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FISHERIES RESEARCH & DEVELOPMENT CORPORATION

CO-ORDINATION OF FISH HEALTH AND NUTRITION RESEARCH FOR THE WESTERN AUSTRALIAN YELLOWTAIL KINGFISH TRIAL 2

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In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

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Abbreviations

YTK – Yellowtail Kingfish

WA – Western Australia

RD&E – Research, Development and Extension

RRG – Research Reference Group

IOFA – Indian Ocean Fresh Australia (Pty) Ltd.

MFA – Marine Fishfarmers Association Inc of Western Australia

ACAAR – Australian Centre for Applied Aquaculture Research

DOFWA – Western Australian Department of Fisheries and its Fish Health Group

MWDC – Mid West Development Commission, Western Australia

YTK2 – Second Yellowtail Kingfish Development Project (WA)

Executive Summary

This project was managed by the Marine Fishfarmers Association Inc. of Western Australia (MFA) and established a multidisciplinary Western Australian (WA) research team that designed and ran a comprehensive suite of industry led research projects. The project also developed a model to guide the process of enabling the effective coordination of research effort to deliver commercially realistic outcomes to support the future success of the emerging Yellowtail Kingfish (YTK) aquaculture industry in WA and elsewhere. The project also built additional technical capacity in WA to support the future Research, Development and Extension (RD&E) activities for this industry.

The project identified the gaps between industry input, research outputs and the end commercial use of research and maximised the available expertise and infrastructure for industry benefit. A Research Reference Group (RRG) was formed that comprised the industry participant Indian Ocean Fresh Australia Pty Ltd (IOFA) supported by the MFA, along with scientists from the Australian Centre for Applied Aquaculture Research of South Metropolitan TAFE (ACAAR) and the WA Department of Fisheries Fish Health Group (DOFWA). Other expertise, including from interstate, was sought as required. The RRG, Chaired by a research co-ordinator outlined a suite of comprehensive research assignments to meet the industry's current needs in developing a sustainable YTK industry in WA. This FRDC project provided coordination of research and industry inputs for a sea cage grow out trial that was conducted at the IOFA site in Geraldton off the WA coast and tank based nutrition research project that was carried out at the ACAAR hatchery in Fremantle. The Project commenced in October 2015 and concluded in October 2016.

Background

The Mid West Development Commission WA (MWDC) commissioned two YTK growout trials supported by the WA Royalties for Regions Program to determine if a sustainable YTK industry was possible in their region. The first successful pilot sea cage grow out trial was conducted off the Geraldton coast in 2011-2012 in conjunction with the MFA and IOFA. The aspirational targets set for this trial were achieved, including high survival and growth rates that demonstrated that the temperature profile and conditions in the Mid West region of WA are highly suitable for the commercial grow out of YTK. The second YTK Development Project (YTK2) commenced in October 2015 and concluded in October 2016. The project reported on here supported this second trial.

It was recognised that the best way to de-risk the emerging marine finfish aquaculture industry sector was to effectively and comprehensively capture the knowledge and experience from this second pilot trial in the Mid-West of WA, since the majority of YTK research to date has been conducted in South Australia in a relatively colder water temperature range. The coast of WA has a different temperature profile to that of South Australia bringing with it advantages as well as its own challenges requiring specific research solutions.

Aims/objectives

The need for a well-coordinated research plan, the development of a comprehensive suite of research projects and the capture of all relevant information resulting from the YTK2 growout trial was highlighted as a priority for the future success of the WA YTK industry.

A secondary priority was the desire to develop a template to assist future industry led research teams in the establishment of relevant research projects for the WA marine finfish aquaculture industry.

The specific objectives of the project were to:

1. Select and appoint a research coordinator for the project;
2. Review the relevant and available YTK research;
3. Develop a coordinated research plan – in conjunction with the RfR Steering Committee and in communication with others engaged nationally in YTK R&D; and
4. Implement the research plan, manage the research program and document the progress.

A major output from this project was to build technical capacity to support the future Research, Development and Extension (RD&E) activities for YTK aquaculture in Western Australia.

