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Final report

Small research and development activity

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1 Acknowledgments

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2 Executive summary

The Australian Centre for International Agricultural Research (ACIAR) through Project FIS 2005/114: "*Building bivalve hatchery production capacity in Vietnam and Australia*" has established hatchery-based bivalve mollusc seed production at Cat Ba Island. This has facilitated the development of small scale bivalve culture businesses in Vietnam through the selection of suitable bivalve species and provision of nursery facilities. Central to continued industry operation and expansion is optimising molluscan health - this is predicated on having in place appropriate biosecurity and disease diagnostic capacity. While there has been advances made through the ACIAR project in bivalve hatchery production in Vietnam, the capacity to diagnose disease and to implement biosecurity measures has until now not been clearly assessed.

This report describes a project which had two primary objectives:

1) To evaluate current diagnostic capacity for bivalve molluscs at the Research Institute of Aquaculture (RIA) No.1, and identify additional diagnostic requirements necessary to develop or implement applied disease diagnosis to support regional biosecurity; and
2) To evaluate current biosecurity for bivalve molluscs, considering the effectiveness of current measures being taken and provide recommendations for functional and operational improvements in hatchery management and design for the control or prevention of molluscan diseases.

Six specific tasks were undertaken to address these objectives. The specific tasks were to:

1. Conduct a review of the diseases of molluscs in Vietnam;
2. Assess the legislative and regulatory framework supporting disease diagnosis (and control);
3. Assess the molluscan diagnostic capacity at RIA No.1 (Facilities and Resources);
4. Assess the diagnostic capabilities of RIA No. 1: Scientific, technical and hatchery staff: molluscan diseases;
5. Assess internal and external biosecurity strategies at RIA No.1;
6. Assess human resources supporting regional biosecurity at RIA No.1.

A three staged process was used, comprising firstly of information collection and review, then an in-country assessment of biosecurity and laboratory diagnostic capacity, and finally the provision of recommendations based on conclusions drawn from first two stages of the investigation.

The following conclusions were identified in this project:

1. Knowledge relating to the occurrence and distribution of diseases of molluscs in Vietnam, at least in the English language, is rudimentary and fragmented particularly prior to 2000. However, the important molluscan pathogens *Perkinsus, Haplosporidium* and *Marteilia* have been reported in the English language as having been identified in Vietnam, although there is little information concerning the disease states with which these agents have been associated. Major disease outbreaks in farmed hard clams, snout clams, edible oysters and in hatchery-reared oyster spat reportedly have occurred but with little information that confirms what the cause of these outbreaks has been.
2. Legislation and regulation exists with respect to diagnosis and control of disease in Vietnam but there appeared to be inconsistencies with apparently different regulatory requirements for Government institutions and private companies. A list of notifiable diseases exists, but discussions indicated that breaches of legislation and regulation occur, especially in relation to illegal imports of live aquatic animals. The Department of Animal Health (DAH) within the Ministry of Agriculture and

Rural Development is responsible for reporting and notification of disease events and has primary responsibility for health certification measures, but the operational relationship between the DAH and the Research Institutes for Aquaculture in the implementation of regulatory requirements for disease investigation and control remains uncertain. No veterinary input into the diagnostic and disease control processes, at least in the RIAs, was apparent.

3. Laboratory infrastructure and resources at RIA No. 1 would allow for basic diagnostic investigations in molluscs. There were a number of factors identified that could potentially compromise diagnostic capacity include the reliance on overseas funded grants for purchase or replacement of equipment, the absence of a laboratory procedures manual, an absence of reference cultures and other materials including reference histopathological sections and difficulties in accessing relevant scientific publications.
4. Scientific and technical staff at RIA No.1 were responsible to the Head of the laboratory who is well qualified. In many cases staff had shared roles between different laboratory areas but it was not possible to directly assess the competency of staff. There appeared to be little veterinary input into the assessment of clinical disease, epidemiology or the diagnostic process by staff in examining samples submitted for laboratory examination. It was of concern that there appeared an absence of formal training in keys areas such as histopathology, which meant that the reliability and/or interpretation of diagnostic findings was questionable.
5. A deficiency of biosecurity measures at the national, regional and local level was apparent. Specifically, no biosecurity measures for finfish or molluscs at the National hatchery or broodstock holding facilities for molluscs and finfish could be identified. There was an absence of sample collection protocols, apparently unregulated sourcing of broodstock, inconsistencies between Government and private hatcheries with respect to health certification, despatch of seed-stock, entry of visitors to facilities, use of protective clothing and a general lack of internal quarantine. In contrast to the mollusc and finfish hatcheries, the adjacent shrimp facility demonstrated a high standard of biosecurity.
6. An awareness of a need for biosecurity measures to limit or contain the spread of disease and to ensure the distribution of seed-stock in optimal health was apparent in senior staff but the implementation of this was not evident at the time of the visit. Hatchery staff exhibited enthusiasm for their work and appeared receptive to new ideas.

A total of 14 recommendations were made in this project:

1. A central database should be developed and maintained as a basis for defining the contemporary disease status of molluscs in Vietnam. The database, preferably in English and Vietnamese should provide information pertaining to the occurrence, nature and distribution of diseases of molluscs throughout Vietnam and be fully referenced;
2. The cause or causes of the losses in oysters, hard clams and otter clams requires urgent investigation;
3. Expanding the review of the current legislation and regulations pertaining to disease diagnosis and the control of disease in molluscs in Vietnam, by identifying, and having translated into English, Vietnamese government documents relevant to this issue;
4. Clarification of the operational relationships between the Department of Animal Health and the Research Institutes for Aquaculture;
5. A review of the functionality of laboratory equipment essential for the accurate and prompt diagnosis of diseases of molluscs should be conducted and equipment found to be non-operational should be replaced;
6. To improve the reliability of diagnostic findings, a system of quality assurance and quality control based on known and unknown samples should be introduced into diagnostic laboratories;

7. A system to evaluate the diagnostic competence of scientific and technical staff involved in diagnostic examinations on diseased molluscs is necessary as a basis for identifying further training needs in specific diagnostic disciplines;
8. Following on from Recommendation (7), specific training in those diagnostic disciplines for which gaps existed is required;
9. In addition, training in comparative histopathology is urgently needed, focussing on the histopathological diagnosis of molluscan diseases;
10. The development, implementation and maintenance of fundamental biosecurity measures;
11. Training workshops for hatchery staff should be undertaken to identify and describe fundamental, practical biosecurity measures which may be introduced into the operations of the mollusc hatchery and broodstock facility.
12. Following on from Recommendations (9) and (10), after the establishment of biosecurity measures, biosecurity audits should be conducted at least annually in both hatchery and broodstock facilities;
13. Sourcing of broodstock should be done from areas of known health status with health examinations of broodstock and quarantine as appropriate;
14. All disease events occurring within the hatchery, either in broodstock or seedstock should be fully investigated.

3 Introduction

Progress in the Australian Centre for International Agricultural Research (ACIAR) Project FIS 2005/114: "*Building bivalve hatchery production capacity in Vietnam and Australia*" has addressed many constraints to the development of small scale bivalve culture businesses in Vietnam. This has been achieved through the establishment of reliable hatchery-based seed production capacity and has highlighted challenges and further opportunities for research and capacity building in northern Vietnam. Specifically the program has:

- Fostered the development of the bivalve hatchery facility at Cat Ba Island;
- Established the knowledge base required for the selection of suitable species and their hatchery production; and
- Assisted the establishment of nursery facilities to bridge the gap between hatchery production and the provision of suitable sized seed to farmers.

In an international environment in which oyster pathogen ranges are expanding (for example, the recent occurrence of Ostreid herpesvirus in Australia and New Zealand in 2010) and a local environment in which unexplained oyster mortality and other unexplained diseases of molluscs have occurred, (for example, mass mortality of triploid Pacific oyster seed in Ha Long Bay, North Vietnam, 2011), there is a clear need to identify and address knowledge gaps and shortfalls to protect existing production from disease and provide assurances for expansion.

The maintenance of molluscan health is central to continued industry operation/expansion and is predicated on:

(1) Biosecurity to successfully exclude pathogens; and

(2) In the event of disease, the capacity to rapidly identify the causative agents as a basis for implementing effective control measures.

Despite the advances in bivalve hatchery production in Vietnam, the capacity to diagnose disease and implement biosecurity measures have not been clearly assessed

As part of FIS-2005/114, the project described here was undertaken to evaluate diagnostic capacity and hatchery based biosecurity measures with respect to diseases of molluscs. This was done as a basis for reinforce the technological progress made within FIS 2005/114, focussing principally on increased institutional biosecurity and veterinary diagnostic capacity. Drs Paul Hardy-Smith and John Humphrey, of Panaquatic Health Solutions Pty Ltd ("Panaquatic") undertook the project in collaboration with Dr Wayne O'Connor, Principal Research Scientist, Industry and Investment NSW.

Two primary objectives were defined in the project to evaluate molluscan biosecurity and disease diagnostic capacity in Vietnam:

Objective 1: Evaluate current diagnostic capacity at RIA No.1 in a national and regional context focusing generally on the diagnosis of diseases of aquatic animal and specifically on the diagnosis of molluscan diseases, and identify additional diagnostic requirements necessary to develop or implement applied disease diagnosis at RIA No.1 to support regional biosecurity, with particular emphasis on diseases of molluscs.

Objective 2: Evaluate current biosecurity for aquatic animals generally and molluscan diseases specifically at RIA No.1 in a local, regional and national context specifically and consider the effectiveness of current measures being taken. Determine whether there is a need to improve current biosecurity in order to reduce risk of aquatic animal disease. If a need is identified, provide

recommendations for functional and operational improvements in hatchery management and design for the control or prevention of molluscan diseases.

To meet these objectives, six specific tasks were undertaken as follows:

Task 1: A review of the diseases of molluscs in Vietnam;

Task 2: An assessment of legislative and regulatory framework supporting disease diagnosis (and control);

Task 3: An assessment of diagnostic capacity at RIA No.1 (Facilities and Resources);

Task 4: An assessment of diagnostic capabilities of RIA No. 1: Scientific, technical and hatchery staff: Molluscan diseases;

Task 5: An assessment of internal and external biosecurity strategies at RIA No.1;

Task 6: An assessment of human resources supporting regional biosecurity at RIA No.1.

This report details the findings of each of these tasks and makes recommendations regarding improving biosecurity measures and diagnostic capacity for molluscan diseases in Vietnam.

Precautionary Note:

It should be pointed out that this report has not been reviewed by any members of the RIA No. 1 or RIA No.3 with whom the Project Leaders were fortunate to spend time with. While considerable effort has been spent in ensuring the information presented in this report is accurate, details on some of the issues discussed may unintentionally have been inaccurately reported due to translation issues and simply due to the fact that health reports available in Vietnamese but not in English were not accessible, where such reports may exist.

4 Approach

A three-staged approach was undertaken to determine biosecurity and veterinary diagnostic capacity relating to molluscs in Vietnam. This approach was implemented as follows:

Stage 1: Information Collection and Review;

Stage 2: In-country Assessment; and

Stage 3: Recommendations and Reporting.

