimal sample preparation, but it requires expertise in reading the slides. Low contrast of most biological samples and low apparent resolution are the limitations. Dark-field microscopy is yet another technique commonly used for improving the contrast of unstained, transparent specimens. But this technique suffers from low light intensity in the final image of many biological samples and continues to be affected by low apparent resolution. Many times for on-farm diagnosis, the presence of virus can be detected by tissue squash preparation and staining. This can then be observed under a microscope for a particular viral infection like Monodon baculovirus (MBV) by hepatopancreas or fecal squash preparation stained with 0.05 % aqueous malachite green for detection of large, single, or multiple roughly spherical, eosinophilic, polyhedral, intranuclear occlusion bodies (OBs). Moreover, microscopy plays a crucial role in the identification of bacterial pathogens by using the special stains like Gram's staining and acid fast staining.

Histopathology

Histopathology holds its importance from the day of its invention in the field of diagnostics. Proper sampling and fixation are the most important steps for correct disease diagnosis. The moribund or very recently dead animals are suitable for histopathology, while putrefied or frozen animals are found unsuitable. Fish/shrimps are usually fixed in 10 % neutral buffered formalin (NBF) fixative in a wide-mouth plastic bottle. The fixative volume should be at least 10 times more than the volume of sample to get the tissues properly fixed. The samples collected should be as small as possible not more than 0.5 cm^2 thickness. For shrimps, Davidson's fixative is also commonly used, and the composition of the common fixatives used is listed below (Bell and Lightner 1988; Lightner 1996).

Common Fixative Used for Histopathology

Davidson's fixative		
95 % ethanol	–	330 ml
37 % formaldehyde	–	220 ml
Glacial acetic acid	–	115 ml
Distilled water	–	335 ml
4 % Formal saline (for parasites)		
37 % formaldehyde	–	40 ml
Distilled water	–	960 ml
Sodium chloride	–	8.5 g
10 % Formal saline (for tissues)		
37 % formaldehyde	–	100 ml
Distilled water	–	900 ml
Sodium chloride	–	8.5 g
10 % neutral buffered formalin		
37 % formaldehyde	–	100 ml
Distilled water	–	900 ml
Sodium dihydrogen phosphate	–	4 g
Disodium hydrogen phosphate	–	6 g

The presence of virus in different tissues can be detected by histopathology. However, proper histopathological techniques and expertise in reading slides are necessary to interpret the results. If properly detected, this will be the most accurate diagnostic method. But it will be difficult to detect any low levels of infections by this method. The most well-defined common viral diseases affecting shrimp and fish are listed below with the details of the inclusion bodies with respect to the specific diseases seen in the histopathology.

In addition, immunohistochemical staining methods have also been developed with paraffin-embedded tissue sections for the detection of viruses such as infectious pancreatic necrosis virus (IPNV), infectious salmon anemia virus (ISAV) and nodavirus (Bondad-Reantaso et al. 2001). Viral antigen is localized by an antibody raised against the virus, and subsequent addition of colored substrate results in a colored product that can be visualized by light microscopy.