Methodology

Upon the establishment of the RRG, its purpose was to understand the objectives of the industry proponents, audit the capacity of the staff and laboratories potentially available to the project; and to propose technically and logistically achievable research assignments that could be run in conjunction with the YTK2 project. The Research Co-ordinator used this information to develop, in collaboration with the Principal and Co-Investigators of this Project, a comprehensive and practical research plan that would maximise the available expertise and available funds and capture the extensive data to be generated throughout the trial for the benefit of the industry partner IOFA. The RRG then met to review progress of the research and add or amend to the projects as required.

Results/key findings

The overall YTK2 pilot project has determined that YTK is a suitable species for sustainable production in the Midwest region of WA with higher than anticipated growth rates and controllable survival. To support the YTK2 pilot project the industry partner was successfully aligned with the research capacity available in WA and identified and prioritised the research required to meet industry's applied needs.

The project developed insight and baseline information on the key performance indicators of potential growth and health of YTK in WA and managed the 'just in time' research resulting from the changing requirements of a real time seacage pilot trial. The co-ordination project allowed for ongoing communications between the multiple agencies and industry to ensure the most valuable data and knowledge was collected and collated in a manner that would assist industry participants moving forward with larger scale production. This project also developed knowledge and skills of key personnel throughout the trial, including on farm staff and researchers and rural personnel indirectly involved in the trial, including local veterinarians.

Implications for relevant stakeholders

One outcome of this highly successful project was the across the board recognition that a prosperous industry relies on the support and assistance of multiple government agencies who understand the applied needs of that industry. This project did not undertake research that did not directly benefit the industry proponent. The primary beneficiary of the project was the industry, with the researchers playing a supporting role.

This project increased the technical capacity and skills extension of rural workers by directly engaging them in the research. This not only developed new skills for the on-farm workers, but also developed an improved understanding of the impacts of feed composition and feed management and the physiological impact of stress on the fish in their cages.

Managers and policy makers were exposed to a research program where the needs of the industry came before the needs of researchers and saw the positive development of multi-disciplinary and multi-jurisdictional relationships and understanding.

Recommendations

This project has demonstrated that multi-agency, multi-disciplinary partnerships that align the applied outputs required by industry with appropriate researchers and their agencies can lead to highly successful outcomes for all parties. In this project, the outcomes needed by industry were the starting point upon which the research programs were built. This may sound logical, however, in Australia, the skills and interests of researchers are all too often the starting point for the generation of projects, with industry being included at a later stage to provide a level of legitimacy to the proposed project.

Researchers commonly instigate projects for industry since the industry proponents are usually busy focussing on their urgent day to day issues. This project has highlighted the additional benefit of industry proponents becoming more engaged in the establishment and running of research aimed at supporting their businesses.

It is therefore recommended that the successful model developed within this project be highlighted to all industry proponents and research agencies so it becomes the new normal. There are many industry proponents and research agencies already moving in this direction, and this push should be accelerated.

This project has also emphasised the need for further research in key areas such as fish health, immunity and responses to disease. Valuable new tools for assessing fish immunity have been developed during this pilot trial and both the research scientists and the industry partner are keen to see these methods develop into reliable indicators for early intervention of disease processes in the sea cage. These tools and methods are easily transferrable to industry participants nationally and progress and funding to these key areas of fish health are supported by the Mid West Development Commission Steering Committee.

Keywords

Yellowtail Kingfish, Research and Development Plan, aquaculture, finfish, Western Australia.

Introduction

Yellowtail kingfish (YTK), *Seriola lalandi*, also known as 'Yellowtail' or 'hiramasa', is highly prized in Japan as a sashimi fish. The *Seriola* species have an estimated culture value of over $1.3 billion USD annually due to the high value of the flesh (Purcell et al. 2015). The limited availability of this popular fish has opened the opportunity for the aquaculture industry to supply a constant fresh fish product, and is a focus of worldwide aquaculture industries (Benetti1997, Nakada 2002, Fowler 2003, Stuart and Drawbridge 2012). The commercial culture of YTK was developed in Australia in the early 2000s and fingerling production is now secured through domesticated broodstock. The YTK aquaculture industry in South Australia provides significant regional employment and has attracted investment and expenditure that supports local economies. As with many emerging aquaculture industries, the South Australian based operator has experienced health issues, unexpectedly high mortality rates and below average growth in the past five years. However, through a better understanding of nutritional requirements and improved husbandry production of YTK has reached 1500 tonnes in 2015.

