

laboratory should check with the client whether they want the health report sent directly to the transfer licensing authorities or the client will send a copy with their request. Copies of the diagnostic report should be kept on file as well as with the specimen data sheets.

## 2.4 HEALTH ZONATION

### Introduction

The concept behind zonation is to establish a reference health profile for a geographic area or facility which can be used as the basis for monitoring any changes in the health status of the culture stock and/or to assess the risk of disease transfers. This method of health management is recommended by the OIE (2006) and is applied on a regional basis for shellfish aquaculture in Canada, parts of the United States of America and the European Union. Stock from facilities or areas with identical health profiles present negligible health risks compared with those with different health profiles. Land-based facilities can be given a health status based on isolation from surrounding facilities. Since this is harder to assume with open-water, health zonation can be conducted on a geographic/hydrographic basis. With pearl oyster farms, the facility-based concept may apply more readily than with other mollusc culture systems, since there is little direct exchange between farms and the number of farms in any given area is usually limited. Where neighbouring farms undertake stock exchange (seedstock, broodstock, etc.), the zonation approach may be more applicable.

### Facility-based health status

In order to establish an accurate health profile, a facility (hatchery, culture production) should have at least four (4) consecutive health checks over a period of 18-36 months and at periods most likely to detect disease agents (e.g. post-transportation, post-spawning, post-surgery). Fewer health checks may be required for land-based facilities with a sterile water supply. Separate samples should be examined for different year-classes, species and stocks from different sources (unless they have been mixed). Numbers of oysters which need to be examined will vary with the number being held at the facility but should comply as closely as possible with 95 percent confidence of detecting an infectious organism at 2 percent prevalence, i.e. a maximum sample size of 150 animals (see Annex 2.3). Once the consecutive checks have been completed, a health status report can be compiled for the facility site. This can be used for requests to transfer stocks to other facilities or to assess risks from importations from other sites or facilities (based upon their health status records, if available).

### Geographic area

The same protocol for establishing a health profile can be applied to a geographic area. This is usually applied to discrete bays or areas with a well-established exchange of oysters, e.g. collection of local source stock or seedstock from a local hatchery, or hydrographic water exchange. Four consecutive health checks are recommended and samples of wild stock should consist of 150 oysters (unless stocks are limited or considered to be endangered). Where several facilities fall within a single geographic zone, the growers may decide to provide broodstock from one farm, seedstock from another, etc. The results from each can then be pooled to provide the health status for the zone.

### Health status maintenance

Following establishment of a health status profile for a facility or geographic zone, this can be maintained by annual samples of stocks.

## 2.5 DISEASE OUTBREAK INVESTIGATION PROCEDURE

### Introduction

A systematic procedure to help identify the cause or causes of disease events and prevent the further transmission of the pathogen is required to control an ongoing and to prevent future disease events. Any disease outbreak investigation will strongly benefit from using an epidemiological approach which assesses the pattern of infection within the affected population (Lilley *et al.*, 1998).

Lilley *et al.* (1998) outlined the nine basic steps to a disease outbreak investigation developed for investigating outbreak of epizootic ulcerative syndrome (EUS) of fresh and brackishwater finfish in Asia. This approach is also applicable to pearl oyster health disease investigations. The nine steps include: (1) establishing a diagnosis, (2) establishing a “case definition”, (3) confirming that an outbreak is actually occurring, (4) characterising the outbreak in terms on time, affected/unaffected species, and place, (5) analysing the data, (6) developing a working hypothesis, (7) making intensive follow-up with different laboratory testing and further epidemiological analysis, (8) establishing control and prevention measures and (9) reporting. These nine steps may not necessarily be included in every investigation, nor will an investigation always follow the same sequence; they may be undertaken simultaneously.

One of the most important criteria to establish, as quickly as possible, is whether or not a disease outbreak is due to an infectious agent or due to stress and/or secondary opportunistic infection. This can be achieved by sending samples of affected and unaffected oysters to a diagnostic laboratory as soon as abnormal mortalities are detected. Since some tests may take weeks, some interim control measures will be required pending diagnostic results.

A careful record of the pattern of mortalities may assist in determining the nature of the problem (although not the cause). Generally-speaking, environmental stress-related mortalities may affect animals throughout the stock. Stocking density effects will tend to cause mortalities at the centre of the cultured population, with peripheral lines/cages showing relatively little effect. Toxins or pollutants may cause the same pattern of mortality or manifest themselves as acute mortalities throughout the stock. Infectious disease agents will show a progressive pattern of mortality spreading between neighbouring lines or nets.