4.1 Stage 1. Information Collection and Review

To assist in the review of biosecurity and diagnostic capacity, a list of known pathogens, parasites and diseases of broodstock, juvenile and larval molluscs in Vietnam was compiled. This list included information obtained from Vietnamese researchers in response to a detailed questionnaire (Appendix A), from literature searches and from disease notifications to the World Organisation of Animal Health (OIE). Based primarily on the response to the questionnaire, an initial assessment of the current laboratory capacity to diagnose disease was made, including an assessment of laboratory equipment and testing methods.

The regulatory environment pertaining to the diagnosis and control of infectious diseases in molluscs was also determined through information obtained from Vietnamese researchers in response to the detailed questionnaire and from literature searches. This regulatory environment specifically addressed:

1) movements of aquatic animals;
2) notification of diseases and disease diagnosis;
3) biosecurity standards; and
4) shellfish quality assurance requirements.

4.2 Stage 2 In-Country Assessment

To further assess biosecurity capacity, Drs John Humphrey and Paul Hardy-Smith of Panaquatic held formal and informal discussions with staff of RIA No.1 and RIA No.3 to discuss and assess the objectives, practical implementation and compliance of the current regulatory environment. This assessment was further assisted by personal inspections of the RIA No.1 hatchery, broodstock facilities and farms in the Cat Ba Island and included an evaluation of day to day biosecurity measures.

Similarly, to further assess diagnostic capacity, Panaquatic held formal and informal discussions with staff of RIA No.1 and RIA No.3, including aquatic health professionals involved in disease diagnosis at both facilities. Further, Panaquatic visited RIA No.1 and RIA No.3 laboratories to assess the laboratories' capacity to diagnose common diseases.

Of particular importance was a meeting with Madam Phan Thi Van, Leader, RIA No.1, at which time a range of issues including biosecurity, diagnostic capacity and future training needs were discussed.

4.3 Stage 3 Recommendations and Reporting

Following formal and informal discussions with RIA staff and an assessment of biosecurity and diagnostic capacities, a series of conclusions were derived in which a number of deficiencies in capacity were highlighted. Specific recommendations to address such deficiencies in the short to medium term were then formulated.

4.4 Itinerary

For a summary of the itinerary, see Appendix 2.

5 Task 1 - Summary Report on Molluscan Diseases in Vietnam

5.1 Introduction

Critical to this project is an understanding of the known occurrence and distribution of diseases of molluscs in Vietnam. This is essential for the establishment of disease control programs, biosecurity and the diagnosis of disease.

The occurrence of pathogens, parasites and diseases of molluscs in Vietnam was ascertained by the following:

i. A review of available literature in the English language;
ii. Response to questionnaire completed by Vietnamese scientists; and
iii. Discussions with Vietnamese scientists during the visit.

5.2 Findings

This report did not seek to review reports and/or publications of disease in bivalve or gastropod molluscs, present in Vietnam, which are published in the Vietnamese language. Hence the completeness of this review cannot be guaranteed.

All of the reports of disease in bivalve or gastropod molluscs in Vietnam in the English language, however, appear to be relatively recent in nature. No reference to the occurrence of pathogens, parasites or diseases in molluscs in Vietnam was identified prior to 2000 and only occasional published and unpublished reports describing diseases were identified since that time.

Historically, NACA[1] (1990) reported that no specific diseases of molluscs could be identified in Vietnam, noting that such diseases were essentially unknown due to the limited farming of molluscs at that time. Subsequently, Hine (1999) presented a comprehensive overview of diseases of molluscs in the Asia-Pacific region, identifying a range of viral, bacterial, protozoan and metazoan diseases but did not identify any diseases or disease agents of molluscs in Vietnam. Bondad-Reantaso *et al* (2001), in the Asia Diagnostic Guide to Aquatic Animal Diseases made no reference to the occurrence of diseases of molluscs in Vietnam. The *Identification Field Guide to Aquatic Animal Diseases Significant to Asia-Pacific* (DAFF 2007) also did not identify any diseases of molluscs in Vietnam. Similarly, Proceedings IV, V and VI of Diseases in Asian Aquaculture (Lavilla-Pitoga and Cruz-Lacierda 2002, Walker *et al* 2005, Bondad-Reantaso *et al* 2008,) made no mention of molluscan diseases in Vietnam.

Formal reporting of diseases of molluscs in Vietnam has occurred through the NACA country disease status reports and include "*sweet snail disease*" (NACA 2000), mass mortality in *Babylonia areolata* (NACA 2006), "*swollen syphon disease*" in *Babylonia areolata* (NACA 2007) and Perkinsosis in the hard clam *Meretrix lyrata* (NACA 2011a).

In addition to the NACA reports, a number of pathogens or potential pathogens have been identified by the Research Institutes for Aquaculture following laboratory examinations of molluscs. These reports do not, however, provide additional information on diseases associated with the described agent and in many cases uncertainty exists as to whether the agent is a primary pathogen, the result of secondary infection or a contaminant.

Table 1 presents a listing of pathogens, parasites and diseases reported in the English literature to occur in molluscs in Vietnam.

[1] *Network of Aquaculture Centres in Asia-Pacific*

5.2.1 Viruses and viral diseases

No reports of viruses or viral diseases of bivalve or gastropod molluscs in Vietnam were identified.

5.2.2 Bacteria and bacterial diseases

Bacteria identified as being recorded from bivalve and gastropod molluscs in Vietnam are shown in Table 1 and include *Vibrio spp*., *Pseudomonas sp*., and unidentified bacteria (Ninh 2012).

Apart from a reference to an association between "*sweet snail disease*" and the presence of *Vibrio sp*., no descriptions of disease accompanying the reports of bacteria were identified.

5.2.3 Fungi and fungal diseases

Fungi identified as being recorded from bivalve and gastropod molluscs in Vietnam are shown in Table 1 and include *Fusarium semitectum*, *Fusarium sp.*, *Aeromonium sp.*, *Exophiala sp.*, *Lagenidium sp*., *Haliphthoros sp.* and an unidentified "*red fungus*" (Ninh 2012). No descriptions of disease accompanying the reported fungi were identified.

5.2.4 Protozoa and protozoan diseases

Protozoa identified as being recorded from bivalve and gastropod molluscs in Vietnam are shown in Table 1 and include *Perkinsus olseni* (NACA 2011b), *Perkinsus sp.* (NACA 2011a, Ninh 2012), *Zoothanmium sp*., *Vorticella sp.*, *Nematopsis sp.*, *Haplosporidium sp.*, *Marteilia sp.* and unidentified ciliates (Ninh 2012). With the exception of Perkinsus, no descriptions of disease accompanying the reported protozoa were identified.

Perkinsosis.

Suspected Perkinsosis due to infection with *Perkinsus olseni* in an undefined mollusc species was reported in 2011 by NACA (2011b).

Subsequently, Perkinsosis due to a *Perkinsus sp.* was reported for the April-June quarter by NACA (2011a) in clams *Meretrix lyrata* with a mortality rate of 15-90% affecting an area of 2054 ha from the Can Gio District (Ho Chi Minh City); Go Cong District (Tien Giang province); Binh Dai and Ba Tri Districts (Ben Tre province); Ngoc Hien District (Ca Mau province); Bac Liue City (Bac Lieu province) in southern Vietnam.

NACA (2011a) reported that samples had been sent to national laboratories for confirmation and that five samples collected from affected areas in Ca Mau and Bac Lieu provinces tested by the laboratory of the National Centre for Veterinary Diagnosis (NCVD) were positive for the disease. NACA (2011a) also noted that the *Perkinsus sp.* was reported to OIE by the Department of Animal Health of Vietnam.

Subsequently, in addition to hard clams (*Meritrix lyrata*), *Perkinsus sp.* has been reported in the otter clam (*Lutraria rhynchaena*), pearl oyster (*Pinctada sp.*) and estuary oyster (Ninh 2012).

Further evidence for the presence of Perkinsus in Vietnam is presented by Shepherd and Phillips (2008) who reported *Perkinsus olseni* in diseased clams *Tridacna crocea* imported into research aquaria in Florida, USA, from a Vietnamese culture facility. Histopathological examination showed the presence of extracellular round organisms consistent with *Perkinsus sp.* trophozoites and PCR assay indicated the presence of *Perkinsus sp.* DNA in these animals. Species-specific assays indicated the presence of *P. olseni*, and possibly other *Perkinsus* species., but not *P. marinus*.

5.2.5 Diseases of uncertain aetiology

Losses due to undescribed or poorly described diseases in molluscs were identified in discussions with RIA No.1 staff. Losses due to unknown causes were commonly identified during the visit when discussing the health problems in molluscs, especially otter clams, hard clams and oysters. It was apparent that in all three mollusc species there have recently been some serious health issues.

Relatively recent losses in mollusc culture (these being otter clams, where millions of dollars have reportedly been lost due to mortalities) were identified with a report the week prior to the visit indicating that the loss approached $10 million in value (USD) (Tai – personal communication).

A report in the Viet Nam News, the National English Language Daily Newspaper, pm Thursday, July 5th titled "Mass Oyster Deaths Leave Farmers Broke" noted that "tens of millions of snout otter clams have died en mass (sic) just weeks before they are harvested…". The newspaper article goes on to report that scientists from the Ministry of Agriculture and Rural Development's National Centre of Vetenerial (sic) Diagnosis named the parasite Perkinsus as the cause of the deaths.

It remained uncertain if any one individual or organisation possessed a complete understanding of the epidemiology and impact of the diseases in question.

"Sweet snail disease" in larval *Babylonia areolata*

"*Sweet snail disease*" was reported by NACA (2000) in what is believed to be larvae of the gastropod *Babylonia areolata*. No details of the disease are given. The disease was reported to be associated with *Vibrio spp*. and a parasitic species of protozoa.

"Swollen syphon disease" in Babylonia areolata

NACA (2007) reported a disease in the gastropod *Babylonia areolata* characterised clinically by a swollen siphon with cessation of feeding, and with deaths scattered or in mass. The disease was reported to have occurred in limited zones in some provinces. It is believed that swollen syphon disease may have been first observed in 2003 (Phuc 2012).

It is possible that the disease referred to by NACA (2005d) in which "*Babylonia areolata* leaves shell and die in mass" is the same condition

Mass mortality in hard clams *Meretrix lyrata*

Mass mortality in the hard clam attributed to Perkinsosis (NACA 2011a) was first recorded in April – June 2011 in southern coastal Vietnam in the Ca Mau, Bac Lieu, Ben Tre, Tien Giang provinces and Can Gio District of Ho Chi Minh City in southern Vietnam. Subsequently mass mortalities occurred in clams in the northern coastal regions of Vietnam extending at least to farms in the Cat Ba Islands and Ha Long Bay.

An association between movements of clams from southern regions and the occurrence of the mortalities in northern regions has been made (Ninh 2012 personal communication).

Clinically, it appears that both mature and immature clams are affected, with deaths exemplified by high numbers of empty shells in the sand at farm sites. No other details of clinical signs or epidemiology can be determined. On one occasion, live crabs inhabiting the same environment as the dead clams were observed. No description of the pathology or histopathology of affected clams is available.

Although Perkinsosis due to infection with *Perkinsus sp*. was reported to the OIE, based on examination of a limited number of samples, the basis on which this diagnosis was made is unknown. The severity and distribution of the mortalities suggests that some other causal factors may be involved.