YTK sea cage culture has been undertaken in WA in pilot scale trials over the past decade. In 2008-2009, Western Kingfish Limited stocked two successive YTK cohorts into seacages in Jurien bay. Unfortunately, the operator reported Cohort 1 losses of up to 70% due to mortality events as the company was running out of funds in the lead up to the Global Financial Crisis, although the exact cause of the mortalities were never fully determined (Stephens and Savage 2010).

The MWDC funded a very successful pilot sea cage grow out trial off the Geraldton coast in 2011-2012 in conjunction with the MFA and IOFA. This trial was supported by the ACAAR and the Fish Health Group of DoFWA. Significant targets were achieved in this trial, including high survival and good growth rates, demonstrating that the temperature profile and conditions in the Mid West region of WA are suitable for the grow out of YTK. Whilst the initial pilot study produced excellent results in terms of growth, survival and a harvestable product that was well accepted in the market, there were many challenges and numerous knowledge gaps concerning YTK sea cage culture in this region.

The MWDC, with support from the WA Royalties for Regions program funded the YTK Development Project No. 2 (YTK2) which commenced in October 2015 and was completed by 31 October 2016. The sea cage grow out trial was conducted by IOFA at their seacage site in Geraldton off the Western Australian coast. Complementary tank based research was carried out by the research provider ACAAR at their hatchery in Perth. The DOFWA provided support from its Fish Health Unit.

The members of the MFA recognised that the future success of the marine finfish industry in Western Australia would rely on well-coordinated research and the collection and collation of the best available information resulting from any particular project. The MFA therefore wished to ensure, in managing this FRDC Research Co-ordination Project, that existing research resources and staff were identified early in the project and were then aligned to a comprehensive suite of research projects to deliver outcomes of commercial

importance to the developing YTK industry in WA. A further outcome of the project, if successful, would be the development of a model, or template, to assist future industry led research teams to ensure that outcomes and outputs are industry relevant, rather than resulting in volumes of reports gathering dust on a shelf.

The YTK industry in WA is relatively new and its rapid growth and potential economic value and employment generation in the region relies on a wide range of research and development issues to be identified and addressed. As with all new and emerging industries, these research issues change as the industry develops and grows.

The 2011-12 pilot trial particularly highlighted the need for well-coordinated research efforts involving multiple agencies across the state addressing industry driven priorities. The culture of YTK in WA as a new industry also requires technical capacity expansion and personnel skills extension to be increased to meet the challenges of this difficult to rear species. Research is necessary to improve the overall health and growth of the animals and increase the capacity of the state to support developing industry participants. This project sought to address the current industry needs of fish health and nutrition issues by developing novel methods to assess the status of the fish health as well as increasing the capacity of research and technical staff of the state. The most meaningful outcome of this industry led research project would be the provision of the necessary research steps needed to support a developing industry sector in regional communities.

This report explains the process undertaken in order to establish an industry led Research Plan for the YTK aquaculture industry in WA.

It is important to note that the majority of the funding utilised to undertake the research projects that were developed within this project was from the MWDC YTK2 project budget. The associated nutrition project is also funded separately and detail of the methods and results of these projects will not be reported on here.

Objectives

The need for a well-coordinated research plan, the development of a comprehensive suite of research projects and the capture of all relevant information resulting from the second growout trial was highlighted as a priority for the future success of the WA YTK industry.

A secondary priority was the desire to develop a template to assist future industry led research teams in the establishment of relevant research projects for the marine finfish aquaculture industry.

The specific Objectives of the FRDC Coordination Project were to:

1. Select and appoint a research coordinator for the project;
2. Review the relevant and available YTK research;
3. Develop a coordinated research plan – in conjunction with the RfR Steering Committee and in communication with others engaged nationally in YTK R&D; and
4. Implement the research plan, manage the research program and document the progress.