### Diagnostic submission

As soon as abnormal rates of mortality are detected, samples of moribund (not dead) and unaffected (if available) oysters should either be preserved on site (see Section 2.2.1) or submitted live to a diagnostic laboratory with molluscan shellfish health expertise. If there is evidence of a microbial (bacterial or fungal) infection, fresh rather than preserved samples should be sent to the laboratory. Phone/Fax the laboratory in advance to let them know the nature of the problem, determine the number of samples they need and ensure that they are prepared for the diagnostic case. The laboratory may need to prepare specialized media for tissue culture and fixatives.

### Quarantine (isolation)

Quarantine is a holding method which prevents the escape or release of shellfish and any of their disease agents. Different periods of quarantine may be required depending on the disease. Quarantine is also applied to shellfish being introduced to a site from an area where there is a disease agent of concern or no health history information (see Section 2.2.1). Quarantine, by definition, is therefore difficult to manage in open-water.

Isolation is used to prevent infection by organisms from the surrounding environment. This may occur within a site where there is a disease outbreak and unaffected stocks are placed under isolation (while the diseased stock fall within quarantine). Isolation can be conducted under two different conditions:

- *Land-based isolation* prevents access to the shellfish by other shellfish or associated disease agents from surrounding waters. Although shellfish may not be introduced to an isolation facility or site, they can be released into the surrounding waters.
- *Geographic isolation* is the spatial separation of shellfish from neighbouring shellfish beds or sites.

### Disinfection

Disinfection involves the application of chemical treatments at sufficient concentrations, and for sufficient periods of time, to kill or inactivate harmful organisms. Since the inherent toxicity of disinfectants negates safe use in open-water, or flow-through systems, disinfection can only be applied with reasonable control within hatcheries, tanks or land-based holding facilities. Disinfectants must be neutralised before release into the surrounding environment, especially seawater treatments, which produce residual oxidants which are particularly toxic to bivalves (OIE, 2007).

If a disease agent is detected, disinfection of tanks, nets and equipment is recommended. Stock infected by an opportunistic pathogen may be destroyed, or transferred following surface disinfection to clean holding tanks at lower stocking densities, water temperatures, and with new food sources.

Where stocking densities are high, shellfish should be rotated between disinfected tanks as frequently as practical. Each new batch of shellfish introduced to a facility should be placed in pre-disinfected tanks. Because the presence of organic matter will reduce the disinfection capacity of most disinfectants, filtering influent water is recommended. In addition, all surfaces should be thoroughly cleaned prior to disinfection. The detergent used for this purpose should be compatible with the disinfectant and both should be compatible with the surface being treated. All waste from washing should be disinfected before disposal. Regular air- or heat-drying of pipelines (daily), tanks and other equipment (e.g. algal culture carboys), in addition to disinfection of their surfaces, is also recommended. All chemical treatments should be undertaken in a manner that prevents their release into the surrounding environment.

### Treatments

Chlorine is usually applied as sodium hypochlorite (Chlorox<sup>®</sup>, household bleach, etc.). Fill all pipelines with 50 ppm chlorine (= 50 mg/l). Allow an exposure time of at least 30 minutes before flushing with clean seawater. This solution is effective against most microbial agents as well as labyrinthid protozoans. Chlorinated seawater should be neutralised prior to release from the holding facility.

Iodophors are applied as alkaline solutions (Wescodyne<sup>®</sup>, Betadine<sup>®</sup>), at an iodine (I<sub>2</sub>) concentration of 200-250 ppm, for a contact time of at least 10 minutes, however, they are not effective against certain protists (Bower, 1989) and may need to be supplemented by air- or heat-drying of tank surfaces and pipelines.

### Testing treatments

Following a disease outbreak, or the initial set-up of a disinfection system and prior to introducing fresh shellfish stock or flushing effluent from the facility, routine bacteriological culture of disinfected surfaces and water for ubiquitous bacteria (such as *Vibrio* spp. and *Pseudomonas* spp.) should be performed. If present, this indicates that the disinfection procedure used was inadequate.

### Residual monitoring

Residual or free chlorine (or other halogen) ions are indicative of the degree of binding to/ oxidation of organic compounds or organisms in the water being treated. If no residual oxidizing ions remain after a set period of treatment, it is assumed that:

- inadequate disinfectant was administered; and
- viable pathogens may have survived the treatment.