Uncorrected Proof

Author's Proof

Sl. No.	Disease	Etiology	Inclusions
1.	Monodon baculovirus (MBV) disease	Family: *Baculoviridae*, dsDNA type A monodon baculovirus (MBV)	Large, single, or multiple roughly spherical, eosinophilic, polyhedral, *intranuclear* occlusion bodies (OBs) in the epithelial cells of the hepatopancreas tubules and the anterior midgut (Lester et al. 1987; Lightner 1988; Vogt 1992; Bondad-Reantaso et al. 2001)
2.	White spot disease (WSD)	Family: *Nimaviridae*, dsDNA *Whispovirus*, white spot syndrome virus (WSSV)	Ectodermal (epidermis, gills, fore and hind gut, antennal gland, and neurons) and mesodermal (hematopoietic tissue, hemocytes, striated muscle, heart, lymphoid organ, and connective tissues) tissues with eosinophilic to basophilic *intranuclear* inclusions (Momoyama et al. 1994; Wongteerasupaya et al 1995)
3.	Infectious hypodermal and hematopoietic necrosis (IHHN)	Family: *Parvoviridae*, ssDNA infectious hypodermal and hematopoietic necrosis virus (IHHNV)	Cowdry type A *intranuclear* inclusion bodies (IBs) in cells of ectodermal and mesodermal origin (Morales-Covarrubias and Chavez-Sanchez 1999)
4.	Hepatopancreatic disease	Family: *Parvoviridae* ssDNA hepatopancreatic parvovirus (HPV)	Single, prominent, basophilic, *intranuclear* inclusion bodies in the hypertrophied hepatopancreatic epithelial cells (Promjai et al. 2002)
5.	Yellowhead disease	Family: *Roniviridae*, ssRNA yellowhead/gill-associated virus/ lymphoid organ virus (YHV/GAV/LOV)	Basophilic, *intracytoplasmic*, Feulgen-positive inclusions in the lymphoid organs, interstitial tissues of the hepatopancreas, connective tissues underlying the midgut, cardiac tissues, hematopoietic tissues, hemocytes, and gill tissues (Chantanachookin et al. 1993)
6.	Taura syndrome	Family: *Dicistroviridae*, ssRNA Taura syndrome virus (TSV)	Eosinophilic then changes to basophilic, *intracytoplasmic*, Feulgen-negative inclusion bodies in the cells in areas of necrosis (Lightner et al. 1995; Lightner 1996; Hasson et al. 1999).
7.	Infectious myonecrosis	Family: *Totiviridae*, dsRNA infectious myonecrosis virus (IMNV)	*Perinuclear*, pale, basophilic to dark basophilic inclusion bodies are evident in muscle cells, connective tissue cells, hemocytes, and cells that comprise lymphoid organ spheroids (Lightner et al. 2004; Poulos et al. 2006)
8.	Monodon slow growth syndrome	Family: Luteoviridae(?), ssRNA Laem–Singh virus (LSNV)	LSNV is detected in the fasciculated zone and in onion bodies of the organ of Bellonci (Sritunyalucksana et al. 2006)
9.	Muscle necrosis disease	Family: *Nodaviridae*, ssRNA *Penaeus vannamei* nodavirus (PvNV)	*Perinuclear*, pale, basophilic inclusion bodies are evident in muscle cells, connective tissue cells, hemocytes, and cells that comprise lymphoid organ spheroids (Melena et al. 2012)
10.	White tail disease (WTD) or white muscle disease (WMD)	Family: *Nodaviridae*, RNA *Macrobrachium rosenbergii* nodavirus (MrNV) and its associate extra small virus (XSV)	Pathognomonic oval or irregular basophilic *intracytoplasmic* inclusion bodies are demonstrated in the target tissues by histology (Arcier et al 1999; Hsieh et al. 2006)

(continued)

Uncorrected Proof

Author's Proof

Sl. No.	Disease	Etiology	Inclusions
11.	Koi herpesvirus disease (KHVD)	Family: *Alloherpesviridae*, DNA herpesvirus	Eosinophilic *intranuclear* inclusions in branchial epithelial cells, leucocytes, kidney, spleen, pancreas, liver, brain, gut, and oral epithelium (Bergmann et al. 2006)
12.	Viral encephalopathy and retinopathy (VER) or Viral nervous necrosis (VNN)	Family: *Nodaviridae*, ssRNA piscine nodavirus of the genus *Betanodavirus*	*Intracytoplasmic* inclusion in nervous cells (Munday et al. 2002).
13.	Iridovirus infection	Family: *Iridoviridae*, dsDNA virus of genera *Lymphocystivirus* and *Ranavirus*	Basophilic *intracytoplasmic* inclusion bodies seen in liver, kidney, heart, pancreas, gastrointestinal tract, gill, and pseudobranch and positive indirect fluorescent antibody test – IFAT in spleen, heart, kidney, intestine, and gill (Jung et al. 1997)
14.	Epizootic hematopoietic necrosis	Family: *Iridoviridae*, dsDNA epizootic hematopoietic necrosis virus of genus *Ranavirus*	Basophilic *intracytoplasmic* inclusion bodies seen in liver, kidney, heart, pancreas, gastrointestinal tract, gill, and pseudobranch (Reddacliff and Whittington 1996)
15.	Infectious hematopoietic necrosis (IHN)	Family: *Rhabdoviridae*, ss RNA infectious hematopoietic necrosis virus	*Intracytoplasmic* inclusion bodies seen in hematopoietic tissues, kidney, spleen, liver, pancreas, and digestive tract (Wolf 1988; Bootland and Leong 1999)
16.	Spring viraemia of carp (SVC)	Family: *Rhabdoviridae*, spring viraemia of carp virus (SVCV), a species in the genus *Vesiculovirus*	*Intracytoplasmic* inclusion bodies seen in hematopoietic tissues, kidney, spleen, liver, pancreas, and digestive tract (Haghighi Khiabanian Asl et al. 2008)
17.	Viral hemorrhagic septicaemia (VHS)	Family: *Rhabdoviridae*, viral hemorrhagic septicaemia virus (VHSV) belonging to the genus *Novirhabdovirus*	*Intracytoplasmic* inclusion bodies seen in hematopoietic tissues, kidney, spleen, liver, pancreas, and digestive tract (Evensen et al. 1994)