A major output from this project was to build technical capacity to support the future Research, Development and Extension (RD&E) activities for YTK aquaculture in Western Australia.

Method

Prior to the commencement of the YTK2 project, potential collaborating agencies were identified, individuals who could directly contribute to the planned outcomes were consulted and an overall Steering Committee was formed.

The Chair of the YTK2 Steering Committee was the CEO of the MWDC (the funder), the members were the Director of IOFA (the industry partner), the Director of ACAAR (the research provider), an independent retired fishery entrepreneur and a local Geraldton business owner (Deputy Chair of the MWDC). The Steering Committee Terms of Reference are at Appendix 2.

The Terms of Reference of the RRG were developed to better understand the objectives of the industry proponents, audit the capacity of the people and laboratories potentially available to the project and to propose technically and logistically achievable research assignments that could be run in conjunction with the YTK2 project. This was to be followed by the development and costing of the Research Plan and responsibility for its implementation and scientific rigour. The RRG Terms of Reference are at Appendix 3.

The RRG reported formally to the Steering Committee and also had the capacity to invite other persons as needed subject to the skills required by the Plan.

The appointment of the Research Coordinator was made just prior to the first meeting of RRG after an advertised recruitment process with 34 applicants. The successful applicant, Dr Woolley completed her PhD within the Seafood CRC on larval rearing of YTK and had four years of experience in marine fish production and research, including in fish health projects.

The members of the RRG included:

Dr Lindsey Woolley (FRDC Project Research Co-ordinator and RRG Chair)

Dr Fran Stephens (Senior Fish Pathologist at the Department of Fisheries)

Dr Gavin Partridge (Principal Research Scientist at ACAAR)

Dr Steve Duda (Veterinarian at the Chapman Animal Hospital, Geraldton)

Justine Arnold (IOFA Technical Officer)

Mike Kendall (Project Officer at the MWDC)

Erica Starling (Director of IOFA)

Most of the RRG members were involved with the previous YTK 1 trial conducted from 2011 to 2012, and understood the challenges and benefits of aquaculture in WA.

The FRDC YTK Co-ordination Project also aimed to increase the capacity in WA to assist in collating, analysing and presenting the data collected in a format to enable assessment of the outcomes of the YTK2 seacage trial as an indicator of future scalability. Data in

itself is limited in its ability to demonstrate key factors in assessment of commercial outcomes. High quality data, generated from commercially targeted research, must be interpreted and presented to industry and judged against key commercial factors in order to critically assess the prospects of a future industry.

The WA aquaculture industry also recognised there is limited local technical and research capacity, particularly targeted at the farm. An important part of this project was to provide the opportunity to increase the knowledge and skills of the on-farm technical team in relation to high level research data collection, and also expose career researchers to the on-farm needs and limitations, thereby facilitating better outcomes of targeted, industry relevant research. This was partly enabled by directly involving on-farm staff in the research activities.

Further limitations lay in the skills and knowledge of the local suppliers, specifically the veterinary support in the rural regions. This project also aimed to upskill the general fish knowledge of the local veterinary clinic and its staff, particularly in the health aspects of YTK culture. The health management support that the veterinary clinic provides is vital both in the day-to-day processing of samples and during unexpected events that require immediate consultation and advice.

The components of the project and associated research activities were:

1. Select and employ the research coordinator for the project.

2. Compile a desktop review highlighting previous YTK research to date.

3. Develop a research plan in association with the RRG that would add to the existing knowledge of the YTK aquaculture industry.

4. Develop working relationships between the research coordinator and management and operational staff in Geraldton. Travel between the cage site and research centres allowed the research coordinator to become familiar with farm practices and highlighted the areas of research that would benefit the commercial production of YTK. It also provided the operational staff with a better understanding of the research and ensured effective data was collected on site.

5. Capacity building and upskilling of personnel and knowledge within WA. As the WA aquaculture industry is still in its infancy, there is a strong need for collaboration across multiple agencies that would support the future industry partners, including fish pathologists, researchers and government agencies. This project assisted in collaborating with these agencies to bridge gaps in the knowledge of sea cage culture of YTK in WA.