The baseline for most disinfection facilities is a free residual chlorine (or equivalent oxidizing ion) level of 5 ppm, following water treatment for a minimum of 10 min. However, the baseline for effective disinfection varies significantly between facilities, depending on water salinity, temperature, turbidity, volume, organic content, pH, flow-rate, etc. Establishing the concentration and contact time for the type of disinfectant chosen requires pre-testing prior to release of the water into the surrounding environment.

### Pre-testing procedure

Treat water with varying concentrations of an appropriate disinfectant (chlorine, iodophor, peroxide, ozone or ultra violet) and graph residual oxidant concentrations after 10, 15, 30, 45 and 60 sec and after 5, 10, 15, 30 and 45 min:

- If concentrations decrease to < 5 ppm within 10 min of water treatment, the concentration or type of disinfectant is ineffective.
- If concentrations are > 5 ppm after 10 min but continue to decrease after the 30 min interval, disinfection is continuing and the starting concentration should be increased or use another type of disinfection because viable pathogens may still be present after 30 min.
- The disinfectant concentration which results in a point where residual oxidant concentrations begin to stabilize at >5 ppm (and can be increased with additional disinfectant) represents the concentration required for disinfection.

### Sterile waste disposal

Both chlorine and ozone produce long-lived residual oxidant compounds in seawater. Seawater at 35 ppt salinity contains 60 ppm bromide ion which produces hypo-bromite in the presence of ozone. Disinfected artificial seawater, at the same salinity, produces bromine and hypobromous acid. Since these are toxic to larval oysters, treated seawater should be passed through an activated charcoal filter before being released or used for live mollusc larvae.

Reducing agents such as sodium thiosulphate or aeration may also be used for halogen neutralisation, but these do not remove toxic chloramines and are not recommended for seawater facilities.

A log of neutralisation of disinfection procedures and monitoring results is highly recommended for ensuring that neutralisation is adequate to prevent negative environmental impacts.

### Chemotherapeutants

To date, there are no chemotherapeutants which are recommended for use in open-water or flow-through mollusc farms. Although effective antibiotics are available, their prophylactic use or use against a build up of opportunistic microbes is not recommended due to the risk and consequences of antibiotic resistance.

### Personnel

Only authorised personnel should be allowed contact with shellfish that are subject to disease investigation. Entry-exit points should be made secure against unauthorized access.

### Log-keeping

Log-books should be kept during a disease investigation to record:

- entry-exit times of authorized personnel
- shellfish mortalities (date, time, method of disposal)
- disinfection administration

- residual levels and neutralisation
- samples sent to laboratories for disease diagnosis.

### Investigation

Mortalities with no immediately apparent cause may take years to investigate, depending on the epidemiology of infection. This means that disease control measures must be balanced in terms of cost and practicality with the continued operation of the farm. Disease control measures and research plans are most effective where the farm management and personnel actively participate. The latter have the day-to-day contact with the shellfish which provides the information essential for accurate interpretation of disease observations and experimental results.

## 2.6 NATIONAL STRATEGIES ON AQUATIC ANIMAL HEALTH

In recent years, countries are developing and implementing “national strategies” on aquatic animal health management (AFFA, 1999; Olivier, 2004; Kanchanakhan and Chinabut, 2004; Amos, 2004). In Asia, the development of the “national strategy” was an outcome of an FAO-supported regional technical cooperation project on “health management for the responsible movement of live aquatic animals” (Bondad-Reantaso, 2004). The “National Strategy on Aquatic Animal Health” contains the actions plans of the government at the short-, medium, and long-term to implement the Asia Regional Technical Guidelines for the Responsible Movement of Live Aquatic Animals (FAO/NACA, 2000). The essential components (or elements) of the “national strategy on aquatic animal health” include the following: (i) policy, legislation and enforcement, (ii) pathogen list and information system, (iii) diagnostics, (iv) health certification and quarantine, (v) risk analysis for aquatic animal movement, (vi) surveillance, monitoring and reporting, (vii) zoning, (viii) response and contingency plans to disease emergencies, (ix) research, (x) institutional structure, (xii) human resources development and (xiii) regional and international cooperation.

There are varying processes involved in the development of the “national strategy”. In some countries, the take-off of national strategy development was immediate; for some others, it went through a slow process for various reasons. Although not aimed specifically at diseases of pearl oysters, the development of such strategies is a valuable framework for enhancing basic health management and biosecurity education and awareness for aquatic animal health at both a national and regional levels. Experience in Asia provided some essential considerations for ensuring success in developing and implementing a “national strategy”. These include: (i) strong national coordination, (ii) a good driver for the process (through the Competent Authority, a commission, a committee, a task force, or a focal person) with clear terms of reference, (iii) needs assessment and prioritization exercise, (iv) active stakeholder consultation, (v) approval from highest authority, (vi) an implementation strategy, (vii) a monitoring and review plan, (viii) development of proposal for addressing the different component (elements) of the national strategy and (ix) resource and funding allocation.