Uncorrected Proof

Transmission or Scanning Electron Microscopy

It requires special methodology to be followed in fixing and processing of tissues for electron microscopy. Transmission electron microscopy (TEM) is very much useful and a great boon to diagnostic pathology to identify and determine the structure of an unknown virus that is characterized for the first time. This can also be used as a confirmatory test for the detection of already known virus or any intracellular parasites. Moreover, it is used in studying the ultrastructural changes during the progress of diseases. Scanning electron microscopy (SEM) is useful in identifying the surface level changes on the cell, and moreover it gives the structure of the cell as a whole in 3D view. The latest technology made scanning transmission electron microscope (STEM) as a dual-mode instrument by combination of both TEM and SEM principles. All of the images seen up to now provide information about the structure of a specimen, but it is also possible to analyze chemical composition of the particles by analytical electron microscopy (AEM) (Egerton 2005). AU4

Antibody-Based Assays

Antibody-based tests for pathogen detection using immune sera polyclonal antibodies

Author's Proof

(PAb's) or monoclonal antibodies (MAb's) can be used in fish disease diagnosis. Since crustaceans do not produce antibodies, antibody-based diagnostic tests are limited in their application to pathogen detection in shrimp diseases. Moreover, since crustacean viruses cannot be routinely produced in tissue culture, purified virus from infected hosts must be used to produce antibody. This has severely limited the development and availability of this diagnostic tool in shrimp disease diagnosis. Antibody-based diagnostic methods have been developed with mouse or rabbit antibodies generated to viruses purified from infected hosts. The recent application of MAb technologies to this problem has begun to provide a few antibody-based tests. MAb's are available for three of the OIE listed crustacean viruses such as TSV, IHHNV, and WSSV (Bondad-Reantaso et al. 2001).

Molecular Methods

Accurate, easy, and convenient availability of rapid and reliable diagnostic methods plays an important role in any disease control and health management programs in aquaculture. Treatment regime is well developed in human, animal husbandry, and agriculture for each and every specific disease, but it is still in growing phase in aquaculture. Proper early diagnosis is as good considered as treatment in aquaculture. So the molecular diagnostics based on polymerase chain reaction (PCR) principles have been extensively used to control the spread of major shrimp and fish pathogens (Ananda Raja et al. 2012), but they have the disadvantage of requiring sophisticated equipment and highly trained personnel. There are so many molecular diagnostics in aquaculture. It is appropriate to use well-proven, validated, and frequently used techniques. Recently, lateral flow chromatographic immunodiagnostic strips similar to common drugstore pregnancy tests have begun to appear for some shrimp diseases (Flegel et al. 2008). Using this kind of strips, unskilled farm personnel can easily diagnose shrimp or fish disease outbreaks at the pond side. The strips are relatively cheap and give an answer within 10 min.