Epidemiological factors associated with the mortality events in discussions with RIA staff include water temperatures outside the optimal range for the species, starvation associated with a massively increased biomass of clams and consequential depleted feed reserves, toxic algal blooms, decreased salinity and exposure to agrichemical runoff, especially cypermethrin, which apparently has been used only in recent years in shrimp farms.

The impact of the losses is high, with some 190,000 tonnes of hard clams exported from Vietnam per year.

Mass mortality events in otter clams *Lutraria rhynchaena*

It is understood that clams started dying in September 2011 and by February 2011, the majority of farmed clams were dead (Ninh – personal communication). The monetary value of the losses in Otter clams was reported to be in the vicinity of 10 million (US$) in the weeks prior to our visit.

The extent of the mortalities in this species was demonstrated by massive numbers of empty baskets piled up on the farms which were reported to have been previously used for holding of the otter clams all of which had died over the past year or so.

Personal observations of photographs of affected clams showed a generalised whitening of the tissues (mouthparts?) protruding from the clam, with apparent tissue bacterial invasion and tissue necrosis observed on photomicrographs, together with large bacterial colonies associated with gills and ovoid basophilic masses possibly representing Rickettsial cysts or rickettsia-like organisms.

Subsequent examination of histological sections of gills of affected clams showed bacterial colonisation of the gills, with bacterial emboli present in some cases. Morphologically, bacteria were large and bacilliform in nature, suggesting terminal colonisation and bacteraemia.

In addition, ovoid basophilic bodies of uncertain nature, possibly representing colonies of rickettsial-like organisms but more likely representing degenerate ova artefactually occurring in the gill tissue, or possibly saprophytic protozoa, were seen in histological sections of affected clams. A low-grade regional inflammatory cell infiltration was also evident, with an occasional poorly defined granulomatous response to the embolic bacteria.

The cause of the deaths could not be determined from the limited gross and histological material examined. Similar epidemiological factors to those described for hard clams were suggested as possible causes.

Mortalities in mature oysters *Crassostrea* spp

Losses in oysters were reported to have occurred along the coastal regions in both southern and northern Vietnam, but mortalities were "*patchy*" in distribution and losses were not as high as in the clams. No further information is available on clinical, epidemiological or pathological findings on affected oysters. It is uncertain if diseased oysters were available or were submitted for laboratory examination.

Mortalities in oyster spat

Recurrent mortalities of 100% were reported in oyster spat at about 7 days post-fertilisation in the oyster hatchery at Cat Ba. Although the deaths had been investigated by staff at CEDMA, the cause remains uncertain.

Vibriosis appeared a likely cause. Apparently, the developing spat grow very well up to this point. Then over a 24 hour period they all become lethargic and die. Sampling by CEDMA was believed to have occurred well after the time of the deaths precluding critical examination and diagnosis

Table 1 - Reported occurrence and distribution of pathogens, parasites and diseases of molluscs in Vietnam

Viruses and viral diseases			
No pathogens or diseases identified			
Bacteria and bacterial diseases			
Identification	**Species affected**	**Disease**	**Reference**
Vibrio sp	Hard clams - *Meretrix lyrata*	Unspecified	Ninh 2012
	Snout Otter clam - Lutraria rhynchaena	Unspecified	Ninh 2012
	Blood cockle - *Anadara granosa*	Unspecified	Ninh 2012
	Abalone – Haliotis spp	Unspecified	Ninh 2012
	Maculated ivory whelk (Babylone snail) – *Babylonia areolata*	Unspecified	Ninh 2012
	Snout oyster (Synonomous with snout clam?)	Unspecified	Ninh 2012
	Pearl oyster – *Pinctada spp*	Unspecified	Ninh 2012
	Estuary oyster (Taxonomy uncertain)	Unspecified	Ninh 2012
Pseudomonas sp	Snout oyster (Snout clam? **Lutraria rhynchaena)**	Unspecified	Ninh 2012
	Pearl oyster – Pinctada spp	Unspecified	Ninh 2012
	Estuary oyster (Taxonomy uncertain)	Unspecified	Ninh 2012
	Hard clam- *Meretrix lyrata*	Unspecified	Ninh 2012
Unspecified bacteria	Snout oyster (Snout clam? **Lutraria rhynchaena)**	Unspecified	Ninh 2012
	Pearl oyster – Pinctada spp	Unspecified	Ninh 2012
	Estuary oyster (Taxonomy uncertain)	Unspecified	Ninh 2012
	Hard clam- *Meretrix lyrata*	Unspecified	Ninh 2012
Fungi and fungal diseases			
Identification	Species affected	Disease	Reference
Fusarium semitectum	Hard clam - *Meretrix lyrata*	Unspecified	Ninh 2012
Fusarium sp	Snout oyster (Snout clam? **Lutraria rhynchaena)**	Unspecified	Ninh 2012
Fusarium sp	Pearl oyster – Pinctada spp	Unspecified	Ninh 2012
Fusarium sp	Estuary oyster (Taxonomy uncertain)	Unspecified	Ninh 2012
Fusarium sp	Hard clam - *Meretrix lyrata*	Unspecified	Ninh 2012
Aerominium sp	Hard clam - *Meretrix lyrata*	Unspecified	Ninh 2012
Exophiala sp	Hard clams - *Meretrix lyrata*	Unspecified	Ninh 2012
Lagenidium sp	Blood cockle - *Anadara granosa*	Unspecified	Ninh 2012
Lagenidium sp	Abalone – Haliotis sp	Unspecified	Ninh 2012
Lagenidium sp	Maculated ivory whelk (Babylone snail) – *Babylonia areolata*	Unspecified	Ninh 2012
Lagenidium sp	Snout oyster (Snout clam? **Lutraria rhynchaena)**	Unspecified	Ninh 2012
Lagenidium sp	Pearl oyster - *Pinctada spp*	Unspecified	Ninh 2012
Lagenidium sp	Estuary oyster (Taxonomy uncertain)	Unspecified	Ninh 2012
Haliphthoros sp	Hard clams - *Meretrix lyrata*	Unspecified	Ninh 2012
Haliphthoros sp	Snout oyster (Snout clam? **Lutraria rhynchaena)**	Unspecified	Ninh 2012
Haliphthoros sp	Pearl oyster - Pinctada spp	Unspecified	Ninh 2012

Table 2 (cont.) - Reported occurrence and distribution of pathogens, parasites and diseases of molluscs in Vietnam

Fungi and fungal diseases (cont.)			
Haliphthoros sp	Estuary oyster (Taxonomy uncertain)	Unspecified	Ninh 2012
"Red fungus"	Blood cockle - *Anadara granosa*	Unspecified	Ninh 2012
"Red fungus"	Abalone – Haliotis sp	Unspecified	Ninh 2012
"Red fungus"	Maculated ivory whelk (Babylone snail) – *Babylonia areolata*	Unspecified	Ninh 2012
Protozoa and protozoan diseases			
Perkinsus sp	Hard clams - *Meretrix lyrata*		Ninh 2012,NACA 2011a.
Perkinsus sp	Snout oyster (Snout clam? *Lutraria rhynchaena* **)**		Ninh 2012
Perkinsus sp	Pearl oyster		Ninh 2012
Perkinsus sp	Estuary oyster		Ninh 2012
Zoothamnium sp	Blood cockle		Ninh 2012
Zoothamnium sp	Babylone snail		Ninh 2012
Vorticella sp	Blood cockle		Ninh 2012
Vorticella sp	Babylone snail		Ninh 2012
Nematopsis sp	Hard clams		Ninh 2012
Nematopsis sp	Snout oyster		Ninh 2012
Nematopsis sp	Pearl oyster		Ninh 2012
Nematopsis sp	Estuary oyster		Ninh 2012
Haplosporidium sp	Hard clams		Ninh 2012
Haplosporidium sp	Snout oyster		Ninh 2012
Haplosporidium sp	Pearl oyster		Ninh 2012
Haplosporidium sp	Estuary oyster		Ninh 2012
Marteilia sp	Hard clams		Ninh 2012
Marteilia sp	Snout oyster		Ninh 2012
Marteilia sp	Pearl oyster		Ninh 2012
Marteilia sp	Estuary oyster		Ninh 2012
Unspecified ciliates	Hard clams		Ninh 2012
Unspecified ciliates	Snout oyster		Ninh 2012
Unspecified ciliates	Pearl oyster		Ninh 2012
Unspecified ciliates	Estuary oyster		Ninh 2012
Diseases of uncertain aetiology			
Swollen syphon disease	Babylonia areolata		NACA 2007d
Sweet snail disease	Taxonomy uncertain		NACA 2000
Mass mortality	Babylonia areolata		NACA 2006
Mass mortalities	Edible oysters		This report
Mass mortalities	Otter clams		This report
Mass mortalities	Hard clams		This report

6 Task 2 -Summary Report on the Regulatory Environment Relating to Infectious Diseases of Molluscs in Vietnam

6.1 Introduction

Legislation and regulation is widely recognised as fundamental to the management, control and prevention of disease, especially disease resulting from trans-boundary movements of aquatic animals in the Asian region. In June 2000, Technical Guidelines for the responsible trans-boundary movement of live aquatic animals in Asia were endorsed by 22 regional countries including Vietnam at Beijing, China ("the Beijing Consensus") (FAO 2000).

Implicit in the Beijing Consensus was a need for health certification and quarantine measures, disease zoning, disease surveillance, disease reporting, import risk analysis, legislative and policy frameworks, and capacity building when considering translocations of aquatic animals.

Legislation and regulation is also a well recognised requirement for the control and containment of disease within a country. FAO (1995) notes "*it is imperative to design successful health management programmes which will not only be effective and environmentally sound, but also economically feasible to implement*".

The determination of the legislative and regulatory framework supporting disease diagnosis and control in Vietnam was undertaken through the following process:

i. Review of available literature in the English language;
ii. Response to questionnaire completed by Vietnamese scientists; and
iii. Discussions with Vietnamese scientists during visit in July, 2012.

6.2 Findings

It is noteworthy that a considerable body of information has accumulated over the past three decades describing legislative and regulatory controls in many Asian regional countries as a result of workshops and meetings (Davy and Chouinard 1982, FAO 1986, Arthur 1987, Subasinge et al 2004). No specific information on legislation and regulations in Vietnam pertaining to aquatic animal biosecurity or disease control, however, could be identified in the English language, with the exception of a list of Notifiable Diseases (Appendix C). Further, a search of FAO databases on national legislation and international agreements concerning food and agriculture (including fisheries, forestry and water) maintained by the FAO Legal Office (available at http://www.fao.org/fishery/legislation/search/en) did not identify legislation or regulations for Vietnam. It is quite possible, however, that such information is present in the Vietnamese language through Government sources.

6.2.1 Responsible authority

In response to the questionnaire completed by Vietnamese scientists and returned by Dr Ninh and in discussions with Vietnamese scientists, it was determined that the Department of Animal Health (DAH) within the Ministry of Agriculture and Rural Development is the key government body responsible for mollusc health issues and reporting and notification of disease events (nationally and internationally). This includes notification of disease events to the World Organisation of Animal Health (OIE). DAH is also apparently responsible for disease investigations on farms. Such investigations utilise DAH regional offices, RIA's and/or universities. DAH also apparently has the primary responsibility for health certification for translocations of live aquatic animals.

