Australian Government

Australian Centre for International Agricultural Research

Final report

project

Improving fish health management and production protocols in marine finfish aquaculture in Indonesia and Australia

project number	FIS/2010/101
date published	1/06/2019
prepared by	Michael A. Rimmer (USYD) Richard Whittington (USYD)
co-authors/ contributors/ collaborators	Joy Becker, Navneet Dhand, Paul Hick (USYD) Ketut Mahardika, Sari B.M. Sembiring, Suko Ismi, Zafran, Haryanti, Des Roza, Daniar Kusumawati, Yasmina Nirmala Asih, Sudewi, Indah Mastuti and Ida Komang Wardana (IMRAFE Gondol) Murwantoko, Cahya Kurnia Fusianto (UGM) Mardiana E. Fachry (UNHAS)
approved by	NA
final report number	FR2019-47
ISBN	978-1-925747-23-2
published by	ACIAR GPO Box 1571 Canberra ACT 2601 Australia

Contents

1 Acknowledgments

Project staff gratefully acknowledge the support of hatchery operators and sea cage farms in northern Bali, who kindly provided access to their hatcheries / farms for sampling.

We thank Alison Tweedie for her support for the ring-testing workshop, and technical assistance at the University of Sydney, and Marion Saddington for administrative support of the project.

We gratefully acknowledge the assistance of the following persons in sampling sea cage farms: Imran Lapong, Baharuddin Angke, Bakhtiar Sah Putra, Arga Kurniawan, Sigit Surya Putra, Afri Herlambang, Desy Putri Handayani, Sabila Rahmawati, and Sri Laksono Condro Negoro.

We thank current and previous staff of IMRAFE Gondol, particularly Dr Tri Heru Prihadi, Dr Rudhy Gustiano, Bambang Susanto, Dr Gede S. Sumiarsa and Jhon H. Hutapea. We also thank the technical staff in the IMRAFE Gondol Pathology and Biotechnology Laboratories and the technical staff of the Biosecurity Hatchery of IMRAFE Gondol. We also thank the following UGM staff: Mr. Indrianto and Mr. Nur Ari Purnomo (project administration) and Mr. Sugiman (transportation and technical support).

We thank the participants in feeds workshop: ABILINDO / P4B members: Wajan Sudja (Secretary General, ABILINDO), Usama Umar Al Hadadi (Oye), Hasnil Hawadis, Rastian A. Nazir, Effendy Wong (Aseng), Bangun Sitepu, Muhibbudin Kotto (Budi Fantygo) and Ketut Brewok (Ketut Bagus Irawan); feed company representatives: Denny D. Indradjaja, Andhikajaya (PT. Cargill Indonesia), Ekky Rizki Abadi, Purnomo, Rudy Purwono (PT. Matahari Cipta Sentosa), Erwin Suwendi (PT Suri Tani Pemuka / JAPFA), Fauzan (PT Skretting / Trouw Nutrition); aquaculture and nutrition researchers: Ketut Sugama (CARD Jakarta), N.A. Giri and Wawan Andriyanto (IMRAD Gondol), Asda Laining and Usman (RICA Maros), Dr Dedy Yusadi (IPB Bogor). Thanks also to Mrs Suliyanti Hakim for logistical support for the workshop.

The success of the ring-test was due to the assistance of staff from LP2IL Serang who helped with communication and distribution of the samples (Pak Toha Tusihadi, Pak Aziz Hakim, and other staff). Dr Murwantoko (Gadja Mada University) provided instructions and discussion in Bahasa Indonesia at the time of sample distribution. We also thank Dr Maskur (Director of Fish Health and Environment) and Mr Slamet Subiyakto (Director General of Aquaculture) for their support of this ring-testing program.

We thank Dr Stewart Fielder and the staff of the Marine Finfish Hatchery at Port Stephens Fisheries Institute, particularly Luke Cheviot, Luke Vandenberg, Joseph Clarke and Brooke McCartin for providing training in best practice for marine finfish hatchery technology and Ms Jo Pickles for administrative support.

We thank David Vaughn, James Cook University, for providing species identification of *Zeylanicobdella arugamensis*

We thank the mid-term reviewers, Dr Stewart Fielder and Prof. Dr Ketut Sugama, for their contribution to the project.

2 Executive summary

Aquaculture of marine finfish (grouper, barramundi, and others) is developing rapidly in Indonesia. For example the government targeted an increase in national production of farmed grouper from 5,300 tonnes in 2009 to 20,000 tonnes in 2014 and ambitious growth targets remain in place. However, fish diseases are a major cause of production losses in marine finfish aquaculture in Indonesia, with typical losses of 40–50%. Thus production could be doubled by removing the burden of disease.

A scoping study was carried out by University of Sydney staff under SRA FIS/2011/038 and the researchers found that grow-out farms had problems with several major disease syndromes, but none were characterised sufficiently so as to make definite recommendations about disease control. A significant issue was lack of capacity for accurate and timely disease diagnosis, and a related problem was over-reliance on PCR and under-utilisation of multi-disciplinary, case-managed, disease investigation tools and epidemiological approaches. There was industry concern regarding the lack of biosecurity in hatcheries in the Gondol area in Bali, and disease transmission from hatcheries. Many of the issues identified by the industry were inter-linked: better quality fingerlings are more robust and demonstrate improved survival and faster growth while the use of pellet feed instead of 'trash' fish may reduce the incidence of disease. Consequently this project adopted a whole-of-production-chain approach to evaluating production losses in marine finfish aquaculture in Indonesia.

The objectives of the project were in four key areas: identifying the key diseases affecting hatcheries and grow-out farms, testing interventions and improved management protocols including compounded diets, evaluating socio-economic aspects of disease and building capacity in fish health management in Indonesia.

A key outcome of this project was improving the diagnostic pathway to properly position PCR results in context with the results of gross pathology, histopathology and parasitological examinations to enable an accurate diagnosis. Single pathogen diseases as well as diseases of uncertain cause associated with combinations of environmental factors, management factors, nutritional factors and background burdens of viruses and parasites were identified at both hatchery and sea cage levels. It is recommended that leadership structures be identified and supported for fish disease diagnosis in Indonesia, together with ongoing training of staff through future projects in fish health.

Nervous necrosis virus (NNV) was a frequent cause of disease and economic loss in grouper hatcheries, where impacts were severe. This led to a major recommendation that control of NNV is a priority for hatcheries; this requires further research on routes of transmission in order to prioritise biosecurity for broodstock (testing prior to entry) or for the hatchery, including treatment of its water supply. However, it is recommended that research be undertaken on improving water treatments to exclude pathogens from hatcheries. In contrast, NNV infection in grower fish in sea cages was mostly incidental, and probably represented harmless residual infections from the hatchery stage. It is recommended that testing for NNV in fish from sea cages be discontinued because the results will not influence disease control recommendations.

The role of iridovirus (megalocytivirus, MCV) in disease outbreaks in both hatcheries and sea cages was unclear, despite this virus being frequently discussed by farmers and others in the context of disease outbreaks. Both viruses were present in the environment (in both the fish and biofouling living on and around the sea cages) and in trash fish, highlighting the importance of biosecurity throughout the susceptible life history stages in hatcheries and nurseries. Further research to clarify the role of MCV in disease outbreaks is recommended.

Ammonia and nitrite toxicity due to poor water quality management were also important causes of morbidity and mortality in hatcheries. It is recommended that research be undertaken on improving water quality management during larval rearing to reduce the

impact of ammonia and nitrite toxicity on fish health. This will improve overall disease resistance. Improvements are needed to reliably and consistently reduce the impact of ammonia-nitrogen toxicity on fish health. In backyard hatcheries protozoans (*Amyloodinium* sp.,*Trichodina* spp.) and a scuticociliate were important and may have had interactions with viruses.

Deformities due to multifactorial nutrition/environment interactions caused losses in hatcheries. It is recommended that the role of micronutrient deficiencies (Vitamins A and C) be investigated specifically, together with a whole system approach to investigate the causes and prevention of skeletal deformities Future research should include a focus on improvements in larval nutrition. Not surprisingly residual deformities from the hatchery stage appeared in fish in sea cages and this issue should be addressed initially by improved nutritional and husbandry practices in the hatchery.

In sea cage grouper, chronic non-specific external and visceral lesions were a problem and were likely of multifactorial aetiology. There was a large difference in the prevalence of these chronic diseases between farms indicating an opportunity to explore management solutions.

Studies are required on hatcheries and sea cage farms in other regions of Indonesia to determine the extent to which the results of this project can be applied elsewhere.

Metazoan ectoparasites (*Neobenedinia* sp., *Benedinia* sp., *Pseudorhabdosynochus* sp. and *Haliotrema* sp.) were important causes of morbidity in sea cage fish. One of the key outcomes from this study was to identify the lack of efficacy of the bathing treatments in eliminating parasites from fish in sea cages. There is a need for research to identify the severity and persistence of parasites, determine the recruitment or re-infestation of key parasites and for clinical trials to identify effective integrated parasite management strategies, such as the use of multiple chemical treatments with changes to farm management (e.g. fallowing, net changes, cleaning, and farm location).

A syndrome called green-eye disease was unrelated to the viruses or parasites, but leeches (*Hirudinea* sp.) were abundant and the syndrome had a complex causation interconnected with treatments applied by farmers. The role of anti-parasitic treatments in sea cages should be investigated in relation to the clinical signs of green-eye disease.

Research is also recommended to identify suitable farm biosecurity practices that can be used alone and in combination with effective parasite management to reduce the introduction and impact of aquatic pathogens. Improving farm biosecurity, including proper disposal of sick and moribund fish, and integrating parasite management strategies will create healthier fish populations, reduce farm chemical inputs and support sustainable production.

Numerous constraints to adoption of compounded diets were identified and pathways must be identified for establishment of demonstration farms on which research to overcome poor growth rates and lack of palatability of pelleted diets could be undertaken. Formal cost-benefit analysis on trash fish and pellet feed, including social and environmental costs should also be undertaken.

The results of socio-economic research on aspects of disease were enlightening. While disease was confirmed to be a major problem at both hatchery and sea cage stages of production, especially for grouper, there was very limited adoption of biosecurity practices and myths abounded about diseases affecting grouper. The views of farmers contrasted dramatically with the objective results from systematic epidemiological research. Farmers believed NNV and MCV (iridovirus) were major problems in sea cages. However, farmers correctly identified parasites as an issue affecting productivity. Only 7% consulted with government technical experts to resolve problems and most disposed of sick and dead fish by dumping at sea, a practice which would ensure ongoing outbreaks of disease on the farms.

During this project Indonesian fish health professionals form a variety of institutions received diverse, intensive training over the life of the project in workshops in both Australia and Indonesia. On-the-job training was also provided during field trials and laboratory work throughout the project, and again this was a highlight for everyone involved. Reciprocal academic visits enabled interactions of university academics from both countries in both undergraduate and post graduate teaching and research in situ. Exchange visits and guest lectures were proposed as follow on activities, as well as student exchanges. At a more technical level, an intensive activity was completed successfully to evaluate PCR test competency in most eligible DGA laboratories in Indonesia and it concluded with strong support expressed for further ring testing and establishment of a reference laboratory network.

3 Background

Aquaculture of marine finfish (grouper, barramundi, etc.) is developing rapidly in Indonesia. The government has targeted an increase in national production of farmed grouper from 5,300 tonnes in 2009 to 20,000 tonnes in 2014 (an increase in production of 30.5% per annum) and barramundi from 4,600 tonnes to 8,500 tonnes over the same period. Kongkeo et al. (2010) note that most marine finfish farms in Indonesia can be categorised as medium-scale (20–100 cages), with fewer small-scale (<20 cages) and large-scale (>100 cages) farms.

Discussions with Ministry of Marine Affairs and Fisheries (MMAF) staff and farmers, including ABILINDO, have confirmed that fish diseases are a major cause of production losses in marine finfish aquaculture in Indonesia. ABILINDO members estimate that survival from stocking fish in the cages through to harvest is 40–50%. A scoping study carried out by University of Sydney staff during October – November 2011 (under SRA FIS/2011/038) found that grow-out farms reported problems with several major disease syndromes: fin rot, likely due to bacterial infection; 'parasit hitam' (black parasite), the cause of which is not clear; and 'iridovirus'. Farmers report that disease-related mortality is highest during the southern hemisphere winter (July – September) when water temperature drops, and during periods of unsettled weather.

Most fingerlings cultured in grow-out cages in Indonesia come from hatcheries in northern Bali or East Java. The Indonesian aquaculture industry estimates that hatcheries in Indonesia currently produce 32 million grouper fingerlings per annum, with an estimated direct value of AUD 6.75 million. Many fingerlings are also exported to other countries in Asia. However, there is a general concern that the 'quality' of fingerlings – particularly in regard to their disease status – is declining. The scoping study found that there was substantial industry concern regarding the lack of biosecurity in hatcheries in the Gondol area in Bali, and disease transmission from hatcheries. Industry representatives described the local hatcheries as being 'full of disease'. One major barramundi grow-out farm in northern Bali has discontinued buying fingerlings from local hatcheries due to disease concerns, and is now buying small fingerlings from Singapore. They report that the Singapore-sourced fingerlings grow much faster and survive better than the locally-produced fish. Some grouper farmers have also discontinued purchasing fish from the Gondol area hatcheries and are instead buying from hatcheries in East Java, which they regard as being better managed and having better quality fingerlings.

Based on this there is a clear need to develop improved management protocols for marine finfish aquaculture in Indonesia, particularly in the hatchery and grow-out sub-sectors. Many of the issues identified by the industry are inter-linked; for example, better quality fingerlings are reported to be more robust and demonstrate improved survival and faster growth; the use of pellet feed instead of 'trash' fish may reduce the incidence of parasite outbreaks (Rückert et al. 2009) and 'trash' fish is also a likely conduit for introducing bacterial and viral diseases to cultured fish. Consequently there is a need to adopt a whole-of-production-chain approach to reducing production losses in marine finfish aquaculture.

In addition to ongoing losses due to regular disease events, there are occasional severe outbreaks of disease that devastate individual farms, or groups of farms. For example, between late 2010 and late 2011 a single large barramundi farm near Batam, Riau Islands, experienced the loss of 30% of its standing stock (300 out of 1,000 tonnes) due to 'scale-drop syndrome' – a disease with unknown aetiology. Direct economic losses on this farm alone are estimated at around AU$ 1 million, and the syndrome has occurred (albeit with less devastating impacts) on other barramundi farms in Indonesia.

At the ACIAR – Indonesia Country Consultation held in Bogor on 30 November 2011, MMAF presented six priorities for Aquaculture R&D, of which one was 'Development of disease management, monitoring, controlling and surveillance systems for fish health and

environment'. This project directly addresses this MMAF priority. In the broader economic development context, the project also supports the Indonesian Government's Masterplan for Acceleration and Expansion of Indonesia Economic Development 2011–2025 (MP3EI), which proposes the development of six economic corridors to support and improve regional economic development. This project will focus its research and development activities in the Bali – NTB corridor. The outcomes of the project will directly support the MP3EI strategies for the Bali – NTB economic corridor relating to increasing fisheries [including aquaculture] production, and developing high-quality seedstock (MP3EI, p.149).

There is an active industry association – the Indonesian Mariculture Association (Asosiasi Budidaya Ikan Laut Indonesia – ABILINDO) comprised mainly of medium-scale farmers, and a few large-scale farmers. ABILINDO provides farmers with a structure to collectively lobby government for legislative change, and provides a forum for distribution of information to members, as well as to non-member farms. The project involved ABILINDO members directly in the research, to provide a conduit for communication and adoption of research results. ABILINDO members are keen to adopt technologies that will improve their profitability and, through industry linkages, will be able to influence non-member farmers.

4 Objectives

1. To identify key diseases through longitudinal epidemiological studies on hatcheries and grow-out farms
    1.1. Determine the key diseases responsible for production losses in marine finfish aquaculture;
    1.2. Plan and, where appropriate, trial interventions to reduce production losses.
2. To develop and test improved management protocols for hatcheries and grow-out farms
    2.1. Test and implement improved hatchery practices, including biosecurity and improved larval nutrition;
    2.2. Improve farm productivity and market access through the adoption of compounded diets.
    2.3. Evaluate socio-economic aspects of practice changes
3. To build capacity in fish health management in Indonesia
    3.1. Provide training in epidemiology and disease diagnosis for Indonesian fish health professionals;
    3.2. Provide improved fish health education services at university level;
    3.3. Support ‘ring-testing’ / validation studies for PCR laboratories in Indonesia.

5 Methodology

5.1 Key diseases responsible for production losses in marine finfish aquaculture

5.1.1 Longitudinal epidemiological study of hatcheries

During the consultation process, it was planned that the longitudinal epidemiological survey would sample fish at two time points, around the time of greatest expected mortality (between 20 and 35 days). Unfortunately, this design was not feasible because obtaining a representative sample of fish prior to metamorphosis was not compatible with acceptable husbandry for these species. Therefore, sampling was limited to grading, which was approximately at 35 days after hatch.

Although sampling at grading provides a representative sample of the population being graded, these populations are not representative of the populations produced at the hatchery. Sampling only at grading would be strongly biased to the most successful production runs (some instances of all-or nothing-production); the healthiest individuals (most mortality occurs < 35 days) and at the exclusion of those which grow the quickest (sold in early grades). Fish that are considered to be sick are not graded for fear of making the disease worse.

Therefore, we used two approaches to address the objective of identifying the key diseases as described below. The target population was defined as all hatcheries producing grouper in the Gondol area and East Java.

a) A longitudinal cross sectional survey using systematic random sampling with a sample size of 36 fish per test per population, which allowed for a design prevalence of 8 to 10%.

b) A targeted sampling of disease events with a goal of 8 events per season. For each disease event, 6 diseased fish and 30 conspecifics per test were selected.

Parasitology

Grouper were returned to the laboratory live and processed in small batches so that fresh preparations were examined immediately. Fish were examined for ectoparasites consisting of a visual examination of external surfaces under a dissecting microscope, a mucus scrape, fin clip and gill mount.

Molecular detection of viral pathogens

Grouper were returned to the laboratory live and processed in small batches. For fish that were 25 days after hatch or older, the head (for NNV testing) and body (for MCV testing) were dissected and stored separately at -80°C. For fish less than 25 days after hatch, they were stored at -80°C as whole fish and tested for NNV and MCV. Nucleic acids were extracted from the pooled tissues for quantitative PCR testing for MCV (Rimmer et al. 2012) and NNV (Hick and Whittington 2010).

Histopathological examination

Grouper were returned to the laboratory live and processed in small batches. Grouper were euthanized and placed as whole fish into 10% neutral buffered formalin (x10 volume) for at least 24 hours. Whole fish or target tissues (eye/brain and internal viscera) were dissected and placed in cassettes for standard H&E processing. Histology sections were examined by an experienced pathologist (Dr Mahardika). The microscopic examination was targeted for NNV and MCV specific lesions and for general pathological changes (e.g. necrosis, presence of melano-macrophage centres, and presence of parasites).

5.1.2 Longitudinal epidemiological study of grow-out farms

Study Design

A repeated cross-sectional survey was undertaken with four sampling events over a period of 12 months. Grouper seacage farms in Pegametan Bay, north Bali formed the sampling frame from which the three farms most suited to the sampling activities were purposively selected for participation. Farm level details were recorded at each visit and the production units at the farm were divided into populations defined as all fish of the same species (or hybrid) of the same age. A representative sample of fish was obtained from 10 populations at each sampling event. The sample size was determined to be 36 fish per population to be 95% confident of detecting diseases present at a prevalence of 10% or more.