Health Management in Aquaculture

The proverb "prevention is better than cure" is well suited to the health management in aquaculture. The disease prevention and control strategy is the best practice for successful hatchery and grow-out culture practices. Quarantine measures should strictly be adopted to import broodstock to avoid entry of existing or emerging pathogen. The following salient points are considered very important to get successful grow-out culture:

- Seasonal factors and crop planning based on the disease incidence.
- Ponds should be dried before starting the culture.
- Strict biosecurity measures should be adopted.
- Sieve should be used at water inlet, and the water should be bleached before stocking to weed out wild shrimp, fishes, and intermediate hosts.
- Good water quality should be maintained throughout the culture.
- Zero-water exchange or minimal-water exchange from reservoir ponds in case of shrimp culture.
- Disease-free stock should be used from good genetic strain of broodstock.
- Development and use of disease-resistant stocks will help in prevention of catastrophic disease outbreak and loss.
- Coastal Aquaculture Authority (CAA) guidelines should be followed for optimum shrimp stocking density in grow-out culture system.
- Quarantine measures should strictly be adopted to import broodstock to avoid entry of existing or emerging pathogen.
- Adequate balanced good nutrition to be made available to avoid problems associated with cannibalism and horizontal spread of diseases.
- Proper destruction and disposal of infected as well as dead animals to be regularly monitored.

Author's Proof

431 • Animals should be handled with good care to
432 avoid unwanted stress.
433 • Proper chemical prophylaxis and vaccine
434 development are needed for immunological
435 protection.
436 • Regulations are required to prevent transfer of
437 pathogens from one host population to
438 another, nationally or internationally.
439 • Sanitation and disinfection of hatchery and
440 equipments are to be strictly followed.
441 • Despite all the precautions, disease outbreak
442 may occur. Handling a disease outbreak with
443 least economic loss is an art of farm manage-
444 ment. Prompt action is essential in such
445 circumstances to rectify the problems, reduce
446 the losses, and minimize the impacts on
447 neighboring farms.
448 • Record keeping is necessary to identify
449 problems in the pond environment and animal
450 health and to rectify those problems at the
451 earliest during the production cycle. It also
452 helps the farmer to learn from the past.