6.2.2 Reporting of disease and disease notification

As determined from the Questionnaire, regulations exist that identify when a disease should be reported. Notification of serious disease events occurs by farmers reporting to government officers, but also sometimes directly to newspapers or web news. The system for reporting of significant disease events to government and the criteria of such events remained unclear.

6.2.3 Assistance with disease control

In discussions with Vietnamese scientists, it was determined that in the event of a disease issue assistance may be sought by farmers from their nearest laboratory, e.g., RIA No.1. Farmers may benefit from reporting problems to government by getting assistance with treatment and control measures.

6.2.4 Movement controls

In discussions with Vietnamese scientists and as determined from the Questionnaire, it was determined that regulations exist for the importation of living aquatic animals from other countries, however, such regulations are difficult if not impossible to enforce due to the widespread (illegal) importation of low-cost seed stock, especially from China but also from elsewhere.

The regulatory requirements for movements of living aquatic animals within Vietnam remain uncertain. There appears to be inconsistent regulatory requirements for movements of living aquatic animals including molluscs within Vietnam. For example, broodstock or seedstock from Government hatcheries or grow-out facilities can be moved without restriction whereas it was understood that health certification was necessary prior to movements of molluscs from non-government sources. As determined from the Questionnaire, it appears that farmers can move stock between farms but the need for a quarantine certificate to do this was uncertain, given the lack of appropriate resources available (both lab and personnel) to provide these certificates.

6.2.5 Submission of laboratory samples

In discussions with Vietnamese scientists, it was determined that submission of samples for laboratory examination is conducted in an ad hoc manner, and samples from disease outbreaks will not necessarily be examined by the competent authority, i.e. the Department of Animal Health.

6.2.6 Disease control

It was determined in discussions with Vietnamese scientists that the laboratory at RIA No.1 at the very least provides information to farmers on diseases and collaborates with regional field staff to assist in disease control measures.

6.2.7 Health certification: role of RIA No.1 and Department of Animal Health

As noted, DAH is the responsible authority for certification and movements of aquatic animals and not RIA1. In discussions with Vietnamese scientists, a clear understanding of the roles and responsibilities for disease diagnosis and health certification matters between DAH and RIA No.1 was not clearly elucidated. It was established that DAH does undertake its own certification procedures and it appeared that these were not necessarily referred to RIA1.

6.2.8 Notifiable diseases list

A list of notifiable diseases of aquatic animals exists in Vietnam (Appendix C). However, there are no diseases of molluscs listed as notifiable.

7 Task 3: Summary Report of RIA No.1 Diagnostic Capacity - Facilities and Resources

7.1 Introduction

The aquatic animal disease diagnostic and research laboratory is incorporated into the overall operational structure of RIA No.1. Physically, the laboratory is located in the ground floor of an independent building. This building also incorporates the environmental testing laboratory and the library. Sample reception and accessions are located in a separate building approximately 100 meters from the laboratory building. The diagnostic laboratory contains dedicated areas for specimen processing and bacteriology, mycology, parasitology, histology, virology and PCR laboratories. Histopathological examination of tissue sections is undertaken using the microscope in the parasitology laboratory.

The laboratory and its facilities were inspected on a tour under the guidance of Dr Lua.

7.2 Findings

7.2.1 Role of the RIA No.1 Laboratory

Discussions with Dr Lua indicated that the laboratory has had a central research role in aquatic animal health, but research depended on donor agency or government funding. The provision of a diagnostic service with follow-up advice to farmers was also apparently a central role of the laboratory.

The research component of the laboratory was unclear. No specific research activities could be indentified at the time of the visit.

Several references were made for the need for funding in order that research could be undertaken. It was indicated that funding for research into clam deaths may be forthcoming.

It also appeared that historically, the laboratory has relied on research projects for the acquisition of its equipment. On each of the main items of equipment (e.g. incubators, centrifuges) there is usually a sticker indicating which project that piece of equipment had been purchased for. It was not apparent that the government supplied some or any of the equipment used for diagnostics.

7.2.2 Submissions and costs of testing

It appeared that most of the samples that come through the laboratory were part of specific projects carried out by RIA No.1staff. It was not possible to obtain a clear understanding of the number or nature of submissions from farmers. A laboratory case load of approximately 100 accessions per year was indicated during discussions which suggest a low level of disease monitoring and surveillance, particularly if this is across all aquatic species.

The distances and costs involved for sample examinations would appear to be active disincentives for submission of samples by farmers. Submissions from government facilities (e.g. the hatchery) for diagnostic services were apparently not charged back to the facility. It appeared that individual farmers were required to pay for diagnostic services, possibly depending on which assays were determined to be necessary although this was uncertain.

In discussions regarding the cost to farmers of the provision of diagnostic services, Dr Ninh indicated that generally the fee was well subsidised by the government except for the

reagents used if these were required. The cost of these reagents would be charged out to the clients.

7.2.3 Equipment and Capital items

As noted, it appeared that many of the capital items including laminar flow cabinets, incubators, microscopes, were a result of grants from overseas funded projects, especially from Denmark.

When questioned about replacements, it was indicated that replacement would rely on similar funding arrangements. It appeared difficult or impossible to acquire capital items from government.

7.2.4 Diagnostic manuals

There was no evidence of diagnostic procedures manuals in laboratories; in fact, the laboratories appeared relatively devoid of texts or manuals.

7.2.5 Accessions and sample reception

A dedicated room was used for recording information on samples submitted to the laboratory. Here farmers bringing samples for examination provided information regarding the history, nature of the samples and the reason for submission.

Details of samples are taken directly from the submitting farmer, or via telephone in the case of samples submitted through a courier. It appears that a number of staff members operate the accessions area. Apparently this information is entered into a computer under a specific accessions number, which accompanied each sample to each respective laboratory.

Laboratory reports detailing the results of laboratory examinations were also collated in this area. There was no laboratory accessions system linking all laboratories.

7.2.6 Bacteriology

No bacteriological isolations were underway at the time of inspection.

The laboratory is appropriately equipped for bacterial isolation. Equipment includes incubators, refrigerator, microscopes, laminar flow cabinet, autoclaves, oven, microwave, centrifuges and balances. Bacteriology on samples of aquatic animals is undertaken using standard culture media, and routine isolation and identification procedures.

Culture media is prepared on site, together with biochemical reagents. Identification of bacteria is based on colonial morphology, Gram staining and biochemical reactions. In some cases the API20 identification system is used for bacterial identification. It appeared that antibiotic sensitivity tests were also conducted

Examination of Gram stained smears showed good staining and good differentiation of bacteria.

Facilities for media preparation were not examined.

Identified shortcomings included:

- An absence of reference or type bacterial cultures, although a collection of typical cultures was held to assist in identification;
- Identification of *Vibrio* spp appeared problematic, with isolates of *V vulnificus*, *V alginolyticus* and *V harveyi* only reported. It was not possible to ascertain exactly how primary infections were differentiated from secondary infections or contaminants.

7.2.7 Parasitology

Basic facilities for conducting parasitological examinations were present, including microscopes (compound and stereo), incubator, refrigerator and computer.

It appeared that wet mounts on skin, gills and intestines of fish were conducted as a routine procedure. Techniques or methods used for the identification of parasites were uncertain.

7.2.8 Histology

Preparation of histological sections was undertaken in a separate laboratory equipped with a tissue processor, tissue embedding station, refrigerator and compound microscope. Staining was conducted manually in reagents (H&E) in a fume cabinet.

It was understood that special staining techniques could be undertaken as required, and reagents for such were present. No special stains were observed.

Random selection of stained histology slides showed good quality sectioning and staining. The slides examined were of diagnostic quality.

7.2.9 Histopathology

A microscope located in the parasitology laboratory was used for histopathological examinations. This Olympus microscope was in good condition with good optics. A digital camera mounted on the microscope was not working.

7.2.10 Mycology

Somewhat paradoxically, a separate, dedicated mycology laboratory was present for the isolation and identification of fungi.

The laboratory was equipped with laminar flow cabinet, reagents, culture media and microscope. The use of a separate laboratory for fungal culture and identification appeared to be a desire to maintain other laboratories free of fungal contamination.

7.2.11 Virology

The virology laboratory was not operational due to the cost and difficulties in maintaining cell lines. Basic equipment was present, however, that would appear to allow for the routine growth of cell lines and virus isolation in cell culture.

Equipment included incubators, refrigerators, liquid nitrogen storage, ultra-deep freezer, microscope, inverted microscope, centrifuges and a laminar flow cabinet.

7.2.12 Polymerase Chain Reaction (PCR) laboratory

The PCR laboratory was divided into two rooms, with preparation of samples conducted in a laminar flow cabinet in an attempt to protect against contamination. Equipment included PCR heat cycling machines, refrigerators, computers, gel electrophoresis system and UV photography, freezers, and centrifuges.

It is understood that tests were available for a limited range of pathogens of fish and shellfish, including WSSV, yellowhead, Taura syndrome,Monodon baculovirus Iridovirus VNN and *Streptococcus iniae* and *S. agalactiae*. The methodologies used were given as the OIE methods or in some cases PCR kits. Exact details of each assay were not obtained.

The use of reference genetic material or validation of the sensitivity or specificity of the tests used was not discussed.

A PCR assay for Perkinsus was also undertaken and it appeared that the Perkinsus was a “screening” assay and that positive identification depended on histopathology.

7.2.13 Water quality laboratory

A water quality laboratory was present in the wing opposite the disease diagnostic laboratory. This laboratory appeared to be part of the Environment and Disease Laboratory but the relationship with CEDMA was uncertain. This laboratory appeared to be capable of testing for ammonia, pH, salinity and dissolved oxygen. Dr Lua indicated that there was a field kit for sampling but that this was in use out in the field.

We did not have time to determine the capabilities of this laboratory in conducting water quality analysis during our visit or how often it is called for. Dr Lua indicated that there are farmers who do their own water quality assessment.

7.2.14 Specimen preparation

Preparation of samples for laboratory examination was undertaken in a room behind the parasitology laboratory. This room did not appear to be set up as a post-mortem room, rather, it was a space for allocating specimens to the different laboratories. It was possible, however, to undertake dissections on samples in this area. No dissections were being carried out at the time of our visit.

7.2.15 Case management

It appeared that all staff could undertake accessions and sample reception duties, however, decisions relating to which laboratory tests would be conducted and final collation of laboratory reports was the responsibility of the head of the laboratory Dr Lua.

7.2.16 Proficiency testing

This was not specifically discussed, however, it appears that there is no proficiency testing undertaken in any of the laboratories at RIA No.1.

7.2.17 Second opinions

Dr Lua indicated that in the past samples have occasionally been forwarded to other laboratories for further identification or confirmation. This was mentioned specifically in relation to identification of fungal isolates.

7.2.18 Library

The library above the laboratory was visited. This has a range of texts in English relating to aquaculture and diseases there-in. No on-line library acquisitions were possible, however, the purchase of key references was possible but only by authorisation from the Director due to the expense of such acquisitions. It was assumed that the cost would be approximately USD$30 or so per article and obtaining articles was unusual due to the expense.

Dr Lua indicated that on occasions references would be obtained from colleagues overseas, presumably who had access to on-line journals.