Sampling

Random selection was achieved by accessing the fish when they were being handled for routine parasite treatment by bathing in freshwater. The fish became unable to move relative to each other as the sea cages were drawn up so that they could be selected at random according to position. The selected fish were euthanized with an overdose of clove oil in seawater.

Parasitology was conducted using samples collected prior to euthanasia. A mucous scrape was prepared from both lateral sides of fish, a gill biopsy was taken from the left second branchial arch and a fin clip included the tail, dorsal and pelvic fin margins. Each specimen was mounted on a glass slide, mixed with a small volume of seawater and a cover slip was applied before examination at 10 - 100 times magnification with a digital microscope at 640x480 pixels resolution. Parasite presence was recorded to genus level and specimens were preserved in ethanol for later speciation if required. Intensity of parasite burdens were scored categorically.

Necropsy was performed immediately after euthanasia using a new disposable sterile scalpel blade for each fish with instruments and a work surface that had been disinfected with 500 ppm sodium hypochlorite and rinsed with water. Detailed observations were recorded on individual necropsy forms. The procedure progressed from noting external lesions to excision of the branchial arch and gills. The left lateral abdominal wall was removed for examination of internal organs. The eyes were sampled before opening the cranial cavity. For each organ a specimen was preserved in 90% ethanol and another in 10% seawater formalin. Specimens were routinely preserved for: gill, heart, liver, spleen, anterior kidney, posterior kidney, eye, and brain. Specimens from the swim bladder, musculature and gastrointestinal tract were sampled when specific gross lesions were observed.

Laboratory tests

Tests for NNV and MCV. Tissue homogenisation and nucleic acid purification was undertaken at UGM by grinding tissues to a 1/10 w/v in PBS. The homogenate was centrifuged at 12,000 g for 1 min and the supernatant was retained. Nucleic acids were purified in duplicate from individual samples using the High Pure Viral Nucleic Acid Extraction Kit (Roche). Nucleic acids were stored at -20°C at UGM and the duplicates were transported on dry ice to the University of Sydney and stored at -80°C.

Real-time PCR (qPCR) assays were conducted on pools of 5 nucleic acids from fish within the same population using an Mx3000 real-time PCR system and fluorescent data analysis software (Stratagene). Tests for MCV were conducted according to the method described by Rimmer et al. (2012). Duplicate 25 µl reactions were prepared with the Quantitect SYBR qPCR kit (Qiagen). The assay for NNV was described by Hick and Whittington (2010) and used hydrolysis probe detection chemistry prepared using the AgPath ID one-step reverse transcriptase qPCR kit (Life Technologies).

A targeted approach was used to select specimens for histopathological analysis based on the presence of patterns of disease identified through analysis of the necropsy data. Formalin preserved tissues were prepared according to standard histological techniques at UGM or IMRAD Gondol and examined under light microscopy by several operators using a multiheaded microscope at the University of Sydney.

Statistical analysis.

Necropsy results were aggregated as the presence or absence of any grossly observed abnormality in an excel spreadsheet. The frequency of lesions with each explanatory variable was compared using the likelihood ratio Chi square tests. The total length and weight of the fish were used to derive Fulton's condition factor: (KF) = $100\times(W\times L^{-3})$ where W = weight in grams and L= total length in cm. These numeric statistics were compared for each explanatory variable using the means procedure and the linear mixed modelling approach in SAS. Values for the explanatory variables for the individual fish in the pools tested by PCR were averaged and used as the input for analysis of NNV and MCV infection prevalence. NNV and MCV infection status were analysed using a generalised linear mixed model using the GLIMMIX procedure with a logit link function for data considered binomial as positive or negative .

5.1.3 Intensive sampling of disease events in sea-cages, targeting the first month after fish are introduced into the cages

Background

Farmers reported that acute outbreaks of high mortality disease accounted for much of the lost production in grouper seacage farms. These high impact disease events were of short duration and not evident during the longitudinal survey that identified chronic production limiting disease syndromes. The acute disease events were reported to occur most frequently in fish within the first month after transfer to the seacages and during the rainy season. A targeted approach was used to collect information and samples suitable for investigating these diseases.

Study design

The sampling frame for the survey was all grouper sea cages in Pegametan Bay during the rainy season of 2016. Farmer reporting of disease outbreaks was encouraged through a series of workshops called to discuss disease problems. Project staff from UGM were stationed in the area ready to implement the sampling strategy immediately when farmers reported disease. Farm visits began by recording farm management information and identification of affected and unaffected populations of fish. Farmer reports of clinical signs and estimates of mortality were recorded for each population.

Sampling

Each population was examined for clinical signs of disease. A sample of 36 fish was obtained using a targeted approach to collect fish showing the typical clinical signs (n=24) and fish from the same cage that were apparently healthy (n=12). These fish were euthanised using clove oil and a complete necropsy was undertaken including photographs to record gross lesions. Microscopic examination and enumeration of ectoparasites was performed by examination of a mucous scrape, gill biopsy and fin clip according to the methods described for the longitudinal disease survey.

Samples from the following tissues were preserved for laboratory tests: brain, heart, gill, retina, liver, spleen, anterior kidney, posterior kidney, stomach, intestine and any cartilaginous, integument or muscle tissue in which lesions were evident. Tissues were divided and preserved in 95% ethanol for molecular tests and in 10% neutral buffered formalin for histopathology.

PCR tests for MCV and NNV were conducted on a pool of kidney-liver-spleen or brain and retina, respectively. Formalin fixed tissues were trimmed, embedded in paraffin and processed for routine H&E staining.

Statistical analysis

Necropsy results were aggregated as the presence or absence of any grossly observed abnormality in an excel spreadsheet. The frequency of abnormalities was determined for individuals classified as affected and unaffected by the disease and compared using the likelihood ratio Chi square tests. The total length and weight of the fish were used to derive Fulton's condition factor: (KF) = $100\times(W/L^{-3})$ where W = weight in grams and L= total length in cm. These numeric statistics were compared for each explanatory variable using the linear mixed model approach.

5.1.4 Interventions to reduce production losses

On-farm parasite reservoirs and the efficacy of the freshwater bathing routine

Protocol to determine the efficacy of freshwater bathing

Grouper were examined immediately before and then again at 1 and 24 hour(s) after a parasite bath treatment. An external parasite examination was completed including a mucus scrape and a gill biopsy. The chemical chosen and its application (e.g. dose and treatment time) was at the discretion of the farm manager. At each farm, the farm manager provided information regarding the fish populations present on the day and the ones selected for sampling (Table A8, Appendix 1). The data were analysed using generalized mixed models with farm and populations random effects. Apparent prevalence was calculated with exact 95% confidence intervals.

At each farm, a population of fish was defined as individuals of the same species and age. Immediately prior to bathing (Time 0), 30 fish were randomly selected in a manner so that there were equal numbers of fish chosen from each cage being bathed on that day (e.g. 5 fish from 6 cages). If more than six cages were being bathed, then a random numbers table was used to choose six cages. In order, each fish was subjected to a gill clip, mucus scrape (left side), total length measurement, external examination for leeches and gross pathology and returned to a holding cage without anaesthesia.

The group of 30 fish was bathed according to the farm practices (Table A8, Appendix 1). At one hour after the bath (Time 1h), the same 30 fish were sampled as above with the right side of the fish scraped for a mucus sample. The samples before and after bath were not matched. At 24 hours following the bath, 30 additional fish were randomly selected from the same cages and sampled as above.

The gill mounts and mucus scrapes were examined on slides under a light microscope for up to 3 minutes per slide and the number observed for each parasite species was recorded. For the gill mount, the number of filaments and the proportion of the filament on the slide was recorded. Representative parasite specimens were fixed in ethanol (70%).

Protocol to identify potential pathogen reservoirs

At each farm, 10 'trash' fish were opportunistically collected from the available 'trash' fish at the farm on the day of sampling. Further, 30 sentinel fish were opportunistically collected using a spear gun and hook and line. Fish were photographed, total length measured and a pool of tissue of brain/eye, spleen and liver (approximately 1-2 g) was dissected and fixed in 70% ethanol. At each farm, 30 samples of biofouling were opportunistically collected. A scalpel blade was used to scrape nets and equipment around cages to attain about 1-2 g each. The material was fixed in 70% ethanol (5 mL tube). Nucleic acids were extracted from the pooled tissues and biofouling samples for

quantitative PCR testing for MCV (Rimmer et al. 2012) and NNV (Hick and Whittington 2010).

5.2 Improved management protocols for hatcheries and grow-out farms

5.2.1 Improved hatchery practices, including biosecurity and improved larval nutrition

During the project, eight batches of hybrid grouper were cultured in the demonstration hatchery at IMRAD Gondol (Table 4). For the most part, the procedures and guidelines published in the best practice manual (Sugama et al. 2012) were followed for feed and water management. Sea water flowed from the estuary to a sedimentation tank and then was drawn to a pressure sand filter, a gravity sand filter and then flowed to the production tanks.

5.2.2 Adoption of compounded diets to improve farm productivity and market access

Workshop on Improved Feeds Adoption for Marine Finfish Aquaculture

A workshop was held in Bali on 8 September 2015 to engage grouper farmers, feed companies and researchers in evaluating commercial grouper feeds and to provide mechanisms for all three stakeholder groups to exchange information about feed performance. Through building this collaboration, we hoped to improve the adoption of compounded feeds by the Indonesian grouper farming industry.

The workshop was attended by 8 experienced grouper farmers (including 7 representatives of ABILINDO with farms located from Medan in the west to Sumbawa in the east), representatives of 5 commercial feed companies, the Chairman of the Indonesian Feedmills Association (GPMT), and nutrition researchers from IMRAD Gondol, RICA Maros and IPB Bogor.

5.2.3 Evaluation of socio-economic aspects of practice changes

The study was conducted in Gerokgak Sub-district, Buleleng Regency, Bali. The study comprised two stages: an initial needs assessment evaluation which took place from January to March 2014, followed by the full survey from October 2015 to January 2016.

The initial study aimed to obtain a general overview of the management of hatcheries and sea cage farms to inform questionnaire development. This was done by conducting interviews with five hatcheries and four sea cage farms. The responses were used to formulate the questionnaire used in the full study. To ensure the validity of the questionnaire, it was pre-trialled with three hatcheries and three sea cage farms in mid-2015. Some minor revision of the questionnaire arose from this pre-test.

The questionnaire was divided into six sections, covering: baseline information (10 questions), production process (10 questions), fish health and disease (16 questions), biosecurity practices (23 questions) and staffing and education (3 questions).

The full survey used the revised questionnaire to interview representatives of 17 hatcheries and 15 sea cage farms. The hatcheries and sea cage farms were randomly selected from a pool of 57 hatcheries and 38 sea cage farms in the Buleleng area.

This socio-economic evaluation uses two data sources: primary and secondary data. The primary data was obtained as described above from the interviews with staff of 17

hatcheries and 14 or 15 sea cage farms. Secondary data was in the form of documents that were obtained from government offices, on-line research, and publications.

5.3 Building capacity in fish health management in Indonesia

5.3.1 Training in epidemiology and disease diagnosis for Indonesian fish health professionals

A range of training covering aspects of epidemiology and disease diagnostic procedures was provided through the project to staff of IMRAD Gondol and UGM in:

- Aquatic Animal Epidemiology, including sampling techniques for hatcheries and sea cages;
- Real-time PCR analysis techniques;
- Histopathology,
- Statistics, focussing on data analysis for large data sets;
- Scientific writing.

In addition, as recommended by the mid-term review, training in best practice management of marine finfish hatcheries was provided to staff of IMRAD Gondol.

Additional training is being provided through the provision of a John Allwright Fellowship to Cahya Kurnia Fusianto (UGM). He will undertake a PhD study on the topic: 'Applications of epidemiology and molecular technology for improved management of MCV infections in aquaculture' at the University of Sydney.

5.3.2 Improved fish health education services at university level

Reciprocal visits of academics from Australia to the university environment in Indonesia, and vice versa were included in the project plan to observe undergraduate and post graduate teaching and research in situ and participate in discussions about organisational structures, physical resources, curriculum and pedagogical approach.

5.3.3 'Ring-testing' / validation studies for PCR laboratories in Indonesia

Study design

Panels of heat inactivated samples were prepared at the University of Sydney by spiking measured amounts cell culture grown virus into fish tissue homogenates. The panel of samples included a known positive and 6 unidentified samples (5 positive and 1 negative) for each of 2 viruses:

1. NNV (NNV) is common in Indonesia where it causes the disease viral nervous necrosis. Many participating laboratories routinely test for NNV.
2. Epizootic Haematopoietic necrosis virus (EHNV) has not been reported in Indonesia and few of the participating laboratories had tested for EHNV prior to this program.

The ring test was designed to be challenging so that factors associated with accurate PCR tests could be identified. This was achieved by:

- using two very different target viruses (RNA or DNA genome) and requiring the implementation of a test that was not in routine use at most laboratories;
- blind coding the unidentified samples so that laboratories could not compare results;
- using samples that resembled real diagnostic samples and required purification of nucleic acids from a fish tissue sample matrix;
- varied amounts of virus in positive samples including some with only small amounts.

The samples were heat inactivated and freeze dried providing a format that was considered ideal for the ring-test because this ensured the samples were:

- stable and could be distributed at low-cost to participating laboratories throughout Indonesia;
- not infectious and posed no biosecurity risk;
- contained complete viral genetic material so that a variety of PCR tests could be used;
- abundant for multiple tests and ongoing staff training.

The samples were distributed at the completion of the “Aquatic pathogen PCR workshop and ring-testing program” hosted by LP2IL, Serang in November 2013. Detailed instructions for transport, storage and reconstitution of the samples were provided by a native speaking workshop facilitator and as English language documents with a summary in Bahasa Indonesia.

6 Achievements against activities and outputs/milestones

Objective 1: To identify and develop fish health management strategies to reduce the impacts of key fish diseases

No.	Activity	Outputs/ milestones	Completion date	Comments
1.1	**Determine the key diseases responsible for production losses in marine finfish aquaculture**			
1.1.1	Undertake a longitudinal epidemiological study of hatcheries (Gondol area, Bali)			
1.1.1.1	Sample hatcheries for PCR, histo, parasitology (IMRAD Gondol)	Sampling completed by Jul-2014	S1: Mar-2014 S2:Jun-2014 S3: Oct-2014 S4: Nov-2014	
	Sample disease events (IMRAD Gondol)	Sampling completed by Jul-2014	Jun-2014 – Jun-2015	Two targeted samplings were undertaken by IMRAD Gondol staff.
1.1.1.2	Sample processing and diagnostics (IMRAD Gondol, USYD)	Sampling processing and diagnostics completed by Oct-2014	S1: Mar-2014 S2: Jun-2014 S3: Oct-2014 S4: Nov-2014	PCR analyses from the epidemiological study were done in conjunction with PCR training (3.1)
1.1.1.3	Data analysis (USYD, IMRAD Gondol)	Data analyses completed	Apr-2017	Complete data sets used for the Data Analysis and Scientific Writing Workshop held at IMRAD Gondol in January 2017.
1.1.1.4	Intensive sampling based on Y1 & Y2 results (IMRAD Gondol)	Additional sampling completed	May-2015 – Apr-2017	Intensive sampling of fish reared in the demonstration hatchery.
1.1.1.5	Data analysis (USYD, IMRAD Gondol)	Data analyses completed	Jun-2017	
1.1.2	Undertake a longitudinal epidemiological study of grow-out farms.			
1.1.2.1	Sample farms for PCR, histo, parasitology (UGM)	Sampling completed by Jul-2014	S1: Mar-2014 S2: Jun-2014 S3: Oct-2014 S4: Dec-2014	
	Sample disease events (UGM)	Sampling completed by Jul-2014	Jul-2016 – Mar-2017	Targeted sampling undertaken on farms during routine sampling events.
1.1.2.2	Sample processing and diagnostics (UGM, USYD)	Sampling processing and diagnostics completed by Oct-2014	S1: Mar-2014 S2: Jun-2014 S3: Oct-2014 S4: Dec-2014	PCR analyses from the epidemiological study were done in conjunction with PCR training (3.1)
1.1.2.3	Data analysis (USYD, UGM)	Data analyses completed	Apr-2017	Complete data sets used for the Data Analysis and Scientific Writing Workshop held at IMRAD Gondol in January 2017.

1.1.2.4	Intensive sampling based on Y1 & Y2 results (UGM)	Additional sampling completed	May-2015 – Apr-2017	Additional targeted sampling of disease outbreaks undertaken by UGM in late 2016.
1.1.2.5	Data analysis (USYD, UGM)	Data analyses completed	Jun-2017	
1.2	**Plan and, where appropriate, trial interventions to reduce production losses**			
1.2.1	Review findings of epidemiological surveys and plan appropriate responses (USYD, IMRAD Gondol, UGM)	Planned interventions for major diseases impacting production.	Apr-2015 – Jun-2015	Undertaken as part of the Project Meeting held at Gadjah Mada University, 10 August 2015
1.2.2	Implement appropriate interventions based on research findings (USYD, IMRAD Gondol, UGM)	Limited interventions undertaken.	Jul-2015 – Jun-2017	Investigation of on-farm parasite reservoirs and evaluation of the efficacy of the freshwater bathing routine currently used by farms undertaken in January 2017.

Objective 2: To develop and test improved management protocols for hatcheries and grow-out farms

No.	Activity	Outputs/ milestones	Completion date	Comments
2.1	**Test and implement improved hatchery practices, including biosecurity and improved larval nutrition**			
2.1.1	Modify hatchery at IMRAD Gondol (IMRAD Gondol)	Hatchery modifications completed.	Nov-2014	Modifications completed November 2014.
2.1.2	Trial improved hatchery production techniques (from FIS/97/73 and FIS/2002/077) (IMRAD Gondol)	Comparative larval rearing trials ('standard' vs. 'best practice') completed.	Jun-2015	A total of 8 trials undertaken using the demonstration hatchery.
2.1.3	Trial interventions to reduce disease-related losses (from 1.1) (IMRAD Gondol)	Trials implementing project findings to improve larval health and production completed.	Jun-2017	A total of 8 trials undertaken using the demonstration hatchery.
2.1.4	Benefit:cost analysis of improved protocols (USYD, IMRAD Gondol)	Benefit – cost analyses completed	Jun-2015 – Jun-2017	
2.2	**Encourage the adoption of compounded diets to improve farm productivity and market access**			
2.2.1	Develop tripartite approach with ABILINDO, feed companies, researcher (ABILINDO)	Hold initial workshop to engage feed companies, develop adoption strategy	Due: Feb-2014	Workshop held in Bali, 8 September 2015. Identified the major factors constraining uptake of pellet feeds: poor performance and high cost.

2.2.2	Undertake feed trials on ABILINDO member farms (ABILINDO)	Feed trials demonstrate continual improvement in performance of pellet feeds	Mar-2014 – Jun-2016	Difficulty in engaging industry in feed trials due to poor performance of commercial pellet feeds (vs. 'trash' fish).
2.2.3	Assess uptake of pellet feeds (UNHAS, ABILINDO)	Increased use of pellet feeds / displacement of 'trash' fish use	Jun-2016	No significant additional uptake – grouper culture primarily reliant on 'trash' fish.
2.3	**Evaluate socio-economic aspects of practice changes**			
2.3.1	Baseline survey of hatcheries and grow-out farms (UNHAS)	Complete baseline study	Due: Dec-2013	Baseline surveys completed.
2.3.2	Follow-up survey (UNHAS)	Complete comparative survey	Jun-2016	Not done.