Development and implementation of national strategies on aquatic animal health within the broader aquaculture development and biosecurity framework should be continuously pursued. The national strategy is comprehensive enough, and using the concept of “*phased implementation based on national needs*”, it could provide a good and strong entry point for capacity building for many countries regardless of aquatic animal health infrastructure or economic development.

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**ANNEX 2.1**

**Shellfish Health Questionnaire**

Name and address of client \_\_\_\_\_  
\_\_\_\_\_

Contact telephone number \_\_\_\_\_ Fax \_\_\_\_\_ E-mail \_\_\_\_\_

Reason for submission:

Stock transfer (indicate from where to where) \_\_\_\_\_

Abnormal mortalities (give approx. percentage of losses) \_\_\_\_\_

Other \_\_\_\_\_

Growing conditions:

Growing Method \_\_\_\_\_

Water temperature \_\_\_\_\_ Salinity \_\_\_\_\_ Turbidity \_\_\_\_\_

Other environmental observations (e.g. predators, high rainfall, wave action, pollution, etc.) \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

General observations on the stock being submitted:

Appearance (gaping, shell damage, fouling, etc.) \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Behaviour (feeding, growth, maturation, movements, etc.) \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Recent handling history of the stock or neighbouring stocks \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Comments

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_



**ANNEX 2.3**

**Sample sizes needed to detect at least one infected host in a population of a given size, at a given prevalence of infection, with 95 percent confidence (Ossiander and Wedermeyer, 1973)**

Population size	Prevalence (%)						
	0.5	1.0	2.0	3.0	4.0	5.0	10.0
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
1000	448	256	136	92	69	55	27
2500	512	279	142	95	71	56	27
5000	562	288	145	96	71	57	27
10000	579	292	146	96	72	29	27
100000	594	296	147	97	72	57	27
1000000	596	297	147	97	72	57	27
>1000000	600	300	150	100	75	60	30



## ANNEX 2.5

### Histology qualitative scales

#### Gonad maturity

- 0 – no gametes (resting or immature)
- 1 – developing gametes attached to gonoduct epithelium (ripening)
- 2 – gametes filling gonoducts (mature)
- 3 – evidence of loss of mature gametes (spawning)
- 4 – residual mature gametes (post-spawning)
- 5 – gamete resorbtion by haemocyte infiltration (recovery)
- 6 – respawning resorbtion (spawning failure)

#### Gut content

- 0 – none
- 1 – small amounts of food in one or other intestinal look
- 2 – food in both intestinal loops or one intestinal loop plus partial stomach
- 3 – food in both intestinal loops and stomach

#### Infiltration

- 0 – no haemocytes present in the connective tissue (rare)
- 1 – haemocytes 1–2 cells deep around the intestine and stomach epithelia
- 2 – thick layer of haemocytes around the stomach and intestine, +/- small numbers of focal aggregations in the connective tissue
- 3 – systemic infiltration of haemocytes throughout the connective tissue

#### Diapedesis

- 0 – none
- 1 – minor diapedesis across the ood duct or intestine wall
- 2 – diapedesis across several epithelial borders
- 3 – extensive diapedesis across stomach, intestine and duct epithelia

#### Metaplasia

- 0 – none
- 1 – < half the tubules with flattened epithelia
- 2 – > half the tubules with flattened epithelia
- 3 – almost all tubules with flattened epithelia
- 4 – chronically flattened epithelia

#### Adipogranular storage tissue

- 0 – none
- 1 – small deposits in mantle only
- 2 – large amounts throughout the mantle
- 3 – mantle and peripheral digestive gland connective tissue
- 4 – mantle and throughout the digestive gland
- 5 – throughout the connective tissue of the whole tissue section.

**Ceroid (connective tissue)/concretions (digestive tubule epithelia)**

- 0 – none
- 1 – minor accumulation in connective tissue/digestive tubules
- 2 – moderate accumulation
- 3 – heavy accumulation

**Necrosis**

- 0 – none
- 1 – focal (limited)
- 2 – focal (moderate)
- 3 – systemic
- 4 – systemic + saprobionts