6. A Fish Health Monitoring Program to be developed by the RRG scientists to optimise the collection of growth and health data alongside monitoring of the marine environment throughout the year long trial. This data was collated and reported as part of the final report to the MWDC to demonstrate the performance of YTK sea cage culture in WA.

7. An ACAAR based series of nutrition trials to benchmark the performance of YTK grown in warmer waters of WA. Harvest trials followed on from the nutrition trial work.

Note that the Fish Health/Monitoring and nutrition trials were funded separately to the Coordination Project and will not be reported on here.

The core of this project involved the design of the well-coordinated Research Plan for the YTK2 trial that encompassed the existing skills and capacity of the research participants and generate meaningful outputs relevant to industry. The Research Plan can be seen at Appendix 5.

The RRG outlined a comprehensive suite of research outlines to meet the industry's current needs in developing a sustainable YTK industry in WA. It was recognised that the research needs may continually change or alter depending on the circumstances, such as the changing environment and the performance of the cohort. The performance of cohorts is complex and multifaceted, dependant on factors such as fingerling quality, immunocompetence at stocking, diet and disease tolerance. Ultimately these factors combine to influence stock survival, growth performance and product quality for the market place.

The Research Co-ordinator used this information to develop, in collaboration with the Principal and Co-Investigators of this Project, a comprehensive and practical research plan that would maximise the available expertise and available funds and capture the extensive data to be generated throughout the trial for the benefit of the industry partner. The RRG then met to review progress of the research and add or amend to the projects as required. It is expected that this planning exercise may be of benefit to aquaculture development projects in other states of Australia as a model or template.

The Research Plan included operational and laboratory based research trials for the duration of the project. The plan guided the activities of the Research Coordinator who implemented and managed the plan in conjunction with the RRG members. The RRG provided oversight to the collation of research data, results and outcomes by the Research Coordinator and ensured the Project Steering Group was informed as required.

The RRG also oversaw the reporting, communication and extension activities as required by the FRDC on behalf of the MFA.

The area of nutrition was identified as a priority, as previous grow out trials for YTK in WA had suffered shortfalls in dietary formulations of commercial feeds. These dietary deficiencies lead to health issues, such as skeletal myopathy and secondary bacterial infections of the intestines, pancreas and liver. It was decided that during the production run the fish in the sea cage would be fed the best possible diets based on previous performances and analyses of currently available diets. As there was only a single production sea cage, replicated sea-based nutrition trials were unable to be conducted and so tank trials were designed to compare multiple commercial feeds at the ACAAR facility. These nutrition trials would investigate the growth and health issues within a land-based tank environment which could be compared to the growth models achieved within

the sea cage over the year-long project. Numerous health parameters were monitored throughout the nutrition study, including histopathology, blood haematology and biochemistry. The blood biochemistry and haematology work was done in collaboration with Dr Jenny Hill, Senior Pathologist at VetPath in Perth. The focus of this work was to develop an automated method for collecting basic haematology data that is repeatable. The development of these techniques could possibly be used for quick diagnosis of disease issues.

A rigorous health and environmental monitoring plan was designed to generate and capture the greatest amount of data possible throughout the trial. This monitoring plan could then be used as a baseline for routine monitoring and also during critical periods for future fish stockings. These data collected from the YTK2 trial could form a baseline health level for fish from stocking to harvest, and indicate potential growth rates achievable for fish grown under similar temperature profiling and fed a species specific diet. These data can then be used to model forecasted growth rates for YTK and feed requirements at the actual ambient temperatures at the site. Monitoring the health of the fish over the year-long commercial trial also highlighted seasonal factors that can cause ill thrift and therefore forecast when close monitoring for signs of ill health and clinical symptoms is required.