Objective 3: To build capacity in fish health management in Indonesia

No.	Activity	Outputs/ milestones	Completion date	Comments
3.1	**Provide training in epidemiology and disease diagnosis for Indonesian fish health professionals**			
	Workshop: Diagnostic sample collection and processing (Bali) (USYD)	Workshop completed; participants demonstrate appropriate skills.	Sep-2013	The workshop consisted of lectures, practical exercises and group activities. IMRAD Gondol and UGM staff engaged enthusiastically with the training. The various practical exercises demonstrated a high level of technical competence by all involved in the project.
	PCR training / sample analysis (Camden) (USYD)	PCR training completed; trainees demonstrated appropriate skills.	Jun-2014	First workshop (June 2014) completed. Two staff from IMRAD Gondol (K. Mahardika and Sari Budi Moria Sembiring) and two staff from UGM (Murwantoko and Cahya Fusianto) participated. Second workshop (March 2015) completed. Three staff from IMRAD Gondol (Zafran, K. Mahardika and Sari Budi Moria Sembiring) and two staff from UGM (Cahya Kurnia Fusianto and Arga Kurniawan) participated.
	Workshop: Results from epidemiological study (Indonesia) (USYD)	Participants demonstrate understanding of results and their application.	May-2015	Completed: 10–12 August 2015.
	Workshop: detailed analysis of project data sets; scientific publication	Workshop completed; participants understand results; draft publications.	Jun-2017	Completed: 24–26 January 2017.
3.2	**Provide improved fish health education services at university level**			

	Indonesian students in online epidemiological courses (USYD)	Successful completion of online epidemiological course.	Jun-2016	None to date. Due to a restructure, administrative support at Faculty level for student e-learning was removed for small cohorts in post graduate coursework, making it risky to enrol Indonesian students at USYD during this project.
	Involvement of postgraduate students (USYD)	Postgraduate students involved in project-related research.	Jun-2016	Bakhtiar Sah Putra (JAF Fellowship to study MVetStudies at USYD) participated in June 2014 routine sampling of sea cage farms and targeted sampling of hatcheries. Bakhtiar also participated in the PCR and histopathology training workshop at USYD Camden in March 2015, and undertook additional histopathology training at USYD Camden in June 2016. Cahya Kurnia Fusianto (UGM) was awarded a JAF to undertake a PhD at USYD, commencing 2017.
	Leadership management courses (Australia) (USYD)	Successful completion of project management courses.	Jun-2016	None to date. Due to a restructure, administrative support at Faculty level for student e-learning was removed for small cohorts in post graduate coursework, making it risky to enrol Indonesian students at USYD during this project.
3.3	**Support 'ring-testing' / validation studies for PCR laboratories in Indonesia**			
	Conduct training workshop and ring-testing exercise (DGA)	Successful completion of workshop; improved skills amongst participants.	Nov-2014	Completed. Excellent participation from Indonesian laboratories with 23 participants, representing 20 laboratories including MMAF Quarantine, University, Private sector, MMAF CARD as well as all DGA TIU laboratories Ring-test results supplied by 18 laboratories by March 2014.

7 Key results and discussion

7.1 Key diseases responsible for production losses in marine finfish aquaculture

7.1.1 Longitudinal epidemiological study of hatcheries

Key Results

- Infection with NNV and MCV are major production limiting pathogens at grouper hatcheries.
- NNV infection was detected in all hatcheries and in all fish populations throughout the year.
- Infections with MCV were highly prevalent between July to October in all fish species.
- Infestations with protozoan parasites were observed from June to October on the gills and skin of fish.
- There may be interactions between these pathogens

Discussion

There were ten major sampling events from March 2014 to June 2015 from a total of 26 hatcheries located around Gondol, Bali, Indonesia. A total of 70 populations and nearly 7,300 fish were sampled with the vast majority being hybrid grouper referred to as 'cantang' and 'cantik'. Each population was categorized as healthy or sick depending on the observation of clinical signs by researchers. Typical clinical signs included mortality, loss of appetite, darkened body colour, reddened mouth and fins and jaw and body deformities. Summaries of the populations are presented in Table 1 and in Table A1 (Appendix1).

Table 1 Summary of the grouper collected from the hatchery survey that were identified as either apparently healthy or showing clinical signs ('sick').

Species	Healthy	Sick	Total
Cantang	2346	1723	**4069**
Cantik	2011	354	**2365**
Tiger grouper	324	108	**432**
Coral trout	324	108	**432**
Total	**5005**	**2293**	**7298**

Infection with NNV was highly prevalent at almost all hatcheries at almost every sampling. Fish tested positive for NNV regardless of hatchery, fish species, the month of sampling, or the presence of clinical signs. Histopathological lesions associated with viral nervous necrosis were observed in association with the positive PCR detections in both healthy and sick populations of fish. Infection with NNV was considered a major production-limiting pathogen for grouper hatcheries.

Infection with MCV was evident from July to October in both 2014 and 2015. MCV was detected in 32% of 70 populations of 'cantang', 'cantik', tiger grouper and coral trout that were considered to be apparently healthy or sick, but at very low prevalence and very low viral load. While infection rates with MCV were considered to be significant, it was not the cause of disease in any populations of fish that were sampled. The role of this virus in limiting production at hatcheries is unclear.

The three most common ectoparasites observed in gills and fins of infested fish were the protozoans *Amyloodinium* sp., *Trichodina* spp., and a scuticociliate. These infestations were mainly observed during the months of March to September. Of note, *Amyloodinium* sp. and *Trichodina* spp. have a broad host and geographic range causing fish mortalities in tropical and temperate environments. Further, they are known to cause disease outbreaks, especially in closed systems because these protozoans can multiply quickly. These parasitic infestations with or without co-infection with viral pathogens would most likely have limited production of the grouper through reduced growth and poor survival.

Based on the results of the cross sectional survey, infection with NNV is endemic in the marine waters of Gerokgak, Bali. Similar to the results from the demonstration hatchery, there is considerable scope for continuing to improve larval fish health and production. Future research should focus on a whole system approach to promote good fish health and improve disease resistance, especially when culturing fish in areas with known endemic viral pathogens such as NNV and MCV. Early detection of viral pathogens is key to effective management of an epidemic and to eliminate the continual infection cycle. Future research is needed to identify and test disinfection and water filtration techniques to prevent viral and parasitic pathogen outbreaks in hatcheries.

7.1.2 Longitudinal epidemiological study of grow-out farms

Key Results

- There was a very high prevalence of chronic low grade disease in grouper grown in sea-cages. External lesions affected 43% of fish and some form of internal abnormality was detected in 94% of fish.
- Abnormalities of the liver or spleen were present in approximately 60% of fish and gastrointestinal or kidney lesions were present in approximately 40% of fish representative of those in routine production.
- These covert, production limiting diseases were characterized by chronic, non-specific histopathological changes. This is consistent with multifactorial aetiology including nutritional deficits.
- A low prevalence of subclinical MCV and NNV infection indicated that these pathogens were present and likely to cause disease outbreaks in naïve batches of fish at various life history stages.
- Ectoparasites infested the gills of 28% and skin of 6% of fish despite a high investment in parasite control. Parasite intensity varied between farms indicating that management influenced the impact of parasites in addition to fish species and seasonal variation.

Discussion

Several chronic, production limiting disease syndromes were identified that were independent of the high mortality outbreaks discussed frequently by famers. These were multifactorial chronic diseases that were notable as a very serious limitation on production without apparent involvement of the frequently implicated viruses and parasites. A very high prevalence of abnormalities was also identified in fish in routine production.

The sampling effort included 965 fish from 32 populations at four time-points across a 1 year period (Tables A2–A7, Appendix 1). External lesions were identified on 43% of fish. There were seasonal changes in incidence with a significant peak of 60% of fish affected in October ($p<0.01$). Progression in the severity of external lesions was a frequent cause of quarantine (Table A2, Appendix 1). The negative impact of more minor skin lesions on health and production was indicated by a lower condition index (CI) in affected fish, after accounting for all other factors that influenced CI ($p<0.05$, Table A3, Appendix 1). Interestingly, there was large difference in the prevalence of external lesions between

farms ($p<0.01$, Table A3, Appendix 1), indicating an opportunity to explore management solutions to these disease syndromes.

Some form of abnormality was identified on internal examination of 94% of fish that were representative of all fish in production (Table A2, Appendix 1). Abnormalities included deformities such as misshapen spine and missing opercula, gastrointestinal disturbance as well as a high prevalence of gross lesions of the internal organs (Table 2).

Table 2 Proportion of fish examined with lesions in internal organs.

Organ / system	Fish with lesions (%)
Liver	61.5
Spleen	53.9
Kidney	41.1
Gastrointestinal	37.9

Interestingly, the lesions of the internal organs were associated with higher condition index (Table A4, Appendix 1). There were several different disease syndromes associated with the abdominal organs including distention of the intestine with watery contents, gall bladder distention, splenomegaly and microhepatica (Figure 1). Histopathological examination of key specimens indicated chronic changes in the liver including fibrosis and loss of tissue architecture that were incompatible with good health. Splenic changes were marked by non-specific reaction to disease processes such as expansion of the number of melanomacrophage centres. These non-specific signs of disease are consistent with multifactorial disease including inadequate nutrition and opportunistic pathogens. The frequency of internal lesions other than those of the spleen varied with species/hybrid, season and farm (Table A1, Appendix 1). This indicates that changes to fish care and farm management can alter the impact of disease.

The overall prevalence of ectoparasite infestation was high despite the heavy investment in parasite control by weekly freshwater bathing (Table 3).

Table 3 Prevalence of ectoparasite infection on the skin, gills, and fins of grouper in this study.

Sample	Parasite	Apparent prevalence (%)
Skin	*Neobenedinia* sp.	6.2
	Benedinia sp.	5.3
	Pseudorhabdosynochus sp.	0.6
	Haliotrema sp.	0.5
Gills	*Pseudorhabdosynochus* sp.	28.2
	Haliotrema sp.	3.8
	other parasite	1.9
Fins	any parasite	0.5

There was a significant difference in the burden of the most prevalent parasites between farms indicating that management could substantially influence this disease (Table A6, Appendix 1). The presence of these parasites was detrimental to the health of fish based on observation of chronic gill damage on histopathology for populations with high prevalence of *Pseudorhabdosynochus* sp. infestations.

The overall prevalence of MCV infection was 2.0% (95% CI: 1.2 – 3.1%) and NNV was 2.5% (95% CI: 1.6-3.8%). For each virus there was a marked association with a specific hybrid and a different seasonal peak (Table A6, Appendix 1). This clustering of infection is consistent with residual infection from a hatchery or nursery source. Each of these viruses was detected only in low quantities which was consistent with residual infection from previous acute disease outbreaks that were not detected with this point-in-time survey. There was no histopathological evidence of the specific diseases caused by these viruses suggesting that the greatest threat they posed was infection of newly introduced, naïve batches of fish (i.e. fish with no prior exposure and therefore potentially weak immunity).

Neither of these viruses was associated with the internal and external lesions that characterised the chronic production limiting disease syndromes identified in this study (Table A7, Appendix 1).

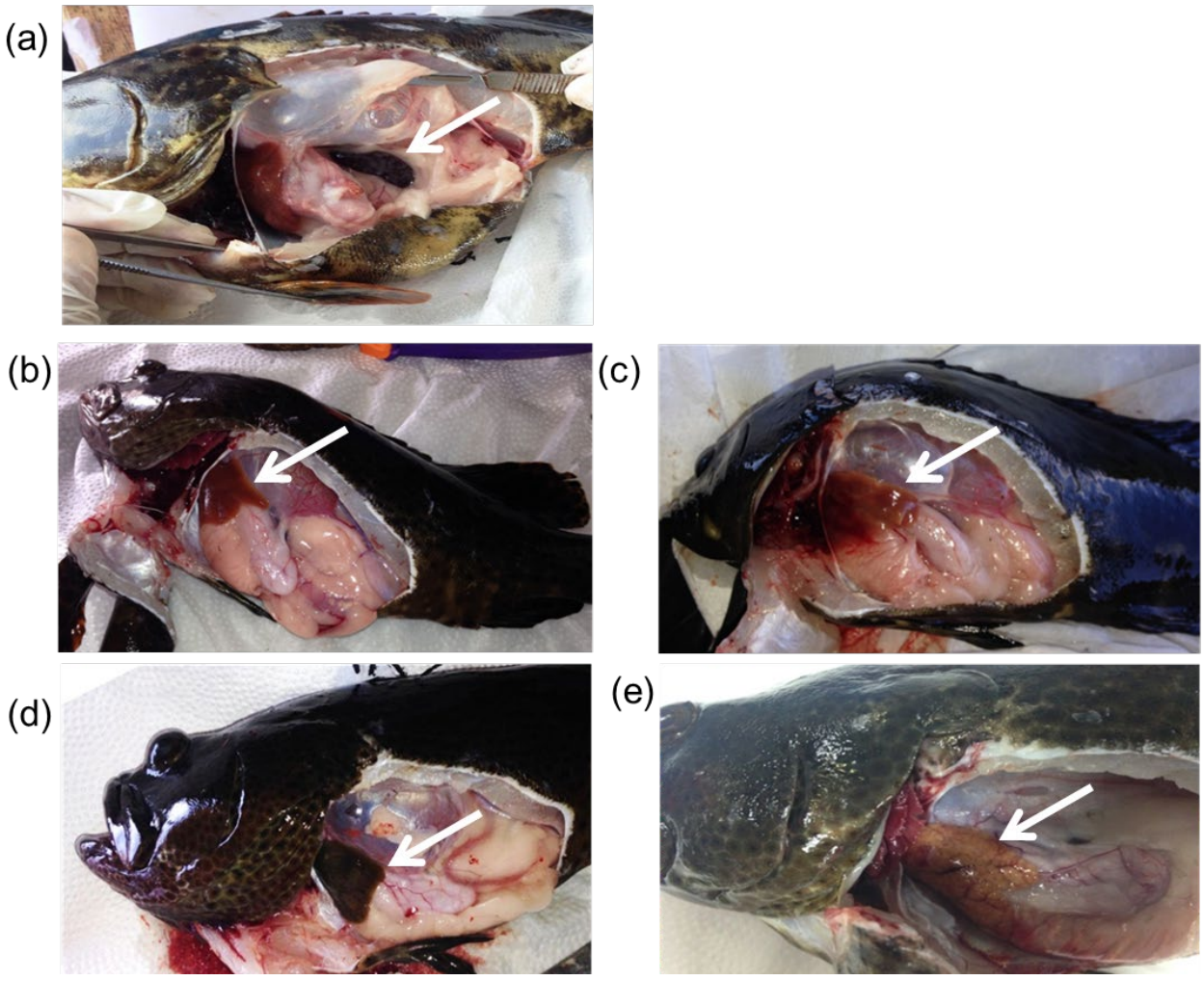

Figure 1 Representative photographs of the most common lesions observed at necropsy. (a) enlarged spleen, (b) normal liver, (c) patchy discoloration of liver, (d) small dark liver (e) pale, granular liver.

7.1.3 Intensive sampling of disease events in sea-cages, targeting the first month after fish are introduced into the cages

Key results

- Substantial losses in sea-cage production of grouper occurred due to acute disease outbreaks; these were unpredictable and resulted in high mortality.
- Farmer reports of 'Green-eye disease' of grouper reflected the occurrence of a complex multifactorial disease syndrome that impacted farmed grouper in Bali for the first time in 2014 and recurred frequently over 2 years. This disease syndrome has a novel aetiology that cannot be explained by the pathogens frequently implicated in disease outbreaks in grouper.

Discussion

A single acute disease syndrome occurred at high frequency during the study period between July 2016 and March 2017 in Pegametan and Sumber Kima Bay, northern Bali. The disease was referred to as 'green-eye disease' because of the appearance of affected fish, which probably reflected attempts at treatment with malachite green. Outbreaks had a sudden onset and progressed to cumulative mortality of 30 – 80% over a course of less than 2 weeks. This disease was not familiar to local farmers and the impact on production was substantial across all age classes.

The survey identified 11 outbreaks of disease on 9 different farms. This enabled a sampling effort that included a complete necropsy of 342 fish collected according to the targeted sampling strategy (Table A8, Appendix 1). Affected fish were lethargic, anorexic and laid on the bottom of the cage. Green discoloration affected the eyes of 85% fish and the entire body surface of 28% of the affected fish (Fig. 2). Treatment with malachite green was reported by some farmers. Interestingly, death predominantly occurred in the very early morning (1–2 am). This disease most frequently occurred in newly introduced batches of fish within weeks of introduction to sea cages from land-based nurseries. The size of the fish was less than 200 mm total length and 100 g weight for 8 / 11 affected populations. The condition index of affected fish was lower than apparently normal fish from the same populations ($p<0.05$), and in many cases a very low score reflected the obvious rapid loss of muscle mass (Fig. 2).

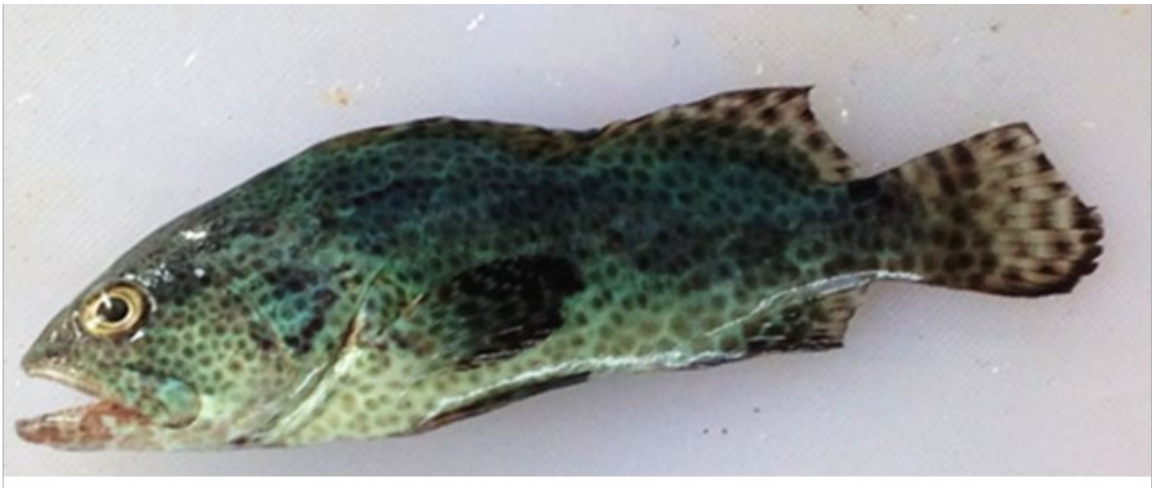

Figure 2 A hybrid grouper affected with green-eye syndrome. This example shows the marked green discoloration of skin which is normally brown; the eyes of this individual are not affected. It was noted that this clinical sign might reflect attempted treatment with

malachite green. The disease syndrome was also characterized by inappetence, inactivity, muscle wasting and lesions in the internal organs (kidney liver and spleen).

At necropsy the stomach in affected fish was frequently empty, consistent with the observation of inappetence. Nearly a quarter of fish had a clear effusion of peritoneal fluid and there was a moderate prevalence of extreme over-distension of the gall bladder, irrespective of the observation of clinical signs (Table A8, Appendix 1). Clinically affected fish differed from apparently healthy individuals by a higher prevalence of abnormalities of the kidney (30%), spleen (29%) and liver (20%). The kidney abnormalities were predominantly pale discoloration of either the anterior or posterior organ with occasional enlargement of the posterior kidney. Splenic abnormalities included an equal number that were enlarged or small and a small proportion that were also darkly discoloured. Liver abnormalities were mixed and included frequent patchy discoloration with equal numbers of enlarged and small liver, and occasionally a misshapen liver with a fibrotic appearance.