8 Task 4: Summary Report of RIA No.1 Diagnostic Capacity - scientific, technical and molluscan hatchery staff

8.1 Introduction

Appropriately trained and experienced veterinary, scientific and technical staff are critical for the effective and accurate diagnosis of disease, for research into the epidemiology, pathogenesis and control of aquatic animal diseases and for the implementation of biosecurity measures to mitigate against disease events. Without such individuals, the diagnosis and management of disease becomes problematic, irrespective of the physical resources and equipment which may be present within diagnostic and research laboratories.

The human resources capacity at RIA No.1 was assessed based on the experience and expertise in (1) aquatic animal disease diagnosis and research and (2) the development, implementation and maintenance of biosecurity. This was evaluated by:

i. Consideration of qualifications and positions held by each of the staff members from questionnaire;

ii. Formal and informal discussions with senior management of RIA No.1;

iii. Discussions with scientific staff during laboratory inspections;

iv. Discussions with scientific and technical staff during hatchery and broodstock facility inspections; and

v. Discussions with Madam Van.

8.2 Findings

8.2.1 Head of laboratory

The Head of CEDMA is Dr Dang Thi Lua. Dr Lua holds the degree of Doctor of Philosophy in the field of molecular biology. Her degree was obtained from a Japanese University. Dr Lua speaks very good English and is clearly a trained and committed scientist who communicates openly and well.

Her expertise and responsibilities include overall responsibility for the administration and operations of the laboratory and supervision of the bacteriology laboratory. She has spent 4 weeks at the CSIRO Australian Animal Health Laboratory (AAHL) in Geelong.

8.2.2 General awareness of need for disease diagnostic capabilities and biosecurity

In discussions arising from the introductory meeting at RIA No.1 and in subsequent discussions with senior management of RIA No.1, there was a clear awareness of the need for disease diagnostic capabilities and for biosecurity measures to mitigate against introduction and spread of disease associated with movements of living aquatic animals.

Unequivocal support for the development of increased capacity in these areas was apparent, especially in relation to mollusc health and biosecurity.

8.2.3 Role of the Aquatic Animal Diseases Laboratory Centre for Environment and Disease Monitoring (CEDMA).

It was determined that CEDMA staff have stated roles and responsibilities in (a) disease diagnosis (b) disease research and (c) extension. It appears though there is limited depth in the capacity of staff to be replaced if any one staff member goes.

The laboratory receives samples for disease investigations from farmers and from field / extension officers. Samples are also received from Departmental hatcheries and broodstock facilities and may also originate from research investigations.

A range of species are received by the laboratory for examination including carp, tilapia, grouper and cobia, hard clams, oysters and otter clams and tiger shrimp, white-leg shrimp and crabs.

CEDMA reports back to farmers and extension / field staff and may follow-up cases to determine the effectiveness of treatment or control measures. It was determined that the head of the laboratory (Dr Lua) recommends treatments as deemed appropriate.

On occasions CEDMA staff may undertake field investigations for the purpose of collecting samples for disease diagnosis

8.2.4 Veterinary input in disease control or management

There were no submissions during the time of our visit and hence we did not witness first hand the handling of specimens or the approach to diagnosis and management of disease. However, there appeared to be little if any veterinary input into the assessment of clinical disease, epidemiology or the diagnostic process. The examination of samples appeared biased towards the individual skills of the scientists in the laboratory. As mentioned though, this was an impression gained during the short period of the visit and may possibly not be representative of what happens during other times.

8.2.5 Assessment of experience and expertise of laboratory staff

Assessment of the diagnostic experience and expertise of laboratory staff depended principally on discussions with Dr Lua during the laboratory inspection, on discussions with Ms Nguyen Thi Thu Ha relating specifically to histopathology, and subsequently on discussions with Madam Van.

8.2.6 Accessions and sample reception

From discussions with Dr Lua and in reference to the earlier questionnaire, it appears that all scientific laboratory staff have the training and ability to receive samples from farmers and enter case details, including case histories.

8.2.7 Case management

It appeared that Dr Lua was the case manager for each case submitted for disease diagnosis. This necessitated making decisions as to which laboratory examinations or tests would be performed and collation of results of laboratory examinations, with subsequent reporting and follow-up as necessary with submitting farmers.

It would seem from our discussions that no other person in the laboratory could undertake this function.

8.2.8 Bacteriological experience and expertise

The bacteriology has three staff members: Pham Thi Yen (MSc), Ms. Nguyen Thi Hanh and Ms. Nguyen Thi Huong. It was apparent that Dr Lua played a supervisory role in the operations and management of the bacteriology laboratory

Assessment of the experience and expertise in the provision of bacteriological diagnostic applications was made in discussions with Dr Lua. Dr Lua exhibited a clear understanding of the basis of bacterial isolation and identification, especially the isolation and identification of select pathogens or potential pathogens of aquatic animals. Other bacteriological procedures were understood and implemented, e.g., media preparation, disinfection.

It was not possible to assess the experience or expertise of the other staff.

Dr Lua indicated an awareness of the difficulties in determining if a bacterial isolate was a primary or secondary pathogen or a contaminant. There appeared however to be a lack of awareness of the diversity and complexity of the *Vibrio* / *Photobacterium* spectrum of bacteria and methodologies whereby these may be differentiated and identified.

8.2.9 Mycological experience and expertise

It was understood that the bacteriology staff also undertook mycological isolations and identifications. Not unsurprisingly, difficulties were evident and were expressed by Dr Lua in the identification of fungal isolates, as well as determining their pathogenic significance, especially in the absence of histopathology. It was clear that, on occasions, fungal isolates had been sent overseas for identification.

No clear indication of the methods or procedures by which mycological identification was attempted was evident. It did though appear that undue emphasis was placed on the isolation and identification of fungi with respect to their role as pathogens of aquatic animals.

8.2.10 Parasitological experience and expertise

The parasitology laboratory has four indentified staff as follows: Bui Ngoc Thanh (MSc), Nguyen Thi Ha (MSc), Ms. Nguyen Thi Thu Ha, and Ms. Tran Thi Hang. Assessment of the experience and expertise of parasitology staff was again dependent on comments from Dr Lua, thus, it was not possible to directly assess the degree of expertise and experience of the staff in the parasitology laboratory.

It was clear that staff undertook wet mounts of skin and gill from fish for parasitological examinations, together with examinations of intestinal contents and occasionally blood. The difficulties and complexities of parasite identification were not discussed in any detail. It was not possible to determine how parasites were identified and the reliability of such identifications.

Discussions on differing levels of parasite burdens suggested difficulties in interpreting the pathogenic significance of such burdens.

8.2.11 Histological slide preparation experience and expertise

The histology laboratory was staffed by two individuals, apparently under the supervision of Ms Ha. These individuals were Ms. Nguyen Mai Phuong and Mr. Dao Xuan Truong. Assessment of the experience and expertise of the staff members in the preparation of histology slides was again dependent on comments from Dr Lua, with assessment assisted by examination of the quality of the histology slides.

Slides selected at random were well sectioned and well stained and were considered of diagnostic quality. It was concluded that staff in the histology laboratory were competent and experienced in producing diagnostic quality sections. It was, however, uncertain who actually selected the tissues for histology slide preparation.

8.2.12 Histopathological experience and expertise

Histopathological examinations were undertaken by Ms Nguyen Thi Thu Ha who was introduced as the pathologist. It was clear following discussions with Dr Lua and Ms Ha

that experience and expertise were lacking at least in the histopathology of molluscs and fish and that training was required in these areas.

Apparently, Ms Ha has no formal training in histopathology and sought information on the histopathology of molluscs from the literature, primarily from the internet. We were advised that Ms Ha had a level of expertise in shrimp histopathology that was supported primarily by reliance on Don Lightner's publications. Reference sources for assistance were described by Dr Lua as the internet and "Shrimp histopathology".

One-on-one discussions of case material with Ms Ha indicated that she had a reasonable understanding of the histology of molluscs and fish and she showed excellent observational skills. It was clear, however, that her experience in identifying and interpreting histopathological changes was limited.

Dedication and application notwithstanding, it was considered unreasonable to expect a young and relatively inexperienced person working in relative isolation to develop the interpretative skills necessary to adequately undertake and report on histopathological examinations of fish and molluscs, especially in cases where disease has severe, adverse socio-economic impacts.

8.2.13 Virological experience and expertise

It appeared that Dr Lua was the only individual within the laboratory that had experience and training in the isolation and identification of viruses. Dr Lua exhibited a sound understanding of cell culture and virus isolation techniques. Dr Lua also indicated that she was waiting for a research grant to activate the virology laboratory.

8.2.14 Molecular diagnostic (PCR) experience and expertise

The PCR laboratory employed three staff members under the direction of Dr Lua. These were identified as Ms. Le Thi May, Ms. Nguyen Minh Thuy and Mr. Nguyen Viet Khue.

Assessment of the experience and expertise in the PCR laboratory was made primarily through discussions with Dr Lua. It was clear that Dr Lua played a leading role in the operations and management of the PCR laboratory. It was also clear that a range of PCR assays were undertaken. It was difficult to ascertain if the limitations and deficiencies of PCR technology were understood and it was unclear if PCR results were ever matched to other laboratory results, e.g., histopathology.

It did appear, however, that PCR was seen as a major diagnostic and research component of the laboratory. Time precluded an in-depth discussion of each assay offered; there was concern expressed by Dr Lua over the reliability of the PCR assay for Perkinsus.

8.2.15 Post-mortem and specimen preparation

Two staff members were identified as working in the post-mortem area. These were Ms. Nguyen Thi Thu Ha and Mr. Dao Xuan Truong. The level of expertise and experience of these individuals was unable to be determined

8.2.16 Extension and training

It was not possible to evaluate the skills sets or effectiveness of individuals in the laboratory involved in extension or training of field staff or farmers.

8.2.17 Capacity building

Training and education through short-term laboratory attachments for laboratory staff were viewed by Dr Lua as a good way of upgrading staff skills in particular areas.

Specific needs expressed by Dr Lua were:

- Training in histopathology, especially with respect to mollusc and fish diseases;
- Short-term laboratory attachments for staff to upskill in particular areas;

9 Task 5. Summary Report on Functional and Operational Biosecurity Policies and Practices at RIA No.1

9.1 Introduction

Biosecurity at the national, regional and local level is an essential component of effective programs to limit or prevent the spread of infectious diseases. The review of internal and external physical and operational biosecurity strategies at RIA1 was undertaken as a basis for determining the need, if any, for cost-effective improvements in biosecurity to support the developing mollusc industry in Vietnam. This includes the potential production of specific pathogen free seedstock from hatcheries.

Internal and external biosecurity strategies supporting the production of specific pathogen free seedstock at RIA No.1 were evaluated through the following mechanisms:

- Personal inspections and observations of the hatchery facilities for finfish, molluscs and shrimp at the National Broodstock Centre for Mariculture Species in Northern Vietnam, facilities on Cat Ba Island;
- Personal inspections of the broodstock holding facilities off Cat Ba Island; and
- Formal and informal discussions with staff at hatchery and broodstock facilities.

9.2 Findings

9.2.1 Collection of samples and submission to CEDMA

It appears that there is no protocol for collection and submission of samples to CEDMA from either the hatchery or broodstock facilities in the event of disease occurrences.