453 ## References

454 Ananda Raja R, Panigrahi A, Kumar S (2012) Epidemio-
455 logical investigation of brackish water culture systems
456 in West Bengal, India. J Appl Aquac 24:49–59
457 Arcier JM, Herman F, Lightner DV, Redman RM, Mari J,
458 Bonami JR (1999) A viral disease associated with
459 mortalities in hatchery-reared post larvae of the giant
460 freshwater prawn *Macrobrachium rosenbergii*. Dis
461 Aquat Org 38:177–181
462 Bell TA, Lightner DV (1988) A handbook of normal
463 shrimp histology, Special publication no. 1. World
464 Aquaculture Society, Baton Rouge, 114 pp
465 Bergmann SM, Kempter J, Sadowski J, Fichtner D (2006)
466 First detection, confirmation and isolation of koi her-
467 pesvirus (KHV) in cultured common carp (*Cyprinus*
468 *carpio* L.) in Poland. Bull Eur Assoc Fish Pathol
469 26:97–104
470 Bondad-Reantaso MG, Mcgladdery SE, East I,
471 Subasinghe RP (2001) Asia diagnostic guide to
472 aquatic animal diseases, FAO fisheries technical
473 paper 402, supplement 2. FAO, Rome, 240 pp
474 Bootland LM, Leong JC (1999) Infectious hematopoietic
475 necrosis virus. In: Woo PTK, Bruno DW (eds) Fish
476 diseases and disorders, vol 3: viral, bacterial and fun-
477 gal infections. CAB International, Oxon, pp 57–121
478 Bruno DW (1996) Cold water vibriosis caused by *Vibrio*
479 *salmonicida*, Aquaculture information series no. 15.
480 The Scottish Office Agriculture, Environment and
481 Fisheries Department. Marine Laboratory, Aberdeen
482 Chantanachookin C, Boonyaratpalin S, Kasornchandra J,
483 Direkbusarakom S, Aekpanithanpong U,
484 Supamattaya K, Sriuraitana S, Flegel TW (1993) His-
485 tology and ultrastructure reveal a new granulosis-like
486 virus in *Penaeus monodon* affected by yellow-head
487 disease. Dis Aquat Org 17:145–157
488 Drinan EM (1985) Studies on the pathogenesis of furun-
489 culosis in salmonids. Ph.D. thesis, National University
490 of Ireland, Dublin
491 Egerton RF (2005) Physical principles of electron micros-
492 copy. An introduction to TEM, SEM, and AEM.
493 Springer, New York, 202 pp
494 Evensen Ø, Meier W, Wahli T, Olesen NJ, Jørgensen
495 PEV, Håstein T (1994) Comparison of immunohisto-
496 chemistry and virus cultivation for detection of viral
497 haemorrhagic septicaemia virus in experimentally
498 infected rainbow trout *Oncorhynchus mykiss*. Dis
499 Aquat Org 20:101–109
500 Flegel TW, Lightner DV, Lo CF, Owens L (2008) Shrimp
501 disease control: past, present and future. In: Bondad-
502 Reantaso MG, Mohan CV, Crumlish M, Subasinghe
503 RP (eds) Diseases in Asian aquaculture VI. Fish
504 Health Section, Asian Fisheries Society, Manila, pp
505 355–378, 505 pp
506 Haghighi Khiabanian Asl A, Azizzadeh M,
507 Bandehpour M, Sharifnia Z, Kazemi B (2008) The
508 first report of SVC from Indian carp species by PCR
509 and histopathologic methods in Iran. Pak J Biol Sci
510 11:2675–2678
511 Hasson KW, Lightner DV, Mohney LL, Redman RM,
512 Poulos BT, White BL (1999) Taura syndrome virus
513 (TSV) lesion development and the disease cycle in the
514 Pacific white shrimp *Penaeus vannamei*. Dis Aquat
515 Org 36:81–93
516 Hsieh CY, Wu ZB, Tung MC, Tu C, Lo SP, Chang TC,
517 Chang CD, Chen SC, Hsieh YC, Tsai SS (2006) In situ
518 hybridization and RT-PCR detection of
519 *Macrobrachium rosenbergii* nodavirus in giant fresh-
520 water prawn, *Macrobrachium rosenbergii* (de Man),
521 in Taiwan. J Fish Dis 29:665–671
522 Jung S, Miyazaki T, Miyata M, Danayadol Y, Tanaka S
523 (1997) Pathogenicity of iridovirus from Japan and
524 Thailand for the red sea bream *Pagrus major* in
525 Japan, and histopathology of experimentally infected
526 fish. Fish Sci 63:735–740
527 Lester RJG, Doubrovsky A, Paynter JL, Sambhi SK,
528 Atherton JG (1987) Light and electron microscope
529 evidence of baculovirus infection in the prawn
530 *Penaeus plebejus*. Dis Aquat Org 3:217–219
531 Lightner DV (1988) Diseases of cultured penaeid shrimp
532 and prawns. In: Sindermann CJ, Lightner DV (eds)
533 Disease diagnosis and control in North American
534 marine aquaculture. Elsevier, Amsterdam, pp 8–127
535 Lightner DV (1996) A handbook of shrimp pathology and
536 diagnostic procedures for diseases of cultured Penaeid
537 shrimp. World Aquaculture Society, Baton Rouge,
538 304 pp