The parasite profile of the fish was different to the baseline established during the longitudinal disease survey with the absence of *Pseudorhabdosynochus* sp. on gills and a heavy infestation of leeches (*Hirudinea* sp.) on skin and fins. The parasite burdens of affected fish were not considered to be contributing to green-eye disease. The ongoing infestations and particularly the leeches were a cause for high concern for the farmers.

The overall prevalence of MCV infection was very high (82%), greatly exceeding the baseline prevalence for this virus in the longitudinal disease survey. MCV was detected at high prevalence in 10 / 11 populations, but there was an inconsistent pattern of association with clinically affected fish (Table A8, Appendix 1). The real-time PCR results indicate that there was a large quantity of MCV DNA present in some fish, consistent with clinical disease. However, the disease could not be definitively attributed to MCV in the absence of the characteristic histopathological findings (megalocytes in tissues). The prevalence of NNV was also higher than previously observed in similar populations of fish (66%). This virus was considered to be an incidental finding reflecting persistent infection from previous exposure because it was not present in large quantities, was not associated with clinical signs and there were no vacuoles in the brain or retina consistent with viral nervous necrosis disease observed on histopathological examination (Table A8, Appendix 1).

Further research is possible in the future using archived samples from this study to determine if MCV is associated with histopathological lesions and unbiased high throughput sequencing can be used to test for a novel pathogen.

7.1.4 Interventions to reduce production losses

On-farm parasite reservoirs and the efficacy of the freshwater bathing routine

Key Results

- Ectoparasite burdens were very high at all farms with 23 to 63% of fish affected with an average of 29 parasites per fish.
- Bathing significantly reduced parasite burdens by about two thirds within 1 hour after treatment. However, 24 hours after treatment, 7 to 73% of fish remained infested with an average of 10 parasites per fish.
- *Benedenia* spp. (monogenean skin fluke) and *Zeylanicobdella arugamensis* (leech) were the most prolific parasites. There is considerable scope to improve farm productivity with the development of targeted intervention strategies.
- Reservoirs for NNV were wild fish caught near cages, ‘trash’ fish, and biofouling from farm infrastructure.
- Reservoirs for MCV were wild fish caught near cages and ‘trash’ fish.

Discussion

The efficacy of the freshwater bathing treatments that are currently used at grouper farms were evaluated along with potential reservoirs for pathogens. Ectoparasites were a serious problem at the farms with 23 to 60% of fish having at least one external parasite immediately prior to treatment. The bath treatments used were a combination of freshwater and the chemical agent acriflavin. We found significant reductions in the number of infested fish and the number of parasites per fish when comparing pre-bath to the 24 hours after bath samples. However, none of the parasite bath treatments were completely effective at removing all parasites. The bath treatments provided a “knock down” effect on the adult parasites but longer term solutions are needed for persistently high parasite burdens.

The most important parasite was *Benedenia* spp. as it was found at all farms with a high prevalence and abundance. While bathing did significantly reduce the numbers of *Benedenia* spp., there was still an average of 10 *Benedenia* spp. per fish 24 hours after treatment. This high number of adult parasites will allow the life cycle to continue. Parasite intervention strategies need to focus on eggs, larvae and adult stages.

Wild fish living around cages and biofouling on nets and equipment were reservoirs for NNV and MCV that could infect fish in the sea cages. The use of low-value ‘trash’ fish was determined to be a direct pathway for these viral pathogens to come on to the farm. Both NNV and MCV were detected in trash fish, which could potentially infect the farmed grouper.

Detailed results of this study are appended (Appendix 1).

7.2 Improved management protocols for hatcheries and grow-out farms

7.2.1 Improved hatchery practices, including biosecurity and improved larval nutrition

Key Results

- Skeletal deformities and disease outbreaks were the biggest limitations to production of healthy and viable juveniles.
- Methods should be developed to culture larval grouper in the face of endemic pathogens.
- Methods for better water quality management are needed to enhance overall fish health and improve production outcomes.

Discussion

For all trials, the proportion of fish hatching was acceptable and ranged between 76% and 90%. The proportion of fish surviving to metamorphosis was poor and ranged between 13 to 42%. The early rearing phase was when high levels of mortality were observed and has the greatest scope for improvement. The two biggest limitations to the production of viable juveniles were skeletal deformities and disease outbreaks. Poor water quality with extremely high levels of nitrogenous waste products was observed in all trials. Ammonia and nitrite toxicity is known to impair immune function, reduce growth and cause mortality in fish. The most successful batch of fish was Trial 7, followed by Trial 6. These trials produced the greatest number of normal fish with no detection of NNV (18,490 and 15,648 fish respectively) but in Trial 7 only 13% of fish survived to metamorphosis and 66% of fish in Trial 6 had skeletal deformity (Table 4).

Figure 3 Deformities in opercula (b and d) were the most common deformity in the juvenile grouper produced at the demonstration hatchery and were removed from the population of fish with a normal appearance (a and c).

Table 4 Summary of larval grouper trials in the demonstration hatchery.

Trial	Fish Species	Start date	Total no. of eggs	Hatching		Metamorphosis		Normal fish		Deformity		qPCR positive batch		Comment
				Number	%	Number	%	Number	%	Number	%	NNV	MCV	
1	Cantik	24 Nov 14	150,000	126,000	84	26,899	21.3	11,270	41.9	15,629	58.1	Yes	No	
2	Cantang	18 Jan 15	150,000	121,500	81	40,100	33.0	31,675	79.0	8,425	21.0	Yes	No	
3	Cantik	20 May 15	200,000	180,000	90	75,600	42.0	65,016	86.0	10,584	14.0	Yes	Yes	
4	Cantik	16 Aug 15	200,000	160,000	80	0	0	0	0	0	0	Yes	No	mortality D27
5	Cantik	20 Oct 15	200,000	152,000	76	0	0	0	0	0	0	Yes	No	mortality D34
6	Cantik	11 Feb 16	200,000	162,400	81	47,420	29.2	15,648	33.0	31,772	67.0	No	No	2nd best trial
7	Cantik	10 Apr 16	200,000	156,000	78	19,968	12.8	18,490	92.6	1,478	7.4	No	No	best trial
8	Cantik	31 Oct 16	200,000	160,000	80	28,572	17.9	7,600	26.6	20,972	73.4	No	No	

Factors limiting production

1. There was a high proportion of fish with deformities observed in all trials (range 7% to 74%). The problems consisted of malformations of jaws and spine, abnormal head shape, missing/open opercula and absence of the tail (Fig. 4). All deformed fish were discarded. Causes of deformity are multi-factorial. They can arise from poor host (e.g. infectious agents, poor nutrition) and environmental (e.g. temperature fluctuations) conditions.

2. Infection with NNV was detected in five trials with two trials having mass mortality events at 27 and 34 days after hatch. It was assumed that infection with NNV significantly reduced fish survival in trials in which it was detected. In the outbreak trials, NNV was first detected at 10 days after hatch. It is notable that NNV was not detected in three trials (Table 4, Trials 6-8). Longitudinal testing indicated that NNV infection was transmitted horizontally rather than vertically.

3. Water temperature, dissolved oxygen and pH remained relatively stable and within acceptable ranges for all trials. However, the nitrogenous waste products greatly exceeded the recommended levels for grouper in all trials. For most trials, toxic levels of ammonia and nitrite exceeding the lethal concentration were observed from about 6 days after hatch and onwards. In some instances, the amount of unionized ammonia was 8 times the maximum recommended level. Nitrate levels were generally below the generic recommendation of <100 mg/L. Ammonia and nitrite toxicity are known to cause mortality, reduce growth and impair immune function.

7.2.2 Adoption of compounded diets to improve farm productivity and market access

Key result

- The workshop on Improved Feeds Adoption for Marine Finfish Aquaculture identified a number of significant constraints to pellet feed adoption by grouper farmers.

Discussion

The one-day workshop identified the following constraints to pellet feed adoption by grouper farmers:

- Cost: pellet diets are usually more expensive than ‘trash’ fish, not only on a per-weight basis, but also after taking into account FCR values.
- Performance: growth rates are usually superior in grouper fed ‘trash’ fish compared with pellets, and survival is usually not significantly different.
- Availability: availability in many parts of Indonesia is poor, and poor transport infrastructure results in wastage due to pellet breakage, degradation of the nutritional value of the feed, and increased costs to cover transport over long distances.
- Acceptability to fish may be poor, particularly when the fish have not been weaned to a compounded diet.
- Acceptability to farmers: many grouper farmers have no experience using pellet feeds.
- The market for feeds for marine finfish generally, and grouper in particular, is small in Indonesia compared with other sectors such as shrimp and freshwater fish. Consequently, feed mills prefer to invest in developing feeds for sectors where there is a larger market and hence higher economic returns.

Overall, the participants at the workshop felt that other species (e.g. barramundi / Asian seabass *Lates calcarifer* and pompano (*Trachinotus* spp.) could more easily adopt compounded feeds, and this is where feed development investment should focus. It was

suggested that semi-moist diets may provide a useful compromise for grouper culture, reducing the amount of 'trash' fish used while providing performance comparable with a 'trash' fish diet.

An additional issue in relation to the adoption of compounded diets is the need to ensure that they are of adequate quality, particularly in the case of marine finfish diets which need to be 'complete'.

7.2.3 Evaluation of socio-economic aspects of practice changes

Key results

- The three main grouper species produced in hatcheries and sea-cage farms in northern Bali at the time of the survey were: tiger grouper *Epinephelus fuscoguttatus*, hybrid grouper 'cantang' ♀ *Epinephelus fuscoguttatus* × ♂ *Epinephelus lanceolatus*, and hybrid grouper 'cantik' ♀ *Epinephelus fuscoguttatus* × ♂ *Epinephelus polyphekadion*. Smaller numbers of humpback grouper (*Cromileptes altivelis* aka mouse grouper) and coral trout (*Plectropomus leopardus*) were also cultured by some hatcheries and farms.
- The three main grouper species reared in these hatcheries are prone to viral diseases (VNN and iridovirus).
- Most hatchery operators categorized their mortality from disease as 'medium' (59%), with only 18% categorizing mortality as 'high'. On the other hand, financial losses associated with disease were mainly categorized as 'high' (47%) or 'low' (47%).
- Common diseases reported by sea cage farmers are: viral nervous necrosis, iridovirus and parasite infestations.
- The experience of sea cage farmers suggests that some grouper species are more prone to disease than others. The grouper hybrid 'cantik' was regarded by most farmers as having a generally high propensity for disease problems, while tiger grouper was rated moderate to high. In contrast, the hybrid grouper 'cantang' and mouse grouper were regarded as having a low propensity for disease.
- Sea cage farmers ascribed disease-related losses to two main factors: limited volume and/or poor quality 'trash' fish feed, and changes in water quality associated with the onset of the rainy season in November – February each year.

Discussion

The survey revealed that disease has a significant impact on grouper production both in hatcheries and in sea cage farms.

Hatcheries

Hatchery operators felt that most mortality was related to viral diseases (VNN and iridovirus) (Fig. 5). Parasitic diseases were identified as a problem only with tiger grouper (Fig. 5).

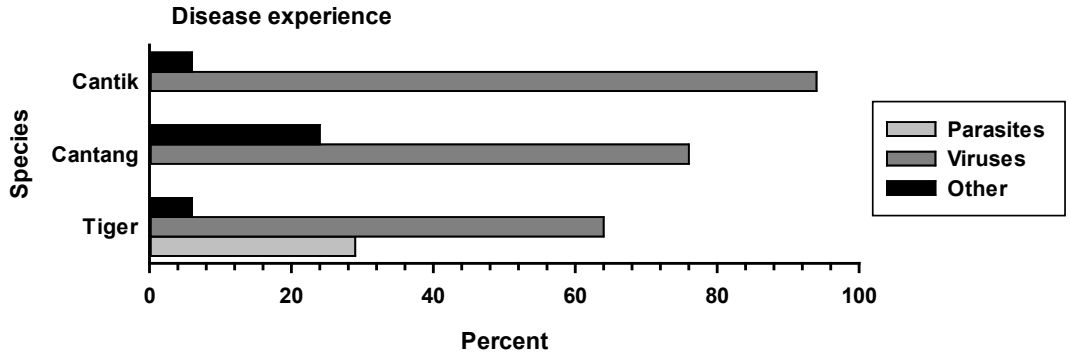

Figure 4 Proportion of hatcheries experiencing various disease problems with the three main grouper species produced in Buleleng hatcheries.

Despite the impacts of disease, there was a general lack of effective biosecurity and health management procedures in place in these hatcheries. Basic biosecurity protocols (such as restricting visitor entry, changing shoes when entering the hatchery, etc.) were used only by few hatcheries.

In the event of a disease outbreak, seventy-one percent of hatcheries separated apparently diseased fish from healthy fish, while 17% treated sick fish with formalin. During a disease outbreak, most hatcheries contacted the owner (53%), other technical staff in the hatchery (35%), or the local research institute (12%).

Sea cage farms

Common diseases reported by sea cage farmers are: viral nervous necrosis (VNN), iridovirus and parasite infestations.

All the farms surveyed use 'trash' or low-value fish as feed for the cultured species. Generally, the 'trash' fish feed was obtained from a business partner in Java. Various species are used as feed, but the main ones are 'biji nangka' (Family Mullidae) and 'lemuru' (*Sardinella* spp.). The price of the 'trash' fish fluctuates between Rp 5,000 and Rp 6,500 per kg. Each farm requires 50 to 250 kg of 'trash' fish feed per day. Five farms 'sometimes' used pellet feed. Generally, pellet feed was used either as a supplement (when there was insufficient quantity of 'trash' fish to satiate the cultured fish) or when 'trash' fish was unavailable due to seasonal availability constraints (such as weather).

Various vitamin supplements (principally vitamins C and B) were added to 'trash' fish by 5 farms, 2 to 4 times per month. Farmers believed that the addition of vitamins would improve fish survival.

Farmer experience suggests that some grouper species are more prone to disease than others. The grouper hybrid 'cantik' was regarded by most farmers as having a generally high propensity for disease problems (Fig. 6), while tiger grouper was rated moderate to high. In contrast, the hybrid grouper 'cantang' and mouse grouper were regarded as having a low propensity for disease (Fig. 6).

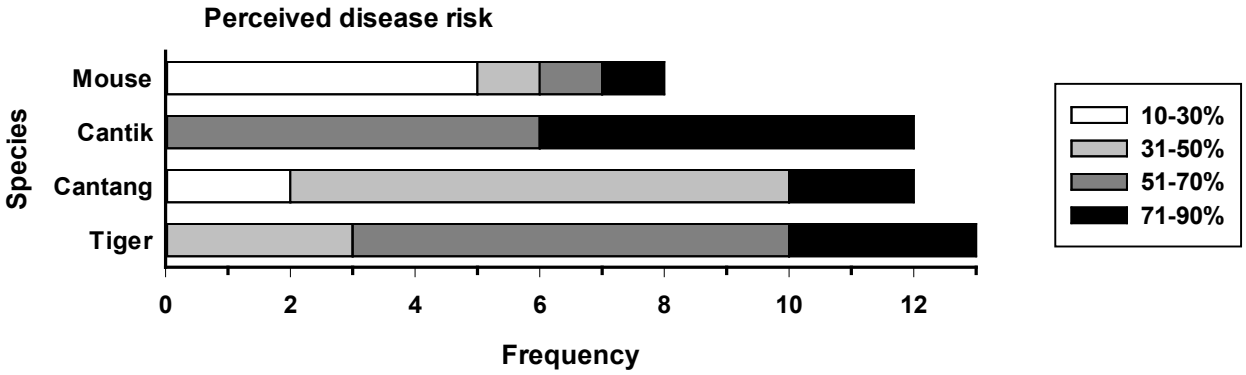

Figure 5 Propensity for different grouper species to be affected by disease, as experienced by farmers.

All the farmers interviewed were confident that they could identify disease outbreaks visually. Swimming activity and loss of appetite were the main clinical signs that farmers used to identify disease outbreaks, followed by changes in skin colour and signs of injury on the body.

During disease outbreaks, the company primarily relies on the owner or technical staff to provide technical advice (93%). Only 7% of farms consulted with government technical institutions.

Ninety-three percent of farms interviewed disposed of sick fish by discarding them to the sea. Only 7% of farms disposed of them by burying them on land.

7.3 Building capacity in fish health management in Indonesia

7.3.1 Training in epidemiology and disease diagnosis for Indonesian fish health professionals

Key results

- The training provided through the project covered a range of topics to provide a fundamental knowledge of epidemiological approaches to fish health for marine finfish hatcheries and sea cage farms.
- A key outcome from the participation of UGM in the project was the acceptance of Cahya Kurnia Fusianto to undertake a PhD study at USYD on a topic related to the findings of the project.
- With support from USYD staff, Joko Santosa (BPBL Lombok) has undertaken a small study of the impacts of disease on grouper newly stocked in sea cages in Lombok.

Discussion

Project training activities

A series of training workshops was held throughout the project to provide project participants with a grounding in epidemiological approaches to fish health management, and testing for viruses using real-time (quantitative) PCR techniques.

At the beginning of the project a series of training workshops linked to practical exercises were held at IMR Gondol (2–11 September 2013). These activities covered training in aquatic animal epidemiology, trial sampling of sea cages and hatcheries and sample processing methods. In addition to providing a theoretical grounding in epidemiological techniques for project participants, this series of workshops enabled the development of

practical yet robust sampling techniques for hatcheries and sea cage farms that subsequently formed the basis of the project's sampling strategy.

Three workshops were held at the University of Sydney's Camden Campus to train project participants from IMR Gondol and UGM in real-time PCR analytical techniques and histopathology: 2–6 June 2014; 2–13 March 2015, and 10–12 May 2017.

In conjunction with the mid-term project meeting and mid-term review, a two-day course on statistics was held for project participants attending the meeting at UGM (11–12 August 2015). This provided participants with a basic grounding in statistical analysis techniques, and introduced them to on-line statistical software.

Participants' education in statistical analysis was strengthened by an additional two-day workshop held towards the end of the project (24–25 January 2017) at IMR Gondol, which focussed on analytical techniques for large data sets and used the longitudinal study data sets from the project sampling as teaching material.

With a view to promoting publication of the project's results in international scientific journals, a one-day workshop on scientific publication was held at IMR Gondol on 26 January 2017.

A recommendation from the mid-term review (#5) was the provision of training in best practice for marine finfish hatcheries. This was undertaken at Port Stephens Fisheries Institute on 14–21 December 2015. The training comprehensively covered the range of practices contributing to the production of high-quality, disease-free fish fingerlings: water intake treatment, broodstock management, egg collection and treatment using ozone, egg incubation and transfer of larvae to rearing tanks, larval rearing procedures, rotifer culture and enrichment and *Artemia* hatching and enrichment. A number of these processes have been implemented at IMR Gondol.

A full list of project training activities and the participants is appended (Appendix 2).

JAF Scholarships

A JAF scholarship was provided by ACIAR to Cahya Kurnia Fusianto (UGM) to undertake a PhD at the University of Sydney. Research topic: 'Applications of epidemiology and molecular technology for improved management of MCV infections in aquaculture'.

Sampling training

The need for assistance with sampling the sea cage farms provided an opportunity to provide training in epidemiological sampling to staff of the Brackishwater Aquaculture Development Centre Ujung Batee, Aceh (Bakhtiar Sah Putra) and the Mariculture Development Centre Lombok (Joko Santosa), as well as Imran Lapong (Counterpart Technical Officer for ACIAR project FIS/2007/124 'Diversification of small-holder coastal aquaculture in Indonesia' based in Makassar.

Both Bakhtiar Sah Putra and Imran Lapong went on to complete post-graduate studies supported by JAF scholarships at University of Sydney and James Cook University, respectively.

Joko Santosa, with support from USYD staff, undertook a study of the impacts of disease on grouper newly stocked in sea cages in Lombok.