It does though appear that when a disease/mortality issue occurred at the hatchery, CEDMA would be notified. Rather than staff at the aquaculture centre collecting and submitting samples that may be of diagnostic value, we were informed that when there was a disease/mortality issue, staff from CEDMA would come out and collect samples themselves. It was noted by hatchery staff that this process may take several days by which time the animals are all dead, or samples are not suitable for diagnostic purposes.

The concept of taking daily or at least regular samples in preservative for retrospective study was discussed with respect to optimising the chances of a reliable diagnosis based on well preserved and/or appropriately collected and retained fresh or frozen material.

This concept appeared to be understood and to be of interest to hatchery staff. It was felt that they would participate in such a program. Such a program though would need to be supported by three actions:

- Good quality fixative and specimen jars to be available at the hatchery;
- Good proficiency in histopathology at RIA No.1;
- The resources to be able to process, read, report and analyse the samples when received.

The nature and value of the diagnostic feedback from CEDMA was uncertain. It was indicated by hatchery staff that any feedback received was of little value because all the animals were dead before samples were taken. There was an awareness that fungi and other organisms would be detected from such samples, but these did not necessarily reflect the actual cause of the disease.

9.2.2 National marine hatchery facility, Cat Ba

General description

The facility was divided into four separate functional units:

1. Administration (Building A);
2. Finfish broodstock spawning and nursery (Building B);
3. Mollusc spawning and nursery (Building C); and
4. Shrimp hatchery (Building D).

Finfish broodstock spawning and nursery building

This was a separate building with large circular concrete broodstock tanks at one end and three large rooms containing multiple square concrete tanks for larval rearing and growth of juvenile fish.

At the time of inspection, relatively low numbers of juvenile cobia, grouper and barramundi cod (Mouse grouper) were present. Fish appeared in good condition.

No broodstock were being held at the time of inspection.

Water for broodstock and juveniles was pumped directly from the sea via what appeared to be sand filters to a holding reservoir. Apart from the sand filtration there did not appear to be any specific treatment of water for the general parts of the hatchery.

A series of structures that looked like sand filters was present in the lines supplying water between the reservoir and the hatchery / nursery, but it was claimed that these were actually pumps for pumping water from the reservoir.

Finfish broodstock for spawning were either spawned at the sea cage site or brought back to the facility depending on the species.

Spawning was induced with hormones (no protocol was described). Eggs were collected and transferred to the nursery tanks. Larvae were fed on live feed (artemia, rotifers?) and weaned onto fine solid feed.

It was unclear at the time how live feeds were produced but a subsequent visit identified an algal culture room that was presumed to be used for production of live feeds.

Health certification on seedstock

No health certification of other routine health examination procedures on seedstock leaving the facility could be identified.

A key comment made by staff was that, as the facility was a government facility, there was no need for stock to be certified.

In comparison, it appeared that other (private?) hatcheries did require stock to be certified although the exact nature of the certification (whether a site visit was required, numbers of animal inspected, who conducted the inspections and signed the certificates etc) was difficult to determine.

Biosecurity measures

Biosecurity measures at the time of the inspection were minimal in the finfish area.

No requirements were evident for (a) signing in for visitors, (b) changing shoes or washing hands and (c) use of protective clothing. This lack of any such requirements was reinforced the following day when we returned from the sea sites with again no request made to change shoes or disinfect. As we were carrying samples we took our own precautions.

There were no footbaths at any point within the facility or on entry or exit. There was no evidence that footbaths were used during spawning/larval rearing.

There was no attempt to isolate tanks holding fish and no restrictions on placing hands in water.

There was no evidence of dedicated equipment, e.g., nets, brushes, for different tanks.

It appeared that live broodstock finfish and dead finfish or their products were moved onto the hatchery site without restriction.

Chlorination of a small number of tanks was being conducted in one area of the facility. It is uncertain why this was being done. The dose and time used was not determined.

Mollusc spawning and nursery building

This was a separate building approximately 750m from the finfish facility and was adjacent to the shrimp hatchery. The building undertook spawning of oysters and of otter clams for subsequent sale to farmers.

No oysters or clams were in the facility a time of visit. Unfortunately we had been told at RIA No.1 immediately prior to our visit to the hatchery that there was a batch of oysters that we would be able to see when we got to the hatchery. On arriving at the hatchery we were informed this batch (approximately 5 million spat) had been sold the previous week.

Unfortunately it was difficult to obtain a clear description of the water source and thus a clear understanding of the water supply from its source, through the pump houses and into the different buildings remains uncertain. It was understood that water was pumped to a reservoir tank and then to individual tanks within the facility.

An algal culture room, insulated and air conditioned with jars of algae (approx. 10 litres) under propagation, including *Chaetocera* and a green algae were inspected. These cultures represented the stock cultures. For feeding of spat, cultures were grown in volume in plastic carboys

Multiple tanks were present within the facility for spat settlement and rearing. Spawning of oysters was apparently achieved by physical dissection of ripe gonads and mixing of egg and sperm to achieve fertilisation. It appeared that this procedure involved collection of broodstock from holding cages and opening them up at the facility until gravid males and females were found, then excising gonads and mixing gonadal fluids. Oyster spat was settled on lines of dead shell.

Batches of oysters were spawned at approximately monthly intervals between April and December, with some 8-9 spawnings per year, with a total of approximately 45 million oyster spat produced.

Spawning of otter clams was achieved by co-habiting males and females in a tank with a bed of sand and inducing spawning by heat shock. Details of the heat shock procedure were not obtained.

There was no spawning of hard clams at the facility; it appears that hard clams for culture are obtained from the wild by netting the sand areas and collecting the wild young spat this way.

Source of broodstock

It appeared that broodstock oysters originated from the broodstock holding facility off Cat Ba. No biosecurity or sanitary measures could be determined for broodstock entering the facility. It was understood that scrubbing of shells was conducted following entry of the broodstock.

Sourcing of otter clam broodstock was problematic at the time due to heavy losses of farmed stocks. Hatchery staff were observed purchasing broodstock from a local market. No biosecurity or sanitary measures were apparent for the otter clam broodstock entering the facility.

Health certification on seedstock

It appeared that no health certification measures were undertaken on oyster or otter clam seedstock leaving the hatchery.

Biosecurity measures

Biosecurity measures were minimal or non-existent at the time of the visit. As described above, unrestricted entry of broodstock was practiced.

No footbaths were evident.

Movement or personnel and visitors into and within the facility was unrestricted and there were no sinks for handwashing apparent, including the entrance into the algal culture room.

There was no indication of dedicated nets or equipment for specific tanks.

Aseptic techniques for algal propagation, if used, were not evident at the time of the visit.

Tank disinfection procedures were not discussed in detail, but it was understood that chlorine disinfection of tanks was practiced.

Washing of jars used for algal culture was evident

Importantly, it appeared that no disinfection or aseptic techniques were practiced for spawning of oysters or clams, with no apparent attempt to dissect gonad in an aseptic manner, or to rinse dissected gonads.

Disease events

It was apparent that disease events in juvenile seedstock occurring approximately 1 week following spawning were common and that the nature of the disease events was undetermined (see comments on sample collection above). It was unclear if any spat survived these events and in the event that they did, there was no indication that these would not be distributed for growout purposes.

Shrimp hatchery

Adjacent to the mollusc hatchery and nursery was the specific pathogen free (SPF) shrimp hatchery.

Paradoxically and in complete contrast to the mollusc and fin-fish hatcheries, the shrimp facility demonstrated a high standard of biosecurity. This was exemplified by:

(a) totally restricted entry to visitors (we were not allowed inside the main facility);

(b) a single entry point;

(c) changes of boots for staff entering the facility;

(d) protective clothing for staff entering the facility;

(e) footbaths on entry (and exit);

(f) use of elbow-length gloves by staff in facility, with disinfection of external surface of gloves before use; and

(g) use of face masks by staff inside the facility.

Notices placed on doors advising of restricted entry were also present. Inspection of broodstock and seedstock was not undertaken because of biosecurity measures in place.

We understand that all water used for the shrimp facility was disinfected prior to use by chlorination. The dechlorination procedure was uncertain, but apparently was effective.

Shrimp broodstock

The shrimp facility is based on *Penaeus vannamei* culture and we understand the broodstock were obtained from Hawaii.

Different biosecurity standards: Molluscs, finfish and shrimp

The reason for the high level biosecurity at the shrimp hatchery and the almost total absence of any such measure in the adjacent mollusc hatchery was not clear. Subsequently, it was determined that the SPF Shrimp facility had been developed under a government project, funded by government and was considered very important to government, indicating the importance of shrimp in Vietnam. It was apparent that such is not yet the case with the finfish and mollusc sections of the hatchery.

10 Summary Report on Human Resources Supporting Regional biosecurity at RIA No. 1Enter Heading 1

10.1 Introduction

Appropriately trained and experienced staff are critical for the effective implementation and maintenance of biosecurity and health management procedures and the production of SPF seedstock for distribution from hatcheries for growout. An understanding of the purpose and need for such practices is fundamental to limiting the spread of disease and ensuring the distribution of seedstock in optimal health and for the ongoing maintenance of biosecurity measures.

Human resources supporting biosecurity at RIA1 were evaluated primarily as a result of personal visits to the hatcheries broodstock facilities at the national Broodstock Centre for Mariculture Specifies, Cat Ba Island.

10.2 Findings

10.2.1 General awareness of need for biosecurity

A general awareness of a need for biosecurity measures to improve the productivity of mollusc and finfish seedstock was apparent and was expressed by senior RIA No.1 staff.

It appeared, however, that there was a disconnect between the expressed need for biosecurity and the actual development, implementation and maintenance of biosecurity measures. Overall, staff exhibited apparent indifference to biosecurity given the absence of biosecurity measures present at the hatcheries or broodstock facilities.

Further, there appeared to be no awareness of the need to avoid transporting potentially diseased material back to the hatchery. This was exemplified by a lack of concern about taking small diseased grouper or slaughtered cobia back to the hatchery

Head of facility

The Vice-Director of the facility is Mr Hoang Nhat Son (MSc). Mr Son holds the degree of Master of Science in an undetermined field. Mr Son speaks good English and appeared to have a sound understanding of the issues discussed. Communication appeared somewhat constrained.

10.2.2 Hatchery and broodstock facility: National Broodstock Centre for Mariculture

Roles of hatchery staff: Finfish and molluscs

It appeared that hatchery staff have responsibility for the production of seedstock finfish and molluscs for distribution for farming purposes.

Assessment of experience and expertise of hatchery staff

Assessment of the experience and expertise of hatchery staff in biosecurity depended principally on discussions with individual staff members and in a general meeting with hatchery staff which may have lost something in the translation. The following points were noted:

- Hatchery staff appeared enthusiastic about their work, appeared to understand the operations of the hatchery and appeared receptive to new ideas;

- Hatchery staff appeared somewhat frustrated at the time it took RIA No.1 laboratory staff to visit the hatchery and collect samples of diseased spat;
- Hatchery staff appeared aware of the futility of collecting samples for disease diagnosis from specimen which had been dead for some time and that reports of fungi present in such samples was not surprising; and
- Hatchery staff appeared to understand the concept of sampling and archiving fixed material for retrospective examination should the need arise and appeared willing and capable of participating in such a program to assist in the accurate diagnosis of disease.