Uncorrected Proof

Author's Proof

539 Lightner DV, Redman RM, Hasson KW, Pantoja CR
540 (1995) Taura syndrome in *Penaeus vannamei* (Crusta-
541 cea: Decapoda): gross signs, histopathology and ultra-
542 structure. Dis Aquat Org 21:53–59
543 Lightner DV, Pantoja CR, Poulos BT, Tang KFJ, Redman
544 RM, Pasos De Andrade T, Bonami JR (2004) Infec-
545 tious myonecrosis: new disease in Pacific white
546 shrimp. Glob Aquac Advocate 7:85
547 Melena J, Tomala J, Panchana F, Betancourt I,
548 Gonzabay C, Sonnenholzner S, Amano Y, Bonami
549 J-R (2012) Infectious muscle necrosis etiology in the
550 Pacific white shrimp (*Penaeus vannamei*) cultured in
551 Ecuador. Braz J Vet Pathol 5:31–36
552 Momoyama K, Hiraoka M, Nakano H, Koube H,
553 Inouye K, Oseko N (1994) Mass mortalities of
554 cultured kuruma shrimp, *Penaeus japonicus*, in Japan
555 in 1993: histopathological study. Fish Pathol
556 29:141–148
557 Morales-Covarrubias MS, Chavez-Sanchez MC (1999)
558 Histopathological studies on wild broodstock of
559 white shrimp *Penaeus vannamei* in the Platanitos
560 area, adjacent to San Blas, Nayarit, Mexico. J World
561 Aquac Soc 30:192–200
562 Munday BL, Kwang J, Moody N (2002) Betanodavirus
563 infections of teleost fish: a review. J Fish Dis
564 25:127–142
AU6 565 Noga EJ (2010) Fish disease diagnosis and treatment, IIth
566 edn. Wiley-Blackwell, Ames, 519 pp
567 Poulos BT, Tang KFJ, Pantoja CR, Bonami JR, Lightner
568 DV (2006) Purification and characterization of infec-
569 tious myonecrosis virus of penaeid shrimp. J Gen
570 Virol 87:987–996
Promjai J, Boonsaeng V, Withyachumnarnkul B, Flegel 571
TW (2002) Detection of hepatopancreatic parvovirus 572
in Thai shrimp *Penaeus monodon* by in situ 573
hybridization, dot blot hybridization and PCR ampli- 574
fication. Dis Aquat Org 51:227–232 575
Reddacliff LA, Whittington RJ (1996) Pathology of epi- 576
zootic haematopoeitic necrosis virus (EHNV) infec- 577
tion in rainbow trout (*Oncorhynchus mykiss* 578
Walbaum) and redfin perch (*Perca fluviatilis* L.). 579
J Comp Pathol 115:103–115 580
Roberts RJ (2012) Fish pathology, IVth edn. Wiley- 581
Blackwell, Hoboken, 590 pp 582
Snieszko SF (1974) The effects of environmental stress on 583
outbreaks of infectious diseases of fishes. J Fish Biol 584
6:197–208 585
Sritunyalucksana K, Apisawetakan S, Boon-nat A, 586
Withyachumnarnkul B, Flegel TW (2006) A new 587
RNA virus found in black tiger shrimp *Penaeus* 588
monodon from Thailand. Virus Res 118:31–38 589
Vogt G (1992) Transformation of anterior midgut and 590
hepatopancreas by monodon baculovirus (MBV) in 591
Penaeus monodon postlarvae. Aquaculture 592
107:239–248 593
Wolf K (1988) Infectious hematopoietic necrosis. In: Fish 594
viruses and fish viral diseases. Cornell University 595
Press, Ithaca, pp 83–114 596
Wongteerasupaya C, Vickers JE, Sriurairatana S, Nash 597
GL, Akarajamorn A, Boonsaeng V, Panyim S, 598
Tassanakajon A, Withyachumnarnkul B, Flegel TW 599
(1995) A non-occluded, systemic baculovirus that 600
occurs in cells of ectodermal and mesodermal origin 601
and causes high mortality in the black tiger prawn 602
Penaeus monodon. Dis Aquat Org 21:69–77 603

Uncorrected Proof

Author's Proof

Author Queries

Chapter No.: 23 0002270968

Queries	Details Required	Author's response
AU1	Please confirm author affiliation.	
AU2	Please check if edit to sentence starting "Confirmatory diagnosis of..." is okay.	
AU3	Please check if "thiosulfate-citrate-bile sucrose agar" should be changed to "thiosulfate–citrate–bile salt–sucrose agar."	
AU4	The reference citation Ray (2005) has been changed to Egerton (2005). Please check if appropriate.	
AU5	Please confirm inserted publisher location for the references Noga (2010), Roberts (2012).	
AU6	Please confirm the publisher's name in Noga (2010).	

Uncorrected Proof