7.3.2 Improved fish health education services at university level

The project enabled numerous visits of academics from Australia to the university environment in Indonesia, and vice versa. Dr Murwantoko (UGM) and a post graduate

student Cahya Fusianto visited USYD on many occasions, with funding from both the Ministry of Research, Technology and Higher Education Indonesia as well as from this project. Dr Madiana E. Fachry (UNHAS) also visited USYD. Drs Richard Whittington, Mike Rimmer, Joy Becker, Paul Hick and Navneet Dhand visited UGM. During each of these reciprocal academic visits both undergraduate and post graduate teaching and research were observed in situ, and there were discussions about the similarities and differences in physical resources, course content, didactic, experiential and other pedagogical approaches. Exchange visits and guest lectures were proposed as follow on activities, as well as student exchanges. It was concluded that project-based collaborative research is an excellent vehicle for insertion of current knowledge in aquatic animal health into the curriculae in both countries.

One particular difference was the inclusion of aquatic animal health education and research in the context of agriculture in Indonesia, while in Australia these are dealt with in the context of veterinary and animal science. A striking similarity was potential for structural change. During the project it was proposed that aquaculture and fisheries be developed as a Faculty separate from Agriculture at UGM. Meanwhile at Sydney, both the Faculty of Agriculture and the Faculty of Veterinary Science were subsumed into the Faculty of Science, and agriculture was re-caste as a university institute with most of its physical and human resources placed in the new School of Life and Environmental Sciences. Due to the observed administrative differences and hierarchies in discipline-structures between the universities in the two countries, and the potential for them both to change further over time, it was concluded that linkages initiated and maintained at academic level that may capture resources available from time to time from varied sources for international linkages, will be more enduring and beneficial than top-down ventures. Support may be sought in future form institutional sources such as the USYD Sydney Southeast Asia Centre, OIE (University twinning program) and Government of Indonesia Ministry of Research, Technology and Higher Education programs for academic enrichment.

7.3.3 ‘Ring-testing’ / validation studies for PCR laboratories in Indonesia

Key results

- A PCR ring-test for fish viruses was a success. Participants from 18 of the 20 eligible DGA laboratories in Indonesia attended the workshop.
- Proficiency with PCR tests for fish viruses was demonstrated with the correct identification of a high proportion (73.1%) of blind coded samples in a very challenging panel.
- Laboratories with prior experience and expert knowledge with a specific PCR test were more successful compared to laboratories with general expertise and no previous experience with the test. The results support the development of a reference laboratory system for ongoing ring-testing and training in specific test protocols.

Discussion

The format of the ring test was a key to the high participation rate and identification of factors associated with accurate PCR tests. Anonymous participation and supportive discussion about the methods, including with scientific experts who were native Bahasa Indonesia speakers, were key features to enable an excellent participation rate. Results were submitted by 18 / 20 laboratories despite using a challenging panel of samples that were prepared to ensure factors associated with accurate results were identified and training opportunities were generated. Ring-testing provided these additional opportunities that are not possible with proficiency tests used for accreditation purposes where there is a bias against discussing difficulties with procedures and reporting results known to be imperfect.

In total, 73.1% (n=238) of the samples were correctly classified as positive or negative under blind testing conditions. The sensitivity and specificity for detection of NNV and

EHNV was similar (Table 4). These aggregated results include laboratories which failed to correctly implement a new assay for an unfamiliar pathogen, as well as 11 panels that were tested with 100% specificity and 100% sensitivity.

Table 5 Overall sensitivity and specificity for NNV and EHNV samples.

	NNV	EHNV
Sensitivity (%) (95% confidence interval)	74.1 (64.8 – 82.0)	75.0 (65.1 – 83.3)
Specificity (%) (95% confidence interval	66.7 (41.0 – 86.7)	62.5 (35.4 – 84.8)

One laboratory achieved grade A for both viruses when the following grading system was used to categorise the results:

A All results correct

B Distinguished the negative sample, but did not detect all positive samples

C Identified some positive samples, but did not distinguish the negative sample

D Invalid run, no correct results

The D-grade results were from laboratories which did not routinely test for these pathogens, and one of the laboratories that received grade D for NNV produced a grade A results for EHNV (Table 5). Familiarity with a specific test method rather than general expertise or availability of equipment was positively associated with competence to provide correct results.

Table 6 Graded results for 16 laboratories which provided results for both virus panels. The results for the 2 laboratories that provided only NNV results were 1 each for grade C and D.

		EHNV results			
		A	**B**	**C**	**D**
NNV results	**A**	1	3	3	0
	B	2	2	1	0
	C	0	1	1	1
	D	1	0	0	0

Interestingly, identification of positive samples was not impacted by the quantity of virus (Table 6). Despite having 10 - 100 times less virus, the same proportion of the low positive samples (71%) were correctly identified as the combined medium and high samples (69%; p=1.0). Nucleic acid purification (extraction) was reported to be a technically challenging aspect of the testing procedure that reduced sensitivity and contributed to at least 1 D-grade result and 1 laboratory not participating. False positive results were attributed to incorrect interpretation of fluorescent data; mislabelling and sample mix-up; and, cross-contamination.

Table 7 Sensitivity for detection of samples with different quantities of virus (EHNV and NNV combined). The overall sensitivity was the same at different viral loads (p=0.941). Real-time PCR was responsible for detecting more of the samples with low viral load, but did not perform better than conventional PCR when the virus load was medium or high combined (p=0.02).

Amount of virus	Overall		Real-time PCR		Conventional PCR	
	No. test results	Correct (%)	No. test results	Correct (%)	No. test results	Correct (%)
high	34	74	39	77	63	73
medium	68	66				
low	102	71	39	87	63	65

Real-time PCR (qPCR) was used by 44% of laboratories to test for NNV and 31% for EHNV. The type of PCR technology did not affect the proportion of correct results or the accuracy grade (Table 7). A higher proportion of correct results were provided by qPCR compared to conventional PCR for NNV (p=0.02), but not for EHNV (p=0.82). This is consistent with intra-laboratory factors including familiarity of technicians with the procedures and equipment being more important determinants of accuracy than the intrinsic characteristics of the PCR technology.

Table 8 Proportion of correct results and distribution of laboratory grade for real-time and conventional PCR methods.

		Conventional PCR		Real-time PCR	
		NNV	EHNV	NNV	EHNV
Sensitivity %	(no. tests)	64 (70)	74 (77)	84 (56)	35 (71)
Grade	A	3	4	4	0
	B	3	4	2	2
	C	2	2	2	3
	D	2	1	0	0

The observations from this study support the recommendation to develop a reference laboratory system. This would provide a focus for ongoing training efforts where highly skilled multi-lingual scientists could assist in training laboratory technicians in specific test procedures. These centres could develop the materials and coordinate ongoing ring-tests to enhance training opportunities and develop test specific competence. This will generate regular communication amongst a network of scientists and assist the implementation of new technology including real-time PCR and automated nucleic acid extraction that will be required to align with changing international standard test procedures and to handle larger numbers of samples.

8 Impacts

8.1 Scientific impacts – now and in 5 years

The MMAF Fish Health and Environment section within the Directorate-General of Aquaculture were very positive about the outcomes of the PCR Proficiency Testing Workshop held at LP2IL Serang in November 2013. The previous Director of the section, Dr Maskur, had requested ongoing support for PCR training and proficiency testing using LP2IL Serang as a reference laboratory to support the other DGA regional laboratories as testing laboratories. USYD was unable to directly support such a scheme without additional funding due to resource limitations. A key scientific impact was the disentangling of PCR results that often are used in isolation to infer disease causation.

8.2 Capacity impacts – now and in 5 years

In addition to IMRAD Gondol and UGM staff directly involved in project activities, the project has involved other MMAF staff in the sampling to help build appreciation of epidemiological approaches to fish health investigation amongst fish health professionals in Indonesia:

- Bakhtiar Sah Putra, Brackishwater Aquaculture Development Centre Ujung Batee, Aceh;
- Joko Santosa, Mariculture Development Centre Lombok.

Data analysis workshops

Two workshops were held with project participants to provide training in data analysis.

Gadjah Mada University, Yogyakarta, 11–12 August 2015

The first workshop covered the following topics:

- Descriptive analysis
    - Identification of variable types
    - Evaluation of the association between two categorical variables
    - Descriptive analysis for individual quantitative variables
    - Evaluation of the association between two quantitative variables
    - Evaluation of the association between a quantitative and a categorical variable
- Chi-squared test
- Two-sample t-test
- Analysis of variance
- Training in the use of a free and user-friendly on-line statistical analysis software package (Statulator)

IMRAD Gondol, Bali, 24–25 January 2017

The second workshop covered the following topics:

- Preparing data for statistical analysis
- Exploratory analysis and recoding variables
- Contingency tables: test associations between two categorical variables
- Comparison of means between two groups
- Comparison of several treatment means simultaneously

Scientific writing workshop

A scientific writing workshop was held at IMRAD Gondol on 26 January 2017. The aim of this workshop was to equip participants with a toolbox of strategies to facilitate the publication of their research in the international scientific literature.

Specifically, the structures and features of original research articles were identified. The writing styles for each element of a paper were discussed. The publication process including the choice of journal and peer review was critiqued.

The content was illustrated by discussion of two sample papers describing recent grouper health research. The results of the data analysis sessions were used to create elements of a publication-ready manuscript from original ACIAR research. The need to identify a package of results that would tell a story, to focus on communicating with the appropriate audience, and to convey key messages for maximum positive impact were highlighted.

The intended outcome of the workshop was to identify publication ideas for ACIAR project FIS2010/101 and to begin to establish collaborative writing teams with enthusiasm to pursue publication. This approach was formalised at the final project meeting which identified the potential publications (refereed scientific publications, brochures or fact sheets, and policy briefs) arising from project research outcomes, identified contact persons in Australia and Indonesia and co-authors for each publication, and nominated writing timelines.

Workshop evaluations

Formal evaluation of the training workshops showed that participants felt that their skills and knowledge had been enhanced by their participation in these workshops. There were requests for additional workshops on data analysis, on scientific writing and publication for international journals.

Long-term capacity impacts

Standard procedures from the project have been used for disease investigations that are not part of the ACIAR-funded research. The epidemiological sampling strategies, incorporation of histopathology in disease investigation and implementation of new real-time PCR assay capacity was reported at Government centres at Gondol, Lombok and Aceh

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

8.3.2 Social impacts

8.3.3 Environmental impacts

8.4 Communication and dissemination activities

Farmer meetings

Farmer meeting, IMRAD Gondol, 18 June 2014

A meeting of sea cage farmers in northern Bali was held at IMRAD Gondol on 18 June. The meeting was suggested by Dr Murwantoko and arranged by IMRAD Gondol, in response to interest amongst local farmers in the sampling being carried out by the project. All local farmers were invited, but only a small number attended. The overall objective of the meeting was to explain to farmers the objectives of the project, and to summarise results from the first sampling. Three short presentations were given:

- an overview of ACIAR project FIS/2010/101 by Mike Rimmer;
- the diagnostic process for investigating the cause of a disease outbreak in grouper at a sea cage farm by Paul Hick;
- a summary of the results of the first sampling from the longitudinal study of sea cage farms by Murwantoko.

During the first routine epidemiological sampling at Pak Harsono's farm provided a sample of moribund *kerapu cantang* with a blue colour from a population of fish which had suffered 90% mortality over the previous month. Results of PCR, parasitology and histopathology on these sick fish were discussed in Paul Hick's presentation.

Following the presentations there was a discussion session. The farmers had specific questions about disease syndromes that they experience and how to treat them.

Farmer meeting, IMRAD Gondol, 11 December 2014

The project invited local farmers and hatchery operators to attend a meeting at IMRAD Gondol on 11 December 2014. The objective of the meeting was for project staff to provide feedback to participating farmers on the results of the project so far. A number of sea cage owners / managers and hatchery operators attended.

The meeting comprised:

- an overview of ACIAR project FIS/2010/101 presentation by Dr Mike Rimmer (USYD);
- a summary of the results of the sea cage study, by Dr Murwantoko (UGM);
- a summary of the results of the hatchery study, and the biosecure hatchery at IMRAD Gondol by Dr Ketut Mahardika (IMRAD Gondol);
- discussion of the project's results and on-farm disease issues.

Farmer meeting, IMRAD Gondol, 27 January 2017

The project invited local farmers and hatchery operators to attend a meeting at IMRAD Gondol on 27 January 2017. The meeting comprised:

- an overview of ACIAR project FIS/2010/101 presentation by Dr Mike Rimmer (USYD);
- a summary of the results of the epidemiological study of hatcheries by Dr Ketut Mahardika (IMRAD Gondol);
- a summary of the results of the epidemiological study of sea cages, by Dr Murwantoko (UGM);
- a summary of the results of the trials undertaken in the demonstration hatchery, by Mrs Suko Ismi;
- discussion of the project's results and on-farm disease issues.

Australian grouper farmers visit

Two representatives of the Australian aquaculture company Torres Strait Sea Harvest visited Gondol to look at small-scale hatcheries and sea cage farms to evaluate the application of the hatchery technology in Torres Strait. The visited IMRAD Gondol, two commercial marine finfish hatcheries and the IMRAD Gondol sea cages. Feedback on the visit was positive and hopefully future collaboration will support the development of Australian commercial grouper farms. However, development of grouper culture in Torres Strait is proceeding slowly due to restrictive legislation, problems in accessing fingerlings, and a difficult site.

Workshop on Improved Feeds Adoption for Grouper Aquaculture

This activity was undertaken to engage grouper farmers, feed companies and researchers in evaluating commercial grouper feeds and to provide mechanisms for all three stakeholder groups to exchange information about feed performance. Through building this collaboration, we hoped to improve the adoption of compounded feeds by the Indonesian grouper farming industry.

The workshop was attended by 8 experienced grouper farmers (including 7 representatives of ABILINDO with farms located from Medan in the west to Sumbawa in the east), representatives of 5 commercial feed companies, the Chairman of the Indonesian Feedmills Association (GPMT), and nutrition researchers from IMRAD Gondol, RICA Maros and IPB Bogor.

The workshop identified a number of significant constraints to pellet feed adoption by grouper farmers:

- Cost: pellet diets are usually more expensive than ‘trash’ fish, not only on a per-weight basis, but also after taking into account FCR values.
- Performance: growth rates are usually superior in grouper fed ‘trash’ fish compared with pellets, and survival is usually not significantly different.
- Availability: availability in many parts of Indonesia is poor, and poor transport infrastructure results in wastage due to pellet breakage, degradation of the nutritional value of the feed, and increased costs to cover transport over long distances.
- Acceptability to fish may be poor, particularly when the fish have not been weaned to a compounded diet.
- Acceptability to farmers: many grouper farmers have no experience using pellet feeds.
- The market for feeds for marine finfish generally, and grouper in particular, is small in Indonesia compared with other sectors such as shrimp and freshwater fish. Consequently, feedmills prefer to invest in developing feeds for sectors where there is a larger market and hence higher economic returns.

Overall, the participants at the workshop felt that other species (e.g. barramundi / Asian seabass *Lates calcarifer* and pompano (*Trachinotus* spp.) could more easily adopt compounded feeds, and this is where feed development investment should focus. It was suggested that semi-moist diets may provide a useful compromise for grouper culture, reducing the amount of ‘trash’ fish used while providing performance comparable with a ‘trash’ fish diet.

An additional issue in relation to the adoption of compounded diets is the need to ensure that they are of adequate quality, particularly in the case of marine finfish diets which need to be ‘complete’.

9 Conclusions and recommendations

In this section the major conclusions and recommendations of the project team are provided in an alignment with the project objectives.

9.1 Conclusions

1. The prime objective of this project was to determine the key diseases responsible for production losses in marine finfish aquaculture through longitudinal epidemiological studies on hatcheries and grow-out farms (sea cages).

Important diseases in hatcheries. NNV was a very frequent infection in grouper in Indonesian mariculture, but disease and economic losses appear to be confined to the hatchery stage, where impacts can be both common and severe. NNV detection in grower fish in sea cages was mostly incidental and probably represented residual infections from the hatchery stage. The role of MCV in disease outbreaks in hatcheries is unclear. Both viruses can be present in the environment (the evidence was in trash fish and in fish and biofouling around sea cages), highlighting the importance of biosecurity throughout the susceptible life history stages in hatcheries and nurseries. Ammonia and nitrite toxicity due to water quality management and deformities due to multifactorial nutrition/environment interaction were important causes of morbidity in hatcheries. In backyard hatcheries protozoans (*Amyloodinium* sp.,*Trichodina* spp.) and a scuticociliate were important and may have had interactions with viruses.

Important diseases in sea cages. Chronic multifactorial diseases were highly prevalent and impacted the production fish considered to be healthy by farmers throughout the production cycle. Residual deformities from the hatchery stage and chronic non-specific visceral lesions were a problem in grouper in sea cages, and were likely of multifactorial aetiology. Metazoan ectoparasites (*Neobenedinia* sp., *Benedinia* sp., *Pseudorhabdosynochus* sp. and *Haliotrema* sp.) were important causes of morbidity of grouper in sea cages. NNV infections were incidental and residual to the hatchery stage while the role of MCV in disease outbreaks in sea cages is unclear. High mortality disease outbreaks also occurred frequently. A syndrome called green-eye disease was investigated and was unrelated to the viruses or parasites mentioned above, but leeches (*Hirudinea* sp.) were abundant and the syndrome clearly had a complex causation.

2. A second aim was to trial interventions to reduce production losses.

Intervention to manage parasites. Routine control of *Benedenia* spp. (the monogenean skin fluke) and *Zeylanicobdella arugamensis* (a leech) was evaluated. The burdens of these parasites varied substantially between farms both before and after treatments were applied by farmers, suggesting that management practices aimed at integrated parasite control could be optimised in future work.

3. In a third objective, improved management protocols for hatcheries and grow-out farms were tested.

Improved hatchery practices. Larval production was hampered by losses due to NNV infection, deformities and poor water quality.

Pellet feed adoption. Numerous constraints to adoption of compounded diets were identified including poor growth rates, cost, poor availability due to small market size for grouper and transport issues, and lack of palatability.

Further work on fish husbandry, biosecurity and nutrition is required to improve farm productivity.

4. The socio-economic aspects of practice changes were evaluated in a fourth objective.

Disease was confirmed to be a major problem among farmers at both hatchery and sea cage stages of production, especially for grouper. There was very limited adoption of basic biosecurity practices. Common myths about diseases affecting grouper in sea cages were well entrenched and contrasted with the findings from the epidemiological surveys, i.e. farmers believed NNV and MCV (iridovirus) were major problems. However, farmers correctly identified parasites as an issue affecting productivity. Although farmers could identify signs of disease, only 7% consulted with government technical experts to resolve problems and most disposed of sick and dead fish by dumping at sea, which may perpetuate pathogen life cycles in the marine environment and ensure ongoing outbreaks of disease on farms.

5. The fifth and final objective was to build capacity in fish health management in Indonesia.

Training. Indonesian fish health professionals form a variety of institutions received diverse, intensive training over the life of the project in workshops in both Australia and Indonesia. The focus was on training in epidemiology and disease diagnosis, data management and analysis and technical writing. These areas had been identified as priorities. Participant feedback from all workshops was extremely positive and this was one of the most rewarding aspects of the project for Australian scientists. On-the-job training was also provided during field trials and laboratory work throughout the project, and again this was a highlight for everyone involved.

Education services at university level. Reciprocal academic visits enabled interactions of academics from both countries in both undergraduate and post graduate teaching and research in situ. Exchange visits and guest lectures were proposed as follow on activities, as well as student exchanges. Project-based collaborative research was an excellent vehicle for insertion of current knowledge in aquatic animal health into the curriculae in both countries. Due to institutional structural changes, both proposed and recently enacted, linkages initiated and maintained at academic level will be more enduring and beneficial than top-down ventures.