11 Conclusions and recommendations

Enter text

11.1 Conclusions

The following are our key conclusions with respect to each of the specific tasks undertaken for this Project.

Task 1: A review of the diseases of molluscs in Vietnam

Based on our review of the literature in the English language and as best as can be determined, knowledge relating to the occurrence and distribution of pathogens, parasites and diseases of molluscs in Vietnam appears rudimentary and fragmented. It is noteworthy that this conclusion is made without reference to literature and laboratory reports which may be present in the Vietnamese language. It is also noteworthy that, with one exception showing what was described as *Nematopsis* in a mollusc, no reports or publications describing pathogens, parasites or diseases in molluscs were presented during our visit.

We must note though that we met only with members of RIA No.1 and RIA No. 3 and not with members of the Department of Animal Health (DAH), which is in the same Ministry (Ministry of Agriculture and Rural Development) and as we understand responsible for overall disease diagnosis, reporting, notification and certification. This caveat notwithstanding, we would be surprised if DAH were privy to information that members of RIA No. 1 and RIA No. 3 were not aware of.

A spectrum of pathogens or potential pathogens have been reported from molluscs in Vietnam, including agents well recognised as primary pathogens of molluscs, e.g., *Haplosporidium*, *Marteilia*. (Table 1). There is little or no information concerning the disease states, if any, with which these agents have been associated and no information on the basis on which these agents have been identified.

Major diseases of molluscs do occur, characterised by massive mortalities, especially in hard clams, snout clams and edible oysters. Despite a published diagnosis of Perkinsosis, the underlying aetiology, pathogenesis and epidemiology remains uncertain based on our conversations with staff at RIA No.1, RIA No.3 and Madame Van.

Similarly, a number of bacteria, fungi and protozoa have been reported from molluscs in Vietnam which may represent secondary or opportunistic infections or post-mortem contaminants. There appears to be no information concerning the disease states, if any, from which these agents have been isolated. No comment can be identified that considers the possibility that these organisms are secondary or opportunistic infections or post-mortem contaminants.

The methodologies used for the identifications of those pathogens, parasites and diseases described in molluscs in Vietnam is uncertain.

We could not identify sufficient health monitoring and surveillance data on which to develop a suggested internal disease control program. Such programs may mitigate against the spread of infectious disease.

Task 2: Legislative and regulatory framework supporting disease diagnosis (and control)

It appears that legislation and regulation does exist with respect to diagnosis and control of disease through standard approaches including biosecurity, quarantine restrictions, health certification, disease notification, sample submission and disease reporting and follow-up. However, no clear understanding of the legislative and regulatory framework supporting disease diagnosis and disease control could be established.

It appears that regulatory requirements for Government institutions are different to those of private companies. No clear understanding of the differences between mandatory biosecurity and disease control and voluntary biosecurity and disease control could be established.

Clearly, breaches of legislation and regulation occur on a regular basis and are well recognised to occur, e.g., illegal imports of live aquatic animals from China and elsewhere. Clearly, RIA staff recognise such breaches occur but do not appear in a position to do anything about it.

Although it was determined that DAH holds overall responsibility for implementation of disease diagnosis and control measures in Vietnam, uncertainty exist with respect to how disease diagnosis is implemented, the coordination of disease investigations and the relationships between the Research Institutes for Aquaculture and the DAH laboratories.

There appears to be lack of certainties regarding the investigation of diseases in the field, what resources are necessary, who should investigate and how collaboration between laboratories may benefit the overall diagnosis.

There also appears to be a reluctance to collaborate between laboratories, e.g., RIA No.1 involving or collaborating with other RIA laboratories and the DAH laboratories in the investigation of disease.

All these factors affect the ability to establish reliable disease lists on which rational disease control programs may be based.

The role of veterinary services in the diagnosis and control of disease remains uncertain. Certainly with respect to the RIA No.1, there was little evidence of veterinary input into the diagnostic and disease control processes.

There appears to be lack of prospective investigation of disease on the part of RIA No.1, e.g., clam mortalities and periodic mortality events in juvenile molluscs over several years at the Cat Ba hatchery

Task 3: Diagnostic capacity at RIA1 (Facilities and Resources)

The laboratory infrastructure and physical resources including equipment present at RIA No.1 would allow for undertaking of basic diagnostic investigations into the occurrence and nature of diseases in molluscs both in samples submitted to the laboratory and in field investigations. This conclusion assumes the presence of competent scientific and technical staff.

Concerns are raised regarding the non-replacement of major items of equipment due to absence of funds. Failure to replace non-functional items of equipment may compromise diagnostic capacity.

The absence of appropriate control specimen for bacteriology, parasitology and histopathology is a cause of concern with respect to the veracity of diagnostic findings on samples, i.e., there is an absence of a quality assurance system.

Similarly, there appeared to be an absence of laboratory notes and methodologies. These may be present but we were not made aware of them.

Task 4: Diagnostic capabilities of RIA No.1: Scientific, technical and hatchery staff: Molluscan diseases

A lack of experience and expertise in laboratory staff was apparent in relation to disease diagnosis in molluscs, especially in the area of histopathology. The lack of experience and expertise was such that it was felt that little confidence could be placed on the reliability and/or interpretation of diagnostic findings.

It was uncertain if laboratory staff understood the importance of access to reference cultures or other reference material for quality assurance purposes and it was unclear if the concept of determining the sensitivity and specificity of assays offered was understood

or indeed applied in diagnostic examinations. It is unreasonable to place the onus of responsibility for disease diagnosis in molluscs on untrained and inexperienced individuals working in relative isolation.

It appeared that there was little concept of a laboratory quality assurance system to support the overall operations of the laboratory and provide a defined basis for scientific and technical staff through the use of laboratory manuals, reference materials and reporting procedures.

It was clear that the RIA No.1 diagnostic laboratory had little if any veterinary input to provide an overview and insight into the nature of disease in the field, the nature of samples required, the nature of laboratory examinations undertaken, interpretation of results and recommendations regarding treatment and control.

Significant training in the areas of mollusc histopathology and bacteriology are necessary in order that disease investigations in molluscs can be conducted in a reliable and competent manner.

Task 5: Internal and external biosecurity strategies at RIA1

Few if any internal controls of movements of live aquatic animals including molluscs appear to exist in Vietnam. Those that do exist appear to be under the jurisdiction of the Department of Animal Health (DAH), however, it appears improbable that any such controls could be, or are being, implemented.

Although regulations appear to exist for the importation of live aquatic animals into Vietnam, it appears that active illegal introduction of live aquatic animals does occur on a regular (routine?) basis but is impossible to stop. Uncertainties exist with respect to what constitutes and illegal importation.

At the regional level, no biosecurity measures could be indentified with respect to the movement of broodstock or seedstock (finfish, molluscs) at the National hatchery or broodstock holding facilities.

No health certification or biosecurity measures were evident for molluscs or finfish entering, leaving or moving within the mollusc or finfish hatchery at RIA No.1.

Laboratory sampling in cases of disease outbreaks within the Hatchery were apparently undertaken well after the event and relied on RIA No.1 staff attending the hatchery for the purpose of sample collection. This process was unlikely to result in an accurate diagnosis of disease. Improved sample collection facilitating rapid diagnosis is required to address spat mortalities within the Hatchery.

The obtaining of broodstock from underdetermined sources, e.g., clams from local markets, and their subsequent introduction into the hatchery in an uncontrolled and unregulated manner present a significant risk of disease incursion into the hatchery.

The situation regarding health certification of broodstock or seedstock remains uncertain. It appears that some form of laboratory examination was undertaken for movements of seedstock from private hatcheries but this was deemed unnecessary from government hatcheries

Despite comments to the contrary (see Task 2 – Regulatory environment), it was clear that there are no internal restrictions on movements of live aquatic animals (fish, crustaceans and molluscs) across Provincial borders within Vietnam as far as we could ascertain. Also despite comments to the contrary (see Task 2 – Regulatory environment), it appears that no requirements exist for quarantine or other risk mitigation measures for aquatic animals coming into Vietnam from other jurisdictions. For example, we were advised that sturgeon which were imported without restriction. It would appear that, although nominal requirements for regulation of imports exists, exact details of any such requirement are uncertain and are not apparently implemented, in part due to the

widespread trade and movement of living aquatic animals which makes practical implementation of quarantine and health certification measures impossible.

Task 6: Human resources supporting regional biosecurity at RIA No.1

Although there appeared to be an awareness of a need for biosecurity measures at the Hatchery and broodstock facilities, at least in senior staff, there appeared to be an overall indifference to the absence of such measures. This was despite there being a strictly controlled and biosecure shrimp production facility on the same site.

There was no perceived expression by RIA No.1 staff of a need for biosecurity within the finfish or mollusc hatcheries in relation to disinfection measures, restriction of visitors, unrestricted movements of broodstock into and within the facility and unregulated release of seedstock.

11.2 Recommendations

1. A central database should be developed and maintained as a basis for defining the contemporary disease status of molluscs in Vietnam. The database, preferably in English and Vietnamese should provide information pertaining to the occurrence, nature and distribution of diseases of molluscs throughout Vietnam and be fully referenced;

2. The cause or causes of the losses in oysters, hard clams and otter clams requires urgent investigation;

3. Expanding the review of the current legislation and regulations pertaining to disease diagnosis and the control of disease in molluscs in Vietnam, by identifying, and having translated into English, Vietnamese government documents relevant to this issue;
4. Clarification of the operational relationships between the Department of Animal Health and the Research Institutes for Aquaculture;

5. A review of the functionality of laboratory equipment essential for the accurate and prompt diagnosis of diseases of molluscs should be conducted and equipment found to be non-operational should be replaced;

6. To improve the reliability of diagnostic findings, a system of quality assurance and quality control based on known and unknown samples should be introduced into diagnostic laboratories;

7. A system to evaluate the diagnostic competence of scientific and technical staff involved in diagnostic examinations on diseased molluscs is necessary as a basis for identifying further training needs in specific diagnostic disciplines;

8. Following on from Recommendation (7), specific training in those diagnostic disciplines for which gaps existed is required;

9. In addition, training in comparative histopathology is urgently needed, focussing on the histopathological diagnosis of molluscan diseases;

10. The development, implementation and maintenance of fundamental biosecurity measures;

11. Training workshops for hatchery staff should be undertaken to identify and describe fundamental, practical biosecurity measures which may be introduced into the operations of the mollusc hatchery and broodstock facility.

12. Following on from Recommendations (9) and (10), after the establishment of biosecurity measures, biosecurity audits should be conducted at least annually in both hatchery and broodstock facilities;

13. Sourcing of broodstock should be done from areas of known health status with health examinations of broodstock and quarantine as appropriate;

14. All disease events occurring within the hatchery, either in broodstock or seedstock should be fully investigated

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13 Appendixes

13.1 Appendix 1: Vietnamese Molluscan Biosecurity and Veterinary Diagnostic Capacity Review QUESTIONNAIRE

13.2 PART 1. REGULATORY INFORMATION REQUEST

i. In Vietnam, which are the key government departments/authorities responsible for fish/mollusc health on a day to day basis?

ii. Does Vietnam have a list of “notifiable diseases”? i.e. diseases of fish/molluscs which if found must be reported to authorities?

iii. Which government department is responsible for the national/international reporting of fish/mollusc disease? For example, if a serious virus was discovered affecting clams, who would report that to the international community or would it not be reported?

iv. Is the same government department(s) responsible for investigating any reported disease outbreak on a fish/mollusc farm?

v. Is there legislation/regulations which identifies when a disease should be reported? If so, is it possible to forward us a copy of this legislation?

vi. Do farmers generally report the finding of a problem on a farm to a government official?

vii. Who do farmers go to if they do have a problem with their fish/molluscs? The laboratory (e.g. RIA #1)?

viii. Are there any benefits for a farmer to report a fish/mollusc problem to a government official?

ix. Can a fish/mollusc farmer move stock from one farm to another farm?

x. Are there any restrictions on movement of fish/molluscs around Vietnam? If there are restrictions, what are the restrictions? Are there documents detailing the nature of these restrictions that we could get a copy of?