During the project, novel methods to determine immunocompetence of fish were investigated. Dr Cecile Dang, the Principal Research Scientist at DOFWA, has extensive experience in the use of flow cytometry as a measure of health and the cell's responsiveness to stress. The flow cytometry methods were adapted during this project by trialling challenge tests on fish blood cells rather than the traditional method of sampling the head kidney from the fish. The traditional method which requires head kidney tissue destroys the fish and also requires a sterile environment, which is not easily established on farm. At present, the methods which only require a sample of fish blood are time sensitive and require analyses the day of sampling or the day after. However, further investigation are still ongoing into developing a cell fixative which would allow this work to be completed as part of the routine fish health assessment and analyses to be completed at a later stage. In parallel with the haematology work, these parameters could potentially be used as early indicators of disease providing invaluable time for the fish farmer to react before major losses occur.

A major limitation lies in the technical capacity in WA, at a researcher and applied researcher level. An important part of this project was to provide an opportunity to increase the knowledge and skills of the on-farm technical team in relation to high level research data collection, and also expose career researchers to the on-farm needs and limitations, thereby facilitating better outcomes of targeted, industry relevant research. To achieve this a major emphasis was ongoing collaboration between on-farm staff and researchers, through day to day communications and monthly onsite visits for 3 – 5 days at a time as well as during critical periods or trials.

Protocols were developed for improved farm management practices for data collection and fish monitoring as well as the development of new protocols for health assessment of fish. Parameters such as blood biochemistry and immunity measurements were

developed through collaboration with industry and government scientists. These set baseline information on the health of the fish at age and weight as well as possible indicator for stress and disease processes. Baseline data sets that were considered the most valuable in terms of monitoring growth, feed consumption, health of the fish and the environment, were outlined in a monitoring plan.

The RRG met on-farm for a fish health workshop shortly after initial stocking with fingerlings. The aim of the workshop was to evaluate the fish health monitoring plan and ensure standardised sampling occurred on site and at the research facilities so to limit the sampling errors for data collection. During the YTK 1 trial, sampling inconsistencies were highlighted as a problem when trying to understand the physiological changes during periods of mortality or poor performance. The RRG continued to meet every three months during the project to discuss the progress of the trials and upcoming events at the cage site and tank trials. The research coordinator was also responsible for monthly written reports to the RRG outlining the progress and/or issues that had arisen during the YTK2 trial.

Previous YTK 1 harvest trials identified numerous harvest techniques and product quality factors that contributed towards the end user product and gaps in this knowledge were identified and earmarked for future work that could potentially improve product quality. Mr Rowan Kleindienst, a former ACAAR and IOFA employee and Seafood CRC Master's graduate, compiled a Harvest Trials Concept Proposal outlining the major findings of his Master's thesis and recommendations of further research that would benefit the industry. Rested harvest was highlighted as an area that warranted further investigation and this particular aspect was better suited to tank-based studies which allowed finer control of the harvest parameters. These trials were completed towards the end of the tank-based nutrition work in the YTK2 project to maximise the amount of data collected from these resources. The rested harvest work was done in collaboration with Mr Andrew Forrest at the Department of Food Technology Queensland. The harvest trials were conducted at the ACAAR facility, the fillets were prepared by Catalano's Seafood in Perth and product quality trials, including a quantitative texture assessments and consumer panel assessment by a trained seafood sensory panel, were conducted in Queensland.

During the early stages of the project, both the fish in the land-based tanks and the sea cage became infected with *Photobacterium* bacteria on multiple occasions. With the immediate assistance of the pathologists and microbiologists at DOFWA, the bacterial infection was identified and treatment advice provided. Whilst the scale of the outbreak was minor in these instances, *Photobacterium* infections are known to cause septicaemia and large stock losses. The development of an autogenous vaccine against *Photobacterium damselae* subsp*. Damselae* was highlighted as an important milestone for the success of the industry moving forward. This was also recognised as a national problem for all industry stakeholders during the Kingfish for Profit meeting in Adelaide in May 2016. A commercial quantity of *Photobacterium* vaccine for WA was produced by Tréidlia BioVet in collaboration with Dr Andrew Barnes at the University of Queensland. The vaccine was produced from several *Photobacterium* strains recovered from moribund fish during the December 2015 and February 2016 outbreaks. This vaccine will be administered to subsequent cohorts of YTK juveniles in WA before stocking to sea.