PCR laboratory ring testing in Indonesia. An intensive highly technical activity was completed successfully to evaluate competency in most eligible DGA laboratories in Indonesia and it was concluded successfully with strong support expressed for further ring testing and establishment of a reference laboratory network.

9.2 Recommendations

9.2.1 Objective 1. Key diseases responsible for production losses in marine finfish aquaculture

Recommendations about diagnosis of disease.

A key outcome of this project was the disentangling of PCR results that often are used in isolation to infer disease causation in Indonesia. PCR results when assessed in combination with the results of gross pathology, histopathology and parasitological examinations enabled an accurate diagnosis of single pathogen diseases as well as diseases of uncertain cause that were associated with combinations of environmental factors, management factors, nutritional factors and background burdens of viruses and parasites. The multidisciplinary approach to diagnosis needs to be reinforced and promoted further in aquatic animal health in Indonesia and more widely in the region. Leadership structures need to be identified and supported, together with ongoing training of staff. Reference laboratories, university laboratories and regional laboratories staffed by scientists with case management skills (which traditionally lie in the discipline of veterinary

pathology in Australia) should be supported through future projects. Studies to identify and quantify risk factors for disease expression will support improved farm management.

Recommendations related to important diseases in hatcheries.

a. Control of disease caused by NNV is a priority. Transmission in Australia was shown to be horizontal, i.e. in the water supply (barramundi hatcheries) and this may also be the case in Indonesia, but it needs to be proven for grouper in order to prioritise biosecurity for broodstock (broodstock testing prior to entry) or for the hatchery and its water supply.
b. The role of MCV in disease outbreaks in hatcheries is unclear and further research during disease outbreaks is recommended, with emphasis on obtaining samples at the peak of outbreaks for histopathology and PCR. Identification of these viruses to species level is also required.
c. Both viruses can be present in the environment (the evidence was in trash fish, wild fish and in biofouling around sea cages), highlighting the importance of biosecurity throughout the susceptible life history stages in hatcheries and nurseries.
d. Ammonia and nitrite toxicity can be detected with routine monitoring and requires improved water quality management
e. In backyard hatcheries protozoans (*Amyloodinium* sp.,*Trichodina* spp.) and a scuticociliate were important and may have had interactions with viruses.
f. Studies are required on hatcheries in other regions of Indonesia to determine the extent to which the results of this project can be applied elsewhere.

Recommendations related to important diseases identified in sea cages.

a. NNV detection in grower fish in sea cages was mostly incidental, and cannot be avoided due to environmental reservoirs. It is recommended that testing for NNV by PCR in fish from sea cages be discontinued because the results will not influence disease control recommendations. It is noted that VNN can occur in grow-out grouper and this would be identified in a case-managed disease investigation with histopathology.
b. Residual deformities from the hatchery stage and chronic non-specific visceral lesions are likely to be of multifactorial aetiology and should be addressed initially by improved nutritional and husbandry practices. Risk factor studies can identify management practices that will minimise the impact of these chronic low grade diseases.
c. The role of MCV in disease outbreaks in sea cages is unclear. Further outbreak investigation research is recommended with emphasis on obtaining samples at the peak of outbreaks for histopathology and PCR. A case management approach to disease investigation with collection of history, clinical, necropsy and histopathology information will determine if bacteriology, virology or specific molecular tests are indicated. Further research is also recommended using the archived samples from this study to determine if MCV is associated with non-specific histopathological lesions, and unbiased high throughput sequencing can be used to test for a novel or unsuspected pathogen.
d. It is recommended that the role of anti-parasitic treatments be investigated in relation to the clinical signs of green-eye disease.
e. Studies are required on sea cage farms in other regions of Indonesia to determine the extent to which the results of this project can be applied elsewhere.

9.2.2 Objective 2. Interventions to reduce production losses.

a. On-farm parasite reservoirs and the efficacy of the freshwater bathing routine. Parasites caused chronic production-limiting problems at sea cage farms but the burdens of these parasites varied substantially between farms both before and

after treatments applied by farmers, suggesting that management practices could be optimised. The bathing schedules are routinely completed weekly to every two weeks, are labour intensive and are considered to be stressful for fish. One of the key outcomes from this study was to identify the lack of efficacy of the bathing treatments in eliminating parasites. There is a need for future research to identify the severity and persistence of parasites at the sea cage farms. Future research activities need to focus on determining the recruitment or re-infestation of key parasites.

b. Clinical trials are recommended to identify effective integrated parasite management strategies, such as the use of multiple chemical treatments targeting various life cycle stages used in combination with changes to farm management (e.g. fallowing, net changes and cleaning and farm location).
c. There was large difference in the prevalence of external and internal lesions between farms), indicating an opportunity to explore management solutions to these non-specific disease syndromes. It is recommended that changes to fish care and farm management be further evaluated to assess the impact of such changes on disease.
d. Research is recommended to identify suitable farm biosecurity practices that can be used alone and in combination with effective parasite management to reduce the introduction and impact of aquatic pathogens. Improving farm biosecurity, including proper disposal of sick and moribund fish, and integrating parasite management strategies will create healthier fish populations, reduce farm chemical inputs and support sustainable production.

9.2.3 Objective 3. Improved management protocols for hatcheries and grow-out farms including biosecurity and improved nutrition

Recommendations for improved hatchery practices.

a. Larval production was hampered by losses due to VNN and poor water quality. It was possible to produce some batches of fish that were not affected by NNV in this project and some that were infected but did not experience high mortality disease. The reasons for biosecurity breakdowns in some intensive hatchery batches were not identified in this study so only general recommendations about water treatment and general biosecurity to exclude pathogens can be made.
b. It is recommended that research be undertaken on improving water treatments to exclude pathogens and on water quality management during larval rearing to reduce the impact of ammonia and nitrite toxicity on fish health. This will improve overall disease resistance. Improvements are needed to reliably and consistently reduce the impact of ammonia-nitrogen toxicity on fish health.
c. For small backyard hatcheries, future research is needed to identify and test suitable biosecurity practices including source of broodstook, personnel management, disinfection and water filtration techniques to prevent viral and parasitic disease outbreaks.
d. Deformities were important causes of morbidity in hatcheries. It is recommended that the role of micronutrient deficiencies (Vitamins A and C) should be investigated specifically together with a whole system approach to investigate the causes and prevention of skeletal deformities. Future research should include a focus on improvements in larval nutrition.

Recommendations for improved sea cage nutrition.

a. Numerous constraints to adoption of compounded diets were identified. It is recommended that pathways be identified for establishment of demonstration farms on which research to overcome poor growth rates and lack of palatability of

pelleted diets could be undertaken. Formal cost-benefit analysis on trash fish and pellet feed, including social and environmental costs should also be undertaken.

b. Further work on fish husbandry, biosecurity and nutrition is required to improve farm productivity. Improved management protocols for hatcheries and grow-out farms

9.2.4 Objective 4. Socioeconomic evaluation.

Common myths about diseases affecting grouper in sea cages were well entrenched and contrasted with the findings from the epidemiological surveys, i.e. farmers believed NNV and MCV (iridovirus) were major problems. However, farmers correctly identified parasites as an issue affecting productivity. Although farmers could identify signs of disease, only 7% consulted with government technical experts to resolve problems and most disposed of sick and dead fish by dumping at sea, which may perpetuate pathogen life cycles in the marine environment and ensure ongoing outbreaks of disease on farms.

9.2.5 Objective 5. Capacity in fish health management in Indonesia

Training.

It is recommended that future projects for capacity building in aquatic animal health build on the model established in this project by including intensive workshops with a low trainee:trainer ratio. Hands on training during both field trials and laboratory work should also be included because the work required for project outcomes and the training are undertaken simultaneously, which provides a high level of motivation.

Education services at university level.

Due to institutional structural changes, both proposed and recently enacted in both countries, it is recommended that linkages be initiated, strengthened and maintained at academic level as this will be a career-long engagement and is likely to be more effective in building expertise in Indonesia than top-down ventures. Applications should be submitted for resources that may become available for international linkages from time to time from institutional sources such as the USYD Sydney Southeast Asia Centre, OIE (University twinning program) and Government of Indonesia Ministry of Research, Technology and Higher Education programs for academic enrichment.

'Ring-testing' / validation studies for PCR laboratories in Indonesia

a. Support for a reference laboratory system. A strong theme emerging in the results was that experience with a specific test procedure was very important in provision of timely and accurate diagnostic results. A reference laboratory system could enhance the capacity of laboratories to perform specific assays. The reference laboratory would be a place to confirm or troubleshoot results from other laboratories. It would also be a focus for investment in the diagnostic network where highly trained multi-lingual scientists gain expertise and in turn provide formal training and support to staff in other laboratories. It was noted that language is a limiting factor in highly technical discussions about PCR methodology so experts communicating in the native language of trainees is desirable.

b. Ongoing ring-testing. A complex range of factors influenced PCR test performance. Regular bench-marking between laboratories using well-characterised standard samples can be achieved through on-going ring-testing.

This will generate regular communication amongst a network of scientists and enable continued performance improvement.

c. Implementation of new technology. It is expected that demand for real-time PCR methods will increase. The impetus for this will be a need to align with changing international standards and the need to handle larger numbers of samples with less risk of cross contamination. Introduction of automated nucleic acid purification technology and procedures is also a high priority.

References

9.3 References cited in report

Hick, P. and Whittington, R.J. 2010. Optimisation and validation of a real-time reverse transcriptase-polymerase chain reaction assay for detection of betanodavirus. Journal of Virological Methods 163**,** 368-377.

Kongkeo, H., Wayne, C., Murdjani, M., Bunliptanon, P. and Chien, T. 2010. Current practices of marine finfish cage culture in China, Indonesia, Thailand and Viet Nam. Aquaculture Asia Magazine. 32-40.

Rimmer, A.E., Becker, J.A., Tweedie, A. and Whittington, R.J. 2012. Validation of high throughput methods for tissue disruption and nucleic acid extraction for ranaviruses (family Iridoviridae). Aquaculture 338–341**,** 23-28.

Rückert, S., Klimpel, S., Al-Quraishy, S., Mehlhorn, H. and Palm, H. 2009. Transmission of fish parasites into grouper mariculture (Serranidae: *Epinephelus coioides* (Hamilton, 1822)) in Lampung Bay, Indonesia. Parasitology Research 104**,** 523-532.

Sugama, K., Rimmer, M.A., Ismi, S., Koesharyani, I., Suwirya, K., Giri, N.A. and Alava, V.R. 2012. Hatchery Management of Tiger Grouper (*Epinephelus fuscoguttatus*): A Best-Practice Manual. ACIAR Monograph No.149. Australian Centre for International Agricultural Research: Canberra, Australia.

9.4 List of publications produced by project

Becker JA. Biosecurity in Aquaculture, International symposium of freshwater and marine fisheries and aquaculture, Universitas Gadjah Mada, Yogyakarta, Indonesia, 5 August 2015.

Fachry, M.E., Sugama, K and Rimmer, M.A. (in press). The role of small-holder seed supply in commercial mariculture in South-east Asia. Aquaculture.

Hick, PM. Nervous necrosis virus and viral nervous necrosis: A pathogen and a disease. International Symposium on marine and fisheries research. Yogyakarta, August 7 2015.

Hick, P., Tweedie, A., Murwantoko, Tosihadi, T., Rimmer, M. and Whittington, R.J. Evaluation of PCR methods used by Indonesian laboratories for aquatic pathogens. 14th International Society of Veterinary Epidemiology and Economics, Merida, Mexico, 2015.

Hick, P., Tweedie, A., Murwantoko, Tosihadi, T., Rimmer, M. and Whittington, R.J. An aquatic pathogen PCR ring-test in Indonesia using Epizootic haematopoietic necrosis virus and nervous necrosis virus as model pathogens. Third International Symposium on Ranaviruses, May 30 – June 1 2015, Gainesville, Florida.

Rimmer, M.A., Fachry, M.E. and Sugama, K. 2017. The role of small-holder seed supply in commercial mariculture in Southeast Asia (abstract). In: Larvi 2017 – 7th Fish and Shellfish Larviculture Symposium, University of Ghent, Belgium, 4 – 7 September 2017; pp. 368–369.

Rimmer, M.A., Sudja, W., Indradjaja, D., Giri, N.A. and Laining, A. 2016. Compound feeds for grouper aquaculture – why has adoption been so poor? Aquaculture Asia-Pacific Magazine 12, 25-28.

Sah Putra B, Hick P, Rimmer M, Khairul R, Evarianti E, Nurbariah N, Royani R, Tajussalatin T, Muslim M and Becker J. Identification of the key pathogens responsible for production losses at grouper nurseries in Aceh Indonesia. *Front. Vet. Sci*. *Conference Abstract: AquaEpi I - 2016*.

10 Appendixes

10.1 Appendix 1 – Additional results tables and figures

Table A1. Summary of each sampling event from the longitudinal cross-sectional hatchery survey.

Sample ID	Farm ID	Sample Date	Fish Species	DAH	Quality of fish	Weight g average	Weight g SD	Total Length mm average	Total Length mm SD	Parasite Proportion	MCV[1]	NNV[1]
1	1	March 2014	Cantang	45	healthy	0.46	0.15	30.95	2.48	0/50	0/50	7PP/50
2	2	March 2014	Cantik	45	healthy	0.40	0.17	28.35	3.43	0/36	0/36	All positive/36
3	11	March 2014	Cantik	47	healthy	0.30	0.08	27.71	2.79	0/36	0/36	All positive/36
4	11	March 2014	Tiger	47	sick	0.39	0.13	29.77	3.06	0/36	0/36	All positive/36
5	37	March 2014	Cantik	47	healthy	1.25	0.24	43.67	5.33	0/36	0/36	1PP/36
6	5	March 2014	Cantang	47	sick	1.69	0.99	43.76	8.93	0/36	0/36	All positive/36
7	6	March 2014	Cantang	47	sick	0.67	0.44	32.95	7.66	0/35	0/35	All positive/36
8	7	March 2014	Cantang	49	healthy	0.49	0.20	30.15	5.02	0/36	0/37	2PP/37
9	8	March 2014	Cantang	50	sick	0.30	0.18	25.84	4.34	0/36	0/35	All positive/35
10	9	March 2014	Coral trout	83	sick	1.42	0.57	47.21	5.59	9/36	0/36	0/36

Sample ID	Farm ID	Sample Date	Fish Species	DAH	Quality of fish	Weight g average	Weight g SD	Total Length mm average	Total Length mm SD	Parasite Proportion	MCV[1]	NNV[1]
11	1	December 2014	Cantang	45	healthy	0.37	0.11	3.00	2.24	0/36	0/36	5PP/36
12	10	December 2014	Cantang	45	sick	0.53	0.25	3.08	0.30	0/36	1PP/36	All positive/36
13	11	December 2014	Tiger	35	healthy	0.16	0.05	2.24	0.20	0/36	0/36	All positive/36
14	11	December 2014	Cantik	45	healthy	0.13	0.07	2.09	0.34	0/36	0/36	All positive/36
15	5	May 2014	Cantik	45	healthy	0.43	0.15	2.98	0.31	0/36	0/36	All positive/36
16	5	May 2014	Cantang	45	healthy	0.93	0.51	3.77	0.59	0/36	0/36	All positive/36
17	12	May 2014	Cantik	45	healthy	0.89	0.28	3.75	0.42	0/36	0/36	5PP/36
18	7	May 2014	Cantang	45	healthy	0.52	0.12	3.00	0.20	0/36	0/36	4PP/36
19	13	May 2014	Cantang	45	healthy	0.31	0.11	2.55	0.23	0/36	0/36	4PP/36
20	14	May 2014	Cantang	45	healthy	0.54	0.12	3.29	0.20	0/36	1PP/36	5PP/36
21	15	June 2014	Cantang	54	sick	0.80	0.41	3.73	0.54	0/30	0/30	All positive/30
22	16	June 2014	Cantang	54	sick	2.35	0.81	4.84	0.56	2/28	0/31	All positive/31
23	17	June 2014	Cantik	54	sick	0.19	0.12	2.38	0.28	23/30	0/30	All positive/30
24	18	June 2014	Cantik	75	sick	2.08	0.76	5.06	0.49	0/16	0/16	All positive/16
25	18	June 2014	Cantang	90	healthy	8.94	3.67	7.91	0.98	12/16	1PP/16	All positive/16
26	19	unknown	Cantang	90	healthy	1.04	0.55	3.85	0.68	0/28	2PP/30	All positive/30

Sample ID	Farm ID	Sample Date	Fish Species	DAH	Quality of fish	Weight g average	Weight g SD	Total Length mm average	Total Length mm SD	Parasite Proportion	MCV[1]	NNV[1]
27	6	August 2014	Cantang	68	sick	2.75	0.86	5.30	0.48	0/35	0/36	All positive/36
28	20	August 2014	Cantang	69	sick	2.75	0.84	5.40	0.52	0/36	0/36	All positive/36
29	10	August 2014	Cantang	70	sick	2.96	1.24	5.05	0.62	34/36	1PP/36	All positive/36
30	21	August 2014	Tiger	68	sick	1.71	0.46	4.82	0.41	4/36	2PP/36	All positive/36
31	21	August 2014	Cantang	68	sick	4.35	0.86	6.26	0.42	5/36	1PP/36	All positive/36
32	19	August 2014	Cantang	97	sick	6.55	1.27	7.39	0.61	0/36	1PP/36	All positive/36
33	22	August 2014	Cantang	69	sick	2.68	0.88	5.26	0.53	19/36	3PP/36	All positive/36
34	9	September 2014	Cantang	71	healthy	4.81	1.58	6.09	0.65	3/36	1PP/36	All positive/36
35	19	October 2014	Coral trout	82	healthy	2.89	0.85	5.76	0.56	0/36	0/36	All positive/36
36	7	October 2014	Cantang	46	healthy	0.26	0.14	2.48	0.40	0/36	1PP/36	7PP/36
37	23	October 2014	Cantik	46	healthy	0.90	0.28	3.79	0.37	0/36	0/36	All positive/36
38	9	October 2014	Tiger	46	healthy	0.14	0.12	1.97	0.52	0/36	1PP/36	All positive/36
39	24	October 2014	Cantang	45	healthy	1.06	0.29	3.84	0.33	0/36	0/36	2PP/36
40	24	October 2014	Cantik	45	healthy	1.03	0.33	4.14	0.36	0/36	0/36	6PP/36
41	25	October 2014	Coral trout	46	healthy	0.09	0.06	1.58	0.24	0/36	0/36	5PP/36
42	26	October 2014	Cantang	46	healthy	2.14	0.41	5.00	0.38	2/36	1PP/36	4PP/36