13.3 PART 2: PRODUCTION INFORMATION REQUEST

xi. We understand that there is a draft Hatchery Manual that has been developed. Is it possible to get a copy of this Manual?

xii. What is the basic way that the oyster industry currently operates? Can a simple description of the current way oysters move around (e.g. from hatchery to grow out operations) be provided?

xiii. Are there any current restrictions placed on this movement?

xiv. Where did the original Pacific oyster broodstock come from?

xv. How big an industry is the clam industry currently? Are wild clams used as broodstock?

13.4 PART 3: LABORATORY INFORMATION REQUEST

We understand that the two key laboratories involved in fish/mollusc disease diagnosis are RIA#1 and RIA#3. For each of these laboratories (and any others that are also important) could the following be provided?

1. Name of the laboratory
2. The location & address of laboratory
3. The head of the laboratory / Director
4. Ideally Name, title, qualifications and contact details
5. The purpose of the laboratory
    - Disease diagnosis (yes / no)
    - Disease research (yes / no)
    - Extension and training (yes / no)
    - Other (specify)
6. **Which aquatic species are examined at the laboratory**
    - Finfish (yes / no) - details
    - Molluscs (yes / no) details
    - Crustaceans (yes / no) details
7. **Where the samples come from that are used for disease diagnosis or research**
    - From an in-house aquaculture research centre or hatchery (yes / no) details
    - From farmers (yes / no) - details
    - From extension staff (i.e. the laboratories own staff that go out into the field to collect the samples (yes / no) - details
    - Other (specify)
8. **Library and IT Resources**
    - What library and Information Technology (IT) resources does the laboratory have access to?
    - Which journal(s) are held or available to the laboratory (please provide list)
    - Which key texts does the laboratory have access to (please provide list)
9. **With respect to the specimen accessions and recording of the laboratory**
    i. Is a specimens accessions/recording system present / absent (delete as appropriate)?
    ii. If present:
    - Staffing: Can you provide the names of staff and their qualifications
    - What is the nature of recording system (Computer based / hand written - describe)
    - Is the specimens accessions in a dedicated area of the laboratory (yes / no)
    - What are the reporting mechanisms i.e. how reports go back out to the submitter (verbal / written / electronic)
    - What facilities are available – describe: e.g., bench, refrigerator, computer, disinfection
    - Any other comments

10. Does the laboratory have a bacteriology capability?

i. If yes, what are the names of the staff and their qualifications in bacteriology?

ii. What facilities are available – can you please describe e.g., incubators, refrigerator, computer, microscopes, laminar flow cabinets, other (describe)

iii. What bacteriological activities are undertaken:

- Bacterial isolation and identification (describe)
- Media preparation (describe)
- Reference cultures (describe)
- Disinfection and sterilisation (describe)
- Laboratory reporting (describe)
- Other (describe)

11. Does the laboratory have a virology capability?

i. If yes, what are the names of the staff and their qualifications in virology?

ii. What facilities are available – can you please describe e.g., incubators, refrigerator, liquid nitrogen storage, ultradeep freeze, computer, microscopes, inverted microscope, cell lines held, centriguges / ultracentrifuges, laminar flow cabinets, other (describe).

iii. What virological activities are undertaken:

- Virus isolation and identification (describe)
- Media preparation (describe)
- Cell culture maintenance and propagation (describe)
- Disinfection and sterilisation (describe)
- Reference viral cultures (describe)
- Laboratory reporting (describe)
- Other (describe)

12. Does the laboratory have a parasitology capability?

i. If yes, what are the names of the staff and their qualifications in parasitology?

ii. What facilities are available – can you please describe e.g., incubators, refrigerators, computers, microscopes, centrifuges, other (describe)

iii. What parasitological activities are undertaken:

- Parasite isolation and identification (describe)
- Disinfection and sterilisation (describe)
- Reference parasites (describe)
- Laboratory reporting (describe)
- Other (describe)

13. Is there a Pathology / post mortem area

i. If yes, what are the names of the staff and their qualifications in pathology?

ii. What facilities are available – can you please describe e.g., refrigerators, computers, microscopes (compound and stereo), centrifuges, other (describe)

iii. Laboratory activities in pathology/post mortem

- Post mortem techniques (describe)
- Disinfection and sterilisation (describe)
- Sample collection – histopathology, bacteriology, virology, molecular biology, other (describe)
- Laboratory reporting (describe)
- Other (describe)

14. Does the laboratory have Histopathology capabilities?

i. If yes, what are the names of the staff and their qualifications in histopathology?

ii. What facilities are available – can you please describe e.g., refrigerators, tissue processors, slide stainers, computers, microscopes (compound and stereo), centrifuges, other (describe)

iii. What histopathology activities is the laboratory capable of:

- Paraffin embedding and staining (describe)
- Special staining techniques (describe)
- Histopathology microscope reading area (describe)
- Laboratory reporting (describe)
- Other (describe)

15. Does the laboratory have molecular biology (PCR) capabilities?

i. If yes, what are the names of the staff and their qualifications in histopathology?

ii. What facilities are available – please describe e.g., PCR machines, refrigerators, computers, other (describe)

iii. What molecular biology activities does the laboratory carry out?

- PCR (describe)
- Other activities
- Laboratory reporting (describe)
- Other (describe)

16. Does the laboratory have access to other specialist laboratories?

E.g., Toxicology, electron microscopy, nutrition (describe)

17. Diseases of molluscs identified by the laboratory

i. In the following section, can you please identify the diseases the laboratory has been involved with over the past few years.

Viruses and viral diseases

Name	Species	Location and date

Bacteria and bacterial diseases

Name	Species	Location and date

Fungi and fungal diseases

Name	Species	Location and date

Protozoa and protozoan diseases

Name	Species	Location and date

Metazoa and metazoan diseases

Name	Species	Location and date

Are there any other diseases that are of concern to the laboratory but which have not been identified over the last few years?

ii. Why are these diseases of concern?

13.5 Appendix 2: Visit itinerary

Day 1. Monday 2nd July, 2012

Attended RIA No.1 at Ta Son, Bac Ninh with welcomed address by Dr Ninh followed by presentations on the structure and activities of RIA No.1 and the ACIAR project. Following was an inspection of the diagnostic laboratory with Dr Lua, specifically assessing the laboratory and discussion its diagnostic capacity and training needs with Dr Lua. Considerable general discussion regarding health, aquaculture, the environment and disease issues was held over lunch with RIA No 1. staff. The tour of the laboratory continued after lunch, with examination of some case material.

Day2. Tuesday 3rd July 2012

Travelled by car to Haiphong and then by ferry to Cat ba Island. Afternoon inspection of hatchery facilities followed by meeting with staff.

Day 3. Wednesday 4th July 2010

Departed Cat Ba to the RIA No.1 grow out and broodstock facility and viewing, but unfortunately not stopping at, numerous farms en route growing a mixture of finfish and molluscs. Inspected broodstock (including king grouper, cobia, mouse grouper but no molluscs) and visited a nearby small farm and had discussions with the farmer here disease issues were discussed. Long periods of inactivity with a short visit to another nearby farm in mid-afternoon. Left the RIA No.1 facility and went to the neighbouring farm where young grouper were dying and held extensive discussions with the farmer, with some difficulty in translation. Farm staff collected samples of grouper for examination and then we returned to the boat harbour, travelled to the hatchery, examined the dead fish collected from the farm, and held with the Vice Director. No oyster broodstock facilities were inspected Apparent arrangements to visit a private hatchery were unsuccessful.

Day 4 – Thursday, 5 July 2012

Returned to mainland and RIA No.1 after significant delays due to non-operation of ferry resulting in an across island bus trip. Met with Ninh, Tai, Lien and Tua (and Du) and talked at length about what their needs were in the area of health management. Met with Madame Van at airport and held wide-ranging discussions relating to losses in shrimp, unregulated imports of shrimp from China, the need for ACIAR support of mollusc aquaculture and health, especially regarding controls on broodstock, training needs and a need to diagnose diseases occurring in clams.

Travelled to Nha Trang that evening.

Day 5 – Friday 6 July 2012

Visited RIA No.3 and met with Mr Bay and Dr Phuc. General discussions regarding mollusc culture and health issues, specifically the purpose of our visit, diagnostic capacity at RIA No.3, species of native oyster in Vietnam, mortality issues in clams and the reports of Perkinsus as the causal agent, limitations to disease diagnosis in Vietnam, shrimp mortality issues, potential collaborative projects, and environmental issues impacting on aquaculture. At the time, the hatchery at RAI No. 3 was currently undergoing renovation and was not in operation. The hatchery site was inspected.

Visited Nha Trang University, met with Dr Binh, Vice Director of the Biotechnology and held discussion regarding the research at the University and the collaboration with RIA No. 3.

13.6 Appendix 3: LIST OF NOTIFIABLE DISEASE IN VIETNAM

(Attached to the circulars: 83/2011/C-MARD December 09th, 2011 of Vietnam MARD)

No.	Name disease	Agent	Culture spesies
1	White spot disease	White spot syndrome virus (WSSV)	Tiger Shrimp *(Penaeus monodon)*, White Shrimp *(Litopenaeus vannamei)*
2	Taura syndrome	Taura syndrome virus (TSV)	White Shrimp *(Litopenaeus vannamei)*
3	Yellow head disease	Yellowhead virus (YHV)	Tiger Shrimp *(Penaeus monodon)*, White Shrimp *(Litopenaeus vannamei)*
4	Infectious Myonecrosis disease	Infectious Myonecrosis Virus (IMNV)	White Shrimp (*Litopenaeus vannamei*)
5	Infectious Hypodermal and Hematopoitic Necrosis desease	Infectious Hypodermal and Hematopoitic Necrosis Virus (IHHNV)	Tiger Shrimp *(Penaeus monodon)*, White Shrimp *(Litopenaeus vannamei)*
6	Spring viraemia of carp	Spring viraemia of carp virus - SVCV	Carp (*Cyprinus carpio*) Carp Koi (*Cyprinus carpio koi*) Gold fish (*Carassius auratus*) Grass Carp (*Ctenopharyngodon idellus*)
7	Koi herpesvirus disease	Koi Herpesvirus (KHV)	Carp (*Cyprinus carpio*) Carp Koi (*Cyprinus carpio koi*)
8	Viral nervous necrosis/ Viral encephalopathy and retinopathy	Betanodavirus	Grouper (*Epinephelus* spp.) Seabass (*Lates calcarifer*)