Results

This pilot trial provided an insight to the synergies that could be created when research is industry driven with the involvement of multiple government agencies.

A Flowchart of the development and implementation of the Research Plan is presented at Appendix 4. The Research Plan (Appendix 5) was designed to support the development of a sustainable YTK industry in WA. The Plan followed the year-long progress of the growout of YTK in open water sea cages and in land based tanks to collect baseline data on the performance of fish within the MidWest region of WA and identify possible issues that would hinder the progress of the industry moving forward. The Fish Health Monitoring Plan was developed from the Research Plan and can be seen at Appendix 6. A list of the management and research staff involved in this project can be seen at Appendix 7.

A major outcome of this project was the recognition that a successful industry relies on the support and assistance of multiple agencies. The nature of this work means time sensitive issues arise without warning and a rapid response is necessary from the farm managers and the pathologists assisting in determination of disease processes. Throughout the project the industry participant has relied on the resources of the government hatchery, ACAAR, and the Fish Health Section of the Department of Fisheries WA. Sound working relationships and seamless collaboration between these agencies were necessary for the success of this project and to provide valuable input into the overarching operations of the farm. This project has highlighted however, that whilst the pilot trial was successful in establishing a fledgling marine finfish aquaculture sector in the Mid West region of WA, the resources across the state and the personnel available to work in the aquaculture industry are limited.

The Research Plan was followed during the YTK2 project to address the objectives and research needs of the industry, however as the industry grows, new challenges become apparent and the research focus must alter to meet the changing the needs of the industry. Whilst the research focus may shift during subsequent stockings, the Research Plan and template used during the pilot project is transferrable to larger, commercial scale business.

The resources and technical capacity in the state was identified as an area that required co-ordination and alignment of industry needs and state capacity. This project provided the opportunity to increase the technical capacity and skills of the on-farm technical staff and government researchers in a number of ways:

- Many of the RRG participants attended the initial ‘Kingfish for Profit’ Research Meeting, as part of the inclusion in the national collaborative FRDC project, in Adelaide in May 2016. During the meeting common problems amongst all industry participants were highlighted, one being the development of autogenous vaccines against *Photobacterium*. Two honour’s students completing YTK research at ACAAR and supervised by Dr Gavin Partridge, and Justine Arnold (IOFA) and Dr Lindsey Woolley (ACAAR) attended the Professional Development workshop as part of the initial meeting.

- The researchers and farm technical staff met with the fish health pathologists from time to time to better understand the histopathology evidence and the consequence of particular findings.
- IOFA Technical staff, Justine Arnold, Lacey McDonald and Stephen Sullivan O' Murphy, travelled to the ACAAR hatchery to assist on the fish health assessment days as part of the nutrition trials.
- Justine Arnold and Drs Gavin Partridge and Lindsey Woolley attended a statistical workshop delivered by the Australian Development Agency for Statistics and Information Systems. The workshop assisted in providing a brief overview into statistical methods and test that could be used to summarise and explore the large volumes of data that was collected during the project.

This co-ordination project successfully:

- Aligned the industry participant with the research capacity available in WA;
- Identified the gaps between industry input, research outputs and the end commercial use of research;
- Prioritised the resultant research to meet industry's practical and applied needs;
- Generated insight and baseline information on the key performance indicators of potential growth and health of YTK in WA;
- Managed the just in time research resulting from the changing requirements of a real time seacage pilot trial;
- Allowed for ongoing communications between the multiple agencies and industry to ensure the most valuable data and knowledge was collected and collated in a manner that would assist industry participants moving forward with larger scale production;
- Developed a model to enable the effective coordination of research effort to deliver commercially realistic outcomes that would support the future success of the emerging YTK aquaculture industry in WA; and
- Maximised the available expertise and infrastructure for industry benefit.

Implications

One outcome of this highly successful project was the across the board recognition that a prosperous industry relies on the support and assistance of multiple government agencies who understand the applied needs of that industry. This project did not undertake research that did not directly benefit the industry proponent. The primary beneficiary of the project was the industry, with the researchers playing a supporting role.