Sample ID	Farm ID	Sample Date	Fish Species	DAH	Quality of fish	Weight g average	Weight g SD	Total Length mm average	Total Length mm SD	Parasite Proportion	MCV[1]	NNV[1]
43	27	October 2014	Cantik	46	healthy	2.22	0.55	5.07	0.46	0/36	1PP/36	All positive/36
44	27	October 2014	Cantang	46	healthy	1.38	0.63	4.27	0.66	0/36	1PP/36	All positive/36
45	28	January 2015	Cantang	36	healthy	0.28	0.18	2.47	0.31	0/36	2PP/36	7PP/36
46	29	January 2015	Cantik	36	healthy	0.23	0.10	2.43	0.35	0/36	0/36	All positive/36
47	30	January 2015	Cantik	36	healthy	0.32	0.08	2.81	0.26	0/36	1PP/36	All positive/36
48	31	February 2015	Cantik	36	healthy	0.34	0.21	2.74	0.54	0/36	0/36	All positive/36
49	1	February 2015	Cantang	36	healthy	0.18	0.06	2.13	0.21	0/36	0/36	5PP/36
50	32	February 2015	Cantang	36	healthy	0.48	0.18	2.99	0.43	0/36	0/36	7PP/36
51	33	February 2015	Cantik	36	healthy	0.46	0.09	3.12	0.23	0/36	0/36	7PP/36
52	32	February 2015	Cantang	36	healthy	0.93	0.48	3.63	0.60	0/36	0/36	All positive/36
53	34	February 2015	Cantik	36	healthy	0.54	0.22	3.19	0.40	0/36	0/36	All positive/36
54	33	May 2015	Cantang	39	healthy	0.18	0.10	2.24	0.33	0/36	0/36	4PP/36
55	35	December 2015	Cantik	25	sick	0.05	0.02	1.30	0.14	not done	0/36	All positive/36
56	35	December 2015	Cantik	35	healthy	0.28	0.26	2.49	0.30	not done	0/36	7PP/36
57	36	February 2015	Cantik	39	healthy	0.20	0.11	2.38	0.20	not done	0/40	6PP/40
58	36	December 2015	Cantik	49	healthy	1.30	0.27	4.35	0.27	not done	0/35	All positive/35

Sample ID	Farm ID	Sample Date	Fish Species	DAH	Quality of fish	Weight g average	Weight g SD	Total Length mm average	Total Length mm SD	Parasite Proportion	MCV[1]	NNV[1]
59	36	November 2015	Cantik	52	healthy	0.12	0.17	1.71	0.43	not done	0/37	6PP/36
60	36	March 2015	Cantik	31	sick	0.05	0.03	1.43	0.18	not done	0/36	All positive/36
61	36	March 2015	Cantik	82	sick	1.10	0.29	3.99	0.37	0/36	0/36	All positive/36
62	36	March 2015	Cantang	38	sick	0.09	0.03	1.92	0.20	0/36	2PP/36	7PP/36
63	7	July 2015	Cantang	47	sick	0.30	0.06	2.62	0.18	0/36	0/36	All positive/36
64	27	July 2015	Cantik	47	sick	0.43	0.07	3.13	0.19	0/36	2PP/36	All positive/36
65	30	July 2015	Cantang	47	sick	1.18	0.17	4.17	0.18	0/36	2PP/36	7PP/36
66	35	August 2015	Coral trout	70	healthy	0.58	0.28	3.34	0.54	0/36	1PP/36	5PP/36
67	30	August 2015	Cantik	48	sick	1.87	0.23	5.04	0.21	0/36	0/36	6PP/36
68	23	August 2015	Cantang	48	sick	0.41	0.17	2.97	0.32	0/36	2PP/36	All positive/36
69	27	August 2015	Cantang	48	sick	0.85	0.12	3.75	0.18	0/36	0/36	7PP/36
70	36	May 2015	Cantik	40	healthy	0.18	0.09	2.40	0.36	0/36	1PP/36	5PP/36

1. Typically, 36 fish were pooled into 8 PCRs with 5 fish in 7 pools and one pool of 1 fish and the number of positive pools (PP) have been reported.

Table A2. Longitudinal epidemiological study of grow-out farms. The frequency of lesions was different between species, farm, season and quarantine. Data are prevalence (%) with p-values determined from the likelihood ratio Chi-square tests.

Explanatory variable	Category	External lesions		Internal lesions									
				Internal lesion - all		Gastrointestinal		Spleen		Liver		Kidney	
		Prev. (%)	p	Prev. (%)	p	Prev. (%)	p	Prev. (%)	p	Prev. (%)	p	Prev. (%)	p
Species	Cantang	43.1	0.47	97.4	0.004	65.5	<0.001	51.7	0.50	53.5	<0.001	40.5	<0.001
	Cantik	41.1		91.1		32.6		52.5		57.2		31.8	
	Macan	45.4		95.8		35.9		56.3		69.8		53.5	
Farm	A	25.4	<0.001	99.2	0.006	31.4	0.002	60.2	0.31	75.4	<0.001	48.3	0.04
	B	47.9		93.2		45.1		52.1		56.3		36.3	
	C	43.6		92.6		34.1		53.6		61.9		42.8	
Season / Month	March	39.5	<0.001	83	<0.001	21.5	<0.001	51.5	0.06	47.5	<0.001	35	<0.001
	June	45.2		96.6		38.8		54.4		63.3		31.2	
	October	58.1		94.5		44.9		48.3		64.8		42	
	December	30.5		98.2		43		59.9		67.3		53.3	
Quarantine	No	38.5	<0.001	93.2	0.02	36.1	<0.001	53.2	0.16	59.9	<0.001	37.6	<0.001
	Yes	91.3		98.8		57.5		61.3		78.8		78.8	

Table A3. Longitudinal epidemiological study of grow-out farms. The total length and weight and the derived condition index for all sampled fish. Data are the minimum, maximum, means and standard deviations of weight, length and condition index. The p-values are based on fitting linear mixed models after including farm as a random effect.

(i): Condition score varied significantly between the species/hybrid after accounting for farm and season and was lower in quarantined fish and with external lesions.

Classification variable	Category	Proportion (%)	n	Weight (g)			Total Length (mm)			Condition Index			
				Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	p
Species	Cantang	12.3	116	10	1090	293 ± 334	100	375	209 ± 90	0.75	3.09	1.85 ± 0.35	<0.001
	Cantik	50.0	472	15	980	288 ± 209	110	380	235 ± 65	1.07	3.20	1.74 ± 0.24	
	Macan	37.8	357	40	960	360 ± 172	135	360	254 ± 48	0.64	2.84	2.02 ± 0.24	
Farm	A	12.5	118	65	980	429 ± 183	165	380	276 ± 40	1.42	3.20	1.88 ± 0.25	-
	B	37.6	355	10	1090	370 ± 267	100	375	247 ± 80	0.75	3.09	1.81 ± 0.29	-
	C	50.0	472	24	770	246 ± 152	122	350	224 ± 49	0.64	2.84	1.90 ± 0.29	-
Season / Month	March	21.2	200	24	910	361 ± 257	122	360	249 ± 74	1.07	2.84	1.78 ± 0.28	<0.01
	June	25.1	237	15	1090	274 ± 238	110	375	226 ± 66	1.02	3.09	1.80 ± 0.28	-
	October	25.0	236	10	735	266 ± 179	100	340	225 ± 64	0.64	2.69	1.89 ± 0.31	-
	December	28.8	272	40	980	362 ± 182	135	380	256 ± 49	1.34	3.20	1.95 ± 0.25	-
Quarantine	No	8.5	865	10	1090	312±218	100	375	237±64	0.64	3.20	1.87±0.29	0.04
	Yes		80	40	980	362±215	135	380	260±64	1.34	2.67	1.80 ±0.28	

Table A4. Longitudinal epidemiological study of grow-out farms. There was a very high prevalence of external and internal lesions in a representative sample of fish from routine production. External lesions were associated with a reduced condition index after accounting for other factors. The condition index was positively correlated with the presence of internal lesions. The p-values are based on linear mixed models after including farm as a random effect.

Lesion	Prevalence (%)	Present (yes/no)	n	Weight (g)			Total Length (mm)			Condition Index			
				Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	p
External - all	43	No	539	10	1090	316± 220	100	375	238±63	0.75	3.20	1.88±0.29	0.04
		Yes	406	15	980	316±217	100	380	240±67	0.64	2.84	1.84±0.29	
Internal - all	93.7	No	60	20	715	248± 217	105	340	217±72	1.17	2.59	1.73 ± 0.28	< 0.01
		Yes	885	10	1090	321± 218	100	380	240±64	0.64	3.20	1.87 ± 0.29	
Gastrointestinal	37.9	No	587	10	1090	331 ± 213	105	380	245±61	0.64	3.20	1.86 ± 0.29	0.43
		Yes	358	15	1075	292 ± 225	100	360	229±68	1.10	2.67	1.86 ± 0.29	
Spleen	53.9	No	436	15	1075	286±213	100	360	230±66	0.64	3.20	1.84 ± 0.3	0.07
		Yes	509	10	1090	342±220	100	380	247±62	0.75	3.09	1.88 ± 0.28	
Liver	61.5	No	364	10	1090	296±228	100	360	231±69	0.75	3.09	1.83 ± 0.29	0.01
		Yes	581	15	1075	329±211	110	380	244±61	0.64	3.20	1.88 ± 0.28	
Kidney	41.1	No	557	10	430	286+219	105	360	230±66	0.64	3.20	1.82 ± 0.29	<0.01
		Yes	388	15	495	359+210	100	380	252±59	1.12	2.84	1.91 ± 0.28	

Table A5. Longitudinal epidemiological study of grow-out farms. Condition index was significantly reduced in the low number of fish with *Haliotrema* sp. infestation of the skin and *Pseudorhabdosynochus sp* infestation of the gill which impacted nearly a third of all fish was associated with a higher condition score. The p-values are based on linear mixed models after including farm as a random effect.

Parasite	Prevalence (%)	Present (yes/no)	n	Weight (g)			Total Length (mm)			Condition Index			
				Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	p
Skin													
Benedinia sp.	5.3	No	895	10	1090	312±219	100	380	237±65	0.64	3.20	1.86±0.29	0.49
		Yes	50	90	900	394.2 ± 198	167	355	272±44	1.39	2.35	1.81±0.22	
Neobenedinia sp.	6.2	No	886	10	1090	304±214	100	380	236±64	0.64	3.20	1.86±0.29	0.12
		Yes	59	20	910	489± 216	112	365	287±52	1.17	2.62	1.87±0.27	
Pseudorhabdosynochus sp.	0.6	No	939	10	1090	315±217	100	380	239±64	0.64	3.20	1.86±0.29	0.11
		Yes	6	39	900	460±380	148	356	267±93	1.20	2.01	1.67±0.33	
Haliotrema sp.	0.5	No	940	10	1090	316±218	100	380	239±64	0.64	3.20	1.86±0.29	<0.01
		Yes	5	27	890	236±372	127	356	198±96	1.07	1.97	1.52±0.4	
Gill													
Pseudorhabdosynochus sp.	28.2	No	678	10	1075	316±224	100	380	239±66	0.75	3.20	1.84±0.28	<.001
		Yes	266	15	1090	317±204	110	360	238±61	0.64	3.09	1.92±0.3	
Haliotrema sp.	3.8	No	909	10	1090	320±218	100	380	241±64	0.64	3.20	1.86±0.29	0.87
		Yes	36	25	815	205±201	113	355	201±68	1.34	2.42	1.85±0.28	
Gill parasite other	1.9	No	927	10	1090	315±219	100	380	238±64	0.64	3.20	1.86±0.29	0.30
		Yes	18	20	655	385±181	112	334	267±59	1.42	2.25	1.76±0.23	
Fin Parasite (any species)	0.5	No	940	10	1090	315±218	100	380	239±64	0.64	3.20	1.86±0.29	0.38
		Yes	5	46	715	466±258	143	340	282±80	1.57	1.85	1.73±0.12	

Table A6. Longitudinal epidemiological study of grow-out farms. Prevalence of parasite infestations varied between species, farm, season and quarantine. Data are prevalence (%) with p-values from the likelihood ratio chi square analysis.

Explanatory variable	Category	Skin parasites								Gill parasites						Fin Parasite	
		Benedinia sp.		*Neobenedinia sp.*		*Pseudorhabdosynochus sp.*		*Haliotrema sp.*		*Pseudorhabdosynochus sp.*		*Haliotrema sp.*		Other species		All species	
		Prev. (%)	p	Prev. (%)	p	Prev. (%)	p	Prev. (%)	p	Prev. (%)	p	Prev. (%)	p	Prev. (%)	p	Prev. (%)	p
Species	Cantang	5.2	<0.001	6.9	0.11	0.9	0.06	0	0.29	11.3	<0.001	8.6	0.01	1.7	0.61	0.9	0.85
	Cantik	8.1		7.6		1.1		0.9		14		2.3		2.3		0.4	
	Macan	1.7		4.2		0		0.3		52.4		4.2		1.4		0.6	
Farm	A	0.9	<0.001	1.7	<0.001	0	0.42	0	0.28	14.4	<0.001	2	0.66	0	<0.01	0	0.38
	B	9.6		15.5		0.9		0.3		26.8		2.5		3.7		0.9	
	C	3.2		0.4		0.6		0.9		32.7		4.2		1.1		0.4	
Season / Month	March	4	0.015	10	<0.001	2	0.02	2.5	0.001	32.5	0.31	32.5	0.10	4.5	<0.01	2.0	0.02
	June	9.3		7.6		0.8		0		29.1		29.1		3.4		0.4	
	October	5.1		8.1		0		0		24.6		24.6		0.4		0	
	December	2.9		0.7		0		0		27.3		27.3		0		0	
Quarantine	No	5.1	0.38	6.5	0.30	0.6	0.52	0.6	0.35	28.4	0.69	3.5	0.11	1.4	<0.01	0.5	0.42
	Yes	7.5		3.8		1.3		0		26.3		7.5		7.5		1.3	

Table A7. Longitudinal epidemiological study of grow-out farms.. Evaluation of associations of weight, length, condition index and lesion presence with infection status for NNV and MCV The p-values are based on linear mixed models or generalised linear mixed models after including farm as a random effect.

Variable		**NNV**			**MCV**		
		Negative	**Positive**	**p**	**Negative**	**Positive**	**p**
Weight (g) (mean ± SD)		317±195	334±255	<0.001	330±198	209±215	<0.01
Total length (mm) (mean + SD)		240±60	239±69	0.70	244±58	196±73	<0.01
Condition index (mean + SD)		1.86±0.23	1.88±0.18	0.60	1.88±0.21	1.66±0.26	<0.01
External lesions		0.42±0.29	0.54±0.32	0.09	0.43+0.3	0.43±0.27	0.70
Internal lesions	all	0.94±0.13	0.95±0.11	0.55	0.94±0.12	0.91±0.17	0.28
	Gastrointestinal	0.35±0.31	0.56±0.32	<0.01	0.37±0.31	0.45±0.38	0.44
	Spleen	0.54±0.26	0.54±0.29	1.00	0.55±0.26	0.49±0.33	0.36
	Liver	0.61±0.28	0.7±0.29	0.07	0.63±0.27	0.51±0.33	0.19
	Kidney	0.41±0.29	0.5±0.31	0.15	0.43±0.3	0.31±0.24	0.13
	Quarantine	0.08±0.26	0.26±0.44	<0.01	0.1±0.29	0.07±0.24	0.66

		NNV					**MCV**			
		Prev. (%)	**OR**	**95% CI**		**p**	**Prev. (%)**	**OR**	**95% CI**	**p**
Species	Cantang	53.2	35.0	7.1	173.0	<0.01	5.0	Model did not converge		
	Cantik	5.7	1.9	0.4	8.1		15.6			
	Macan	3.1	ref	-	-		0			
Season / Month	March	2.9	0.19	0.04	0.97	0.11	6.98	Model did not converge		
	June	8.1	0.60	0.17	2.1		26.0			
	October	3.5	0.24	0.06	1.0		6.12			
	December	12.9	ref	-	-		0			

Table A8. Summary data for populations affected by Green-eyed grouper syndrome, stratified according to presence of clinical signs. Data are: mean and standard deviation for length, weight and condition index; prevalence (%) for lesions identified on necropsy; prevalence of ectoparasite infestation; and individual prevalence for the viruses NNV (NNV) and MCV (MCV). Factors that are significantly associated with clinical disease are indicated * ($p<0.05$).

Population hybrid, farm ID month/year	Health status	n	Size			Surface lesions (%)			Internal lesions (%)					
			Length (mm)	Weight (g)	Condition Index	Green eyes	Green body	Fin / skin	Peritoneal fluid	Kidney	Liver	Gall bladder	Large spleen	Small Spleen
1. Cantik 1, 8/16	Normal	12	169.7 +/- 9.3	84.6 +/- 11.5	1.74 +/- 0.23	67	42	17	0	0	33	0	8	17
	Sick	24	176.7 +/- 19.3	71.8 +/- 28.3	1.24 +/- 0.21	88	79	38	0	46	75	0	4	50
2. Cantik 2, 9/16	Normal	12	144.6 +/- 7.2	45.7 +/- 6.4	1.51 +/- 0.15	25	0	8	8	0	0	0	0	0
	Sick	24	155.4 +/- 9.4	58.6 +/- 10.4	1.55 +/- 0.17	38	0	21	4	13	13	0	0	4
3. Cantik 3, 9/16	Normal	12	173.8 +/- 8.8	88.3 +/- 15.7	1.67 +/- 0.15	50	0	0	17	0	17	25	17	8
	Sick	24	123.1 +/- 14.1	26.4 +/- 8	1.40 +/- 0.26	79	33	4	8	33	8	4	17	17
4. Cantang 4, 9/16	Normal	12	157.9 +/- 10.1	65.3 +/- 20.7	1.61 +/- 0.27	58	0	0	58	0	8	8	0	8
	Sick	24	171.3 +/- 8.6	73.4 +/- 10.7	1.46 +/- 0.2	83	0	0	63	17	13	21	38	4
5. Cantik 5, 9/16	Normal	12	189.2 +/- 10.6	104.8 +/- 14.5	1.55 +/- 0.17	50	0	8	42	0	17	33	8	0
	Sick	24	168.2 +/- 11.4	70 +/- 15.8	1.47 +/- 0.24	79	0	38	25	38	13	21	17	0
6. Cantik 6, 9/16	Normal	12	167.5 +/- 8.4	66.8 +/- 7.8	1.43 +/- 0.21	25	0	25	0	0	17	8	0	8
	Sick	24	151.7 +/- 12.4	55.5 +/- 7.9	1.65 +/- 0.59	96	0	4	29	13	0	8	4	0
7. Cantang 6, 9/16	Normal	6	200 +/- 7.7	113.7 +/- 7.7	1.43 +/- 0.14	100	33	0	100	17	17	33	50	17
	Sick	12	212.4 +/- 13.5	105.3 +/- 14.1	1.10 +/- 0.16	100	33	8	25	42	17	42	17	17
8. Cantik 7, 10/16	Normal	12	156.3 +/- 6.4	56.5 +/- 5.5	1.48 +/- 0.13	75	0	25	33	0	0	17	0	8
	Sick	24	145.6 +/- 12.8	47 +/- 12.9	1.49 +/- 0.18	96	13	50	21	21	13	21	33	4
9. Cantang 8, 1/17	Normal	6	219.2 +/- 13.2	170 +/- 34	1.61 +/- 0.23	50	17	17	0	50	17	17	17	33
	Sick	12	213.3 +/- 12.1	123.8 +/- 28.3	1.29 +/- 0.3	100	67	17	42	58	42	33	0	42
10. Cantik 10, 2/17	Normal	6	283.3 +/- 27.3	376.5 +/- 68.5	1.68 +/- 0.37	17	0	0	0	0	0	50	0	0
	Sick	12	335.8 +/- 30.6	512.7 +/- 152.2	1.34 +/- 0.28	92	67	0	25	33	42	58	8	8

Population hybrid, farm ID month/year	Health status	n	Size			Surface lesions (%)			Internal lesions (%)					
			Length (mm)	Weight (g)	Condition Index	Green eyes	Green body	Fin / skin	Peritoneal fluid	Kidney	Liver	Gall bladder	Large spleen	Small Spleen
11. Cantik 8, 3/17	Normal	12	143.8 +/- 8.6	44.9 +/- 9.7	1.52 +/- 0.3	58	0	0	8	25	0	50	0	17
	Sick	24	141.9 +/- 15.2	47.9 +/- 14.8	1.65 +/- 0.31	100	58	17	21	42	8	46	17	17
All popualtions (combined)	Normal	114	174.1 +/- 34.1	93.4 +/- 76.1	1.57 +/- 0.23	52	7	10	23	6	11	20	7	10
	Sick	228	170 +/- 47.9	86.5 +/- 109.6	1.45 +/- 0.32	85*	28*	19*	23	30*	20*	20	15*	14
	Total	342	171.3 +/- 43.8	88.8 +/- 99.6	1.49 +/- 0.3	74	21	16	23	22	17	20	12	12

Population hybrid, farm ID month/year	Health status	n	Ectoparasites (%)					qPCR for viruses (%)	
			Fin *Hirudinea* sp.	Skin *Hirudinea* sp.	Gill *Benedenia* sp.	Gill *Haliotrema* sp.	Skin *Benedenia* sp.	NNV	MCV
1. Cantik 1, 8/16	Normal	12	0	0	0	0	0	100	100
	Sick	24	0	4	13	0	0	93	93
2. Cantik 2, 9/16	Normal	12	0	50	0	0	0	50	100
	Sick	24	0	25	0	0	0	60	75
3. Cantik 3, 9/16	Normal	12	100	100	0	0	0	0	100
	Sick	24	79	29	4	0	0	58	83
4. Cantang 4, 9/16	Normal	12	50	8	8	42	75	17	100
	Sick	24	50	21	4	58	46	8	100
5. Cantik 5, 9/16	Normal	12	42	8	0	0	0	83	67
	Sick	24	54	25	8	8	0	83	58
6. Cantik 6, 9/16	Normal	12	25	8	0	0	0	100	100
	Sick	24	88	67	8	0	0	0	0
7. Cantang 6, 9/16	Normal	6	50	0	0	0	0	83	100
	Sick	12	0	0	0	0	0	75	100
8. Cantik	Normal	12	0	8	0	0	0	33	83

Population hybrid, farm ID month/year	Health status	n	Ectoparasites (%)					qPCR for viruses (%)	
			Fin *Hirudinea* sp.	Skin *Hirudinea* sp.	Gill *Benedenia* sp.	Gill *Haliotrema* sp.	Skin *Benedenia* sp.	NNV	MCV
7, 10/16	Sick	24	0	13	0	0	0	33	67
9. Cantang 8, 1/17	Normal	6	0	17	33	0	0	83	33
	Sick	12	8	33	75	0	0	50	0
10. Cantik 10, 2/17	Normal	6	0	17	33	0	0	83	17
	Sick	12	8	42	42	0	0	92	86
11. Cantik 8, 3/17	Normal	12	0	0	42	0	0	100	67
	Sick	24	0	0	38	0	0	100	86
All popualtions (combined)	Normal	114	25	21	9	4	8	67	81
	Sick	228	29	23	14	7	5	66	83
	Total	342	28	23	12	6	6	66	82

Detailed results: On-farm parasite reservoirs and the efficacy of the freshwater bathing routine

A total of six populations of grouper were sampled from three different farms (Table A8). The leech *Zeylanicobdella arugamensis* was observed at Farms 2 and 3 with 17% to 47% of the fish infested prior to the bath treatment (Table A8). Prior to bathing (T0), 23 to 60% of the fish had at least one parasite observed from the mucus scrape and gill mount (Table A9). The parasites observed (in order of significance) included *Benedenia*, *Z. arugamensis*, and a parasitic egg that could not be identified (Table A10, Figure A1). We found significant reductions in the number of fish infested and the number of parasites on fish when comparing pre-bath to the 24 hours after bath samples. However, no parasite treatment was completely effective at removing all parasites at one hour or 24 hours after bathing. At 24 hours after bathing, 6 to 73% of fish had at least one ectoparasite.

From the logistic regression model with farm and population as random effects, there was a significant reduction ($p = 0.0021$) in the number of fish with at least one parasite following bathing at one hour (T1) and 24 hours (T24) after bathing (Table A9). The odds of a fish having at least one parasite at the T1 sample was one third (0.302; 95%: 0.173-0.528) of that compared to the T0 sample. After 24 hours, the odds of a fish having one parasite was approximately half (0.46; 95%CI: 0.27-0.784) compared to the T0 sampling point. Similarly, there was a reduction in the number of parasites observed following bathing at T1 and T24 (0.0182). From the model with farm and population as random effects, prior to bathing, the (geometric) mean (±SE) number of parasites per fish was 29 (± 14.8) and the burdens were significantly reduced to 11.6 (± 5.9) and 9.7 (±4.9) parasites per fish at T1 and T24 ($p = 0.0012$ and 0.007).

Benedenia was considered the most important parasite as it was observed in all populations with the majority of parasites observed in the gills (Table 3). None of the treatments were completely effective at eliminating *Benedenia* at 1 hour after the bath. Further, with the exception of Population #6, all populations were infested with *Benedenia* at 24 hours with 7 to 53% of fish affected (Table A10). There was a significant reduction in the number of fish with Benedenia at T1 and T24 ($p = 0.0048$). The odds of having *Benedenia* at T1 and T24 was 0.394 of that compared to T0. The mean number (±SE) of *Benedenia* per fish was significantly reduced ($p = 0.0214$) from 25.3 (±12.9) at T0 to 9.0 (±4.5) at T1 and T24.

Viral pathogen reservoirs

Wild fish living in and around cages (Table A11)

A variety of sentinel fish were collected at each farm with the most common being a cardinalfish. Fin erosion and gross pathological changes to the liver and spleen were observed in the sentinel fish. NNV was detected in 10-13% (95% CI: 2.1-30.7%) of wild fish collected from Farm 1 and Farm 2. Six cardinalfish and one parrotfish were positive for NNV and some fish had Ct values around 20 indicating a clinical infection. MCV was detected at one farm in 3% (95% CI: .01-17.2%) of wild fish.

Biofouling on Farm Infrastructure (Table A12)

NNV was detected in the biofouling samples at all three farms. The apparent prevalence at Farm 1 and 3 was 30% (95% CI: 14.7-49.4) and was 63.3% (95% CI: 43.9-80.1) at Farm 2. The biofouling on farm infrastructure (e.g. nets, equipment, buoys, and anchor ropes) is a vector for NNV. MCV was not detected in any of the biofouling samples collected at the three farms.

'Trash' fish (Table A12)

At Farm 2, NNV was detected in 40% (95% CI: 12.2-73.8%) of the ‘trash’ fish (*Sardinella lemuru*). At Farm 1, MCV was detected in 10% (95% CI: 0.03-44.5) of the ‘trash’ fish (*Terapon jarbua*). The samples were opportunistically collected from dead fish without knowledge of prior handling (e.g. freezing or cold storage). Trash fish are a vector for NNV and MCV for grow out production of grouper.

Table A9. Summary of the populations of juvenile grouper sampled at seas cages for the parasite study.

Farm ID	Farm Location	Population ID	Grouper species	Age (months)	Total length (mean ± SD cm)[1]	Percent of fish with no external abnormality[1]	Percent of fish with frayed fins at T0	Percent of fish with leeches at T0 (sum of parasites)[2]	Bath description
1	8° 7' 16.3" S 114° 37' 5.1" E	1	Cantik	6	23.6 ± 1.1	41.7 (25/60)	60 (18/30)	0	freshwater; 8m30s
		2	Macan	7	28.1 ± 1	75 (45/60)	10 (3/30)	0	freshwater; 11m0s
		3	Cantik	2.5	18.7 ± 1.1	86.7 (52/60)	13.3 (4/30)	0	freshwater; 11m0s
2	8° 7' 52.0" S 114° 36' 8.9" E	4	Cantik	6	21.1 ± 2.1	15 (9/60)	50 (15/30)	20 (11)	freshwater with acriflavin[3]; 30s
		5	Cantik	1	15.1 ± 0.69	3.3 (2/60)	60 (18/30)	46.6 (31)	freshwater with acriflavin[3]; 21s
3	8° 7' 8.7" S 114° 36' 45.7" E	6	Cantik	4	16.8 ± 1	40 (24/60)	43.3 (13/30)	16.6 (6)	sea water with acriflavin[3] for 2m20s

[1] includes the 30 fish randomly selected before the bath and the 30 fish selected 24 hours later

[2] as observed during gross external examination

[3] Acriflavin is a mixture of euflavine and proflavine used to treat bacterial and parasitic pathogens (Noga, 2011)

Table A10. Percentage of grouper with at least one observed parasite on the gill mount and mucus scrape at each of the three sample points.

Population ID	Percent of fish with at least one parasite (95% CI) (n=30)		
	T0	T1	T24
1	33.3 (17.3 ± 52.8)	13.3 (3.8 ± 30.7)	6.7 (0.8 ± 22.1)
2	63.3 (43.9 ± 80.1)	20 (7.7 ± 38.6)	43.3 (25.5 ± 62.6)
3	23.3 (9.9 ± 42.3)	3.3 (0.1 ± 17.2)	13.3 (3.8 ± 30.7)
4	60 (40.6 ± 77.3)	36.7 (19.9 ± 56.1)	33.3 (17.3 ± 52.8
5	60 (40.6 ± 77.3)	56.7 (37.4 ± 74.5)	73.3 (54.1 ± 87.7)
6	36.7 (19.9 ± 56.1)	10 (2.1 ± 26.5)	13.3 (3.8 ± 30.7)

Table A11. Summary of parasites observed from gill mount and mucus scrape in juveniles grouper.

Population ID	Percentage of gill mounts with parasites (parasite count)			Percentage of mucus scrapes with parasites (parasite count)		
	T0 (n=30)	**T1 (n=30)**	**T24 (n=30)**	**T0 (n=30)**	**T1 (n=30)**	**T24 (n=30)**
Benedenia						
1	13.3 (5)	13.3 (4)	6.7 (2)	13.3 (6)	0	0
2	53.3 (79)	20 (26)	40 (40)	13.3 (4)	0	0
3	6.7 (2)	3.3 (1)	6.7 (4)	16.7 (5)	0	0
4	26.7 (18)	30 (14)	13.3 (7)	26.7 (22)	6.7 (3)	20 (10)
5	30 (9)	53.3 (35)	53.3 (37)	40 (26)	3.3 (1)	16.7 (7)
6	16.7 (16)	6.7 (2)	0	16.7 (5)	0	0
Leech						
1	10 (3)	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	3.3 (1)	3.3 (1)	0
5	0	0	3.3 (1)	0	0	10 (3)
6	0	0	0	3.3 (1)	0	0
Egg						
1	0	0	0	0	0	0
2	0	0	3.3 (1)	0	0	0
3	0	0	3.3 (2)	0	0	0
4	6.7 (2)	0	0	3.3 (1)	3.3 (1)	3.3 (1)
5	3.3 (1)	3.3 (2)	16.7 (11)	0	0	3.3 (1)
6	3.3 (1)	0	0	0	0	0
Haliotrema						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	3.3 (1)	0	6.7 (2)	0	0	0
Neobenedenia						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	3.3 (6)	0	0	3.3 (4)	0	3.3 (4)
6	0	0	0	0	0	3.3 (1)
Unknown						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	3.3 (1)
4	6.7 (2)	0	0	0	0	0
5	0	0	0	0	0	0
6	3.3 (1)	3.3 (1)	3.3 (1)	0	0	0

Table A12. Summary of wild caught sentinel fish collected near the sea cages.

Farm ID	Fish species collected (number)[1]	Proportion of fish with an abnormality			Proportion with positive test (Ct range[5])	
		fin erosion[2]	liver[3]	spleen[4]	NNV	MCV
1	**cardinal** (14), *Abudefduf vaigiensis* (9), Stripey (2), Banded grouper (1), goby (1), yellow striped (1), *Terapon jarbua* (1), Pink fins (1)	8/30	6/30	3/30	3/30 (20.4-38.1)	0/30
2	**cardinal** (25), **Banded grouper** (1), *Thalassoma* wrasse (1), Lyre-tail damsel (1), Yellow tail (1), **Parrotfish** (1)	10/30	16/30	8/30	4/30 (21.8-33.1)	1/30 (38.1)
3	cardinal (26), *Abudefduf vaigiensis* (4), Pomacentrid (1), Striped (1)	15/30	13/30	11/30	0/30	0/30

[1] species in bold had a positive test for NNV or MCV

[2] at least one fin was recorded as frayed or eroded

[3] at least one of these changes was recorded in the liver, colour change (dark or pale), granular, petechial hemorrhage

[4] at least one of these changes was recorded in the spleen, colour change (dark or pale), size change (enlarged or reduced), petechial hemorrhage

[5] Ct (cycle threshold) ranges under 30 were considered clinical infections and over 30 were considered persistent sub-clinical infections.

Table A13. Summary of trash fish and biofouling samples collected at each farm.

Farm ID	Proportion with positive test (Ct range[1])			
	Trash fish		Biofouling	
	NNV	MCV	NNV	MCV
1	0/10	1/10 (34.4)	9/30 (30.5-37.3)	0/30
2	4/10 (31.6-35.2)	0/10	19/30 (30.4-37.7)	0/30
3	Samples not available		9/30 (30.5-37.3)	0/30

[1] Ct: cycle threshold

Figure A1. Indicative images of observed parasites from grouper held at sea cage farms in Bali. (a) *Benedenia* (mucus), (b) *Haliotrema* (gill), (c) and (d) *Zeylanicobdella arugamensis* (mucus) (40X).

10.2 Appendix 2: Table of project training activities

Topic(s)	Date and location	Participants
Aquatic Animal Epidemiology	IMR Gondol, Bali, 2–6 September 2013	IMR Gondol: Zafran, Suko Ismi, Haryanti, Fris Johnny, Des Roza, Retno Andamari, Ketut Mahardika, Sari Budi Moria Sembiring, Mujimin, Ni Wayan Widya Astuti UGM: Murwantoko, Cahaya Kurnia Fusianto
Trial sampling of sea cages and hatcheries	IMR Gondol, Bali, 9–10 September 2013	
Sample processing	IMR Gondol, Bali, 11 September 2013	
Real-time PCR analysis	2–6 June 2014; University of Sydney Camden campus	IMR Gondol: Ketut Mahardika, Sari Budi Moria Sembiring UGM: Murwantoko, Cahya Kurnia Fusianto
Histopathology, PCR, data management	2–13 March 2015; University of Sydney Camden campus	IMR Gondol: Zafran, Ketut Mahardika, Sari Budi Moria Sembiring UGM: Cahya Kurnia Fusianto, Arga Kurniawan
Supplementary analyses (PCR and histopathology)	10–12 May 2017, University of Sydney Camden campus	Murwantoko, Cahya Kurnia Fusianto, Afri Herlambang
Statistics	11–12 August 2015, UGM Yogyakarta	IMR Gondol: Gede Sumiarsa, Jhon Hutapea, N.A. Giri, Suko Ismi, Des Roza, Zafran, Daniar Kusumawati, Yasmina Nirmala Asih, Wawan Andriyanto, Sudewi UGM: Murwantoko, Cahya Kurnia Fusianto, Arga Kurniawan BPBL Lombok: Joko Santosa UNHAS: Mardiana E. Fachry, Wahyudin Putra Sasmita
Best practice marine finfish hatchery techniques	14–21 December 2015, Port Stephens Fisheries Institute	IMR Gondol: Suko Ismi, Yasmina Nirmala Asih
Data analysis for large data sets	24–25 January 2017, IMR Gondol	IMR Gondol: Gede S. Sumiarsa, I Nyoman Adiasmara Giri, Haryanti, Des Roza, Zafran, Suko Ismi, Ketut Mahardika, Sari Budi Moria Sembiring, Ketut Maha Setiawati, Wawan Andriyanto, Daniar Kusumawati, Eko Prianto, Ahmad Muzaki, Sudewi, Yasmina Nirmala Asih UGM: Murwantoko, Desy Putri Handayani, Sabila Rahmawati, Afri Herlambang BPBL Lombok: Joko Santosa
Scientific writing	26 January 2017, IMR Gondol	
Publications planning	16 May 2017, University of Sydney	IMR Gondol: Suko Ismi, Zafran, Ketut Mahardika, Daniar Kusumawati, Yasmina Nirmala Asih UGM: Murwantoko, Cahya Kurnia Fusianto

10.3 Appendix 3: Mid-term review recommendations

The mid-term review provided several recommendations for ACIAR (Recommendations 1–3) and the project team (Recommendations 4–5):

Recommendation 1: It is recommended that ACIAR consider a no cost 6 month extension to the project with the termination date rescheduled to 31 December 2016

A 12-month extension (to 30 June 2017) was agreed with ACIAR in April 2016. Details of additional activities funded through this extension are provided below.

Recommendation 2: It is recommended that ACIAR consider a follow-on project to investigate policy development for Viral Nervous Necrosis through SEAsia.

Recommendation 3: It is recommended that ACIAR consider a follow-on project for extension of project outcomes to end-users, including hatchery and farm operators.

It is intended that concept note development for a follow-on project will form part of the end-of-project meeting at Camden in early 2017.

Recommendation 4: It is recommended that clarification be made of the publications and communication strategy

Development of a publications and communication strategy will be incorporated in the workshop proposed for late 2016 at IMRAD Gondol which will cover detailed analysis of project data sets and approaches to scientific publication.

Recommendation 5: It is recommended that key hatchery technicians from IMRAD Gondol receive training in operation of biosecure hatcheries and live feed enrichment/handling

This training was completed in December 2015.

Project extension

In response to the recommendations of the mid-term review of FIS/2010/101, ACIAR agreed to fund a 12-month extension to the project. This extension also supports the following additional activities:

Objective 1: To identify key diseases through longitudinal epidemiological studies on hatcheries and grow-out farms

1.1 Determine the key diseases responsible for production losses in marine finfish aquaculture

New activity: Undertake intensive sampling of disease events in sea-cages, targeting the first month after fish are introduced into the cages.

Description: More information will be gathered about causes of disease in sea cages by examining fish during disease outbreaks. Targeted sampling during the appropriate season and stage in the production cycle is still required to define the disease syndromes

that cause mortality in sea-cages that were not captured during the longitudinal sampling program that has been conducted so far.

1.2 Plan and, where appropriate, trial interventions to reduce production losses

New activity: Based on the results of the sea cage epidemiological survey, a trial will be undertaken to investigate on-farm parasite reservoirs and evaluate the efficacy of the freshwater bathing routine that is currently used by Indonesian farms.

Description: This is an intervention study investigating the efficacy of the current practice of regular freshwater baths and to identify potential reservoirs for parasites at the sea cage. It will involve mainly UGM and Gondol staff on sea cage farms in Bali. This is a short duration trial, and is low cost. The fish would be existing stock and no costs are associated with purchase of fish or feed. Farmer incentives may be required.

Objective 2: To develop and test improved management protocols for hatcheries and grow-out farms

2.1 Test and implement improved hatchery practices, including biosecurity and improved larval nutrition

New activity: Following from earlier trials incorporating elements of biosecurity, two additional trials will be undertaken with additional treatment of the hatchery water supply. These trials will also allow full adoption by IMRAD Gondol of the training in nutritional enhancement of live prey undertaken at Port Stephens Fisheries Institute in December 2015.

Description: This is an intervention trial at the demonstration hatchery to research methods to exclude viable NNV from incoming seawater.

Objective 3: To build capacity in fish health management in Indonesia

3.1 Provide training in epidemiology and disease diagnosis for Indonesian fish health professionals

New activity: A third training workshop will be held at IMRAD Gondol to cover detailed analysis of the project data sets, and to train Indonesian scientists in scientific writing for publication in international journals.

Project administration

New activity: an end-of-project meeting will be held in Camden to:

- Review and discuss project outcomes;
- Provide a forum for a formal end-of-project review;
- Plan joint publications arising from the project;
- Allow Australian and Indonesian project participants to plan a follow-on project.