This project increased the technical capacity and skills extension of rural workers by directly engaging them in the research. This not only developed new skills for the on-farm workers, but also developed an improved understanding of the impacts of feed composition and feed management and the physiological impact of stress on the fish in their cages.

Managers and policy makers were exposed to a research program where the needs of the industry came before the needs of researchers and saw the positive development of multi-disciplinary and multi-jurisdictional relationships and understanding.

Recommendations

This project has demonstrated that multi-agency, multi-disciplinary partnerships that align the applied outputs required by industry with appropriate researchers and their agencies can lead to highly successful outcomes for all parties. In this project, the outcomes needed by industry were the starting point upon which the research programs were built. This may sound logical, however, in Australia, the skills and interests of researchers are all too often the starting point for the generation of projects, with industry being included at a later stage to provide a level of legitimacy to the proposed project.

Researchers commonly instigate projects for industry since the industry proponents are usually busy focussing on their urgent day to day issues. This project has highlighted the additional benefit of industry proponents becoming more engaged in the establishment and running of research aimed at supporting their business.

This project has also emphasised the need for further research in key areas such as fish health, immunity and responses to disease. Valuable new tools for assessing fish immunity have been developed during this pilot trial and both the research scientists and the industry partner are keen to see these methods develop into reliable indicators for early intervention of disease processes in the sea cage. These tools and methods are easily transferrable to industry participants nationally and progress and funding to these key areas of fish health are supported by the Mid West Development Commission Steering Committee.

It is therefore recommended that the successful model developed within this project be highlighted to all industry proponents and research agencies so it becomes the new normal. There are many industry proponents and research agencies already moving in this direction, and this push should be accelerated.

Further development

The RRG met towards the end of the YTK 2 project to discuss and prioritise future research that was needed to further assist the industry. Research areas that are currently high risk and potentially costly to the industry, requiring immediate investigation and solutions were highlighted for further research projects. The next step is assisting industry from its current project scale towards a profitable business model.

Extension and Adoption

The industry and government participants in this project have been informed of the outputs. This report is part of the extension to industry and researchers in the broader aquaculture field in Western Australia and in other states of Australia.

The Communication and Extension Plan for the Western Australian Yellowtail Kingfish Trial 2 can be seen at Appendix 1.

Project coverage

Nil on this project.

Project materials developed

See Appendices 2 – 6

Appendices

APPENDIX 1. COMMUNICATION AND EXTENSION PLAN FOR THE WESTERN AUSTRALIAN YELLOWTAIL KINGFISH TRIAL 2

Objectives

- To communicate the process involved in developing a comprehensive research strategy and plan.
- To communicate the process involved in co-ordination and implementation of above
- To facilitate an increase of available technical capacity to support future R & D in YTK aquaculture.
- To facilitate dialogue with key researchers and industry across Australia re YTK research and areas of potential collaboration
- To communicate results of any shared projects to key researchers and industry across Australia re YTK

Target Audience/s

Potential WA, SA and NWS Aquaculture businesses.

Key Message/s

How to maximise and build on collective research outcomes utilising effective research co-ordination and creating a methodology for a well- coordinated research programme.

How to develop a simple but effective process for sharing of research aims, work together in common areas and share results of such shared projects

How to build technical capacity to support future R & D in YTK aquaculture in WA.

Communication/Extension Methods

A steering group will be formed to oversee the overarching YTK 2 project,
A subgroup (Research Reference Group) also formed including industry, government and researchers.
Research co-ordinator employed will liaise with Working Group, who will liaise with steering group for formal communication.

MFA to be included where relevant

Informal communication between industry operator and research group members to be managed by Co-ord in conjunction with Principal investigator of project , Greg Jenkins

DURING THE PROJECT

Email Updates
Information emails at key times, including relevant matters.

Meetings
Regular meetings of research group will facilitate communication.
Presentations and reports to Steering Group

Media Releases

Approved Media Releases will be issued only as required in conjunction with MFA, FRDC, MWDC.

AFTER THE PROJECT
Evaluation
The communication plan will be deemed effective if all parties in the project have been provided with enough relevant information to evaluate the success of the project.

The FRDC/CRC contractual arrangements have been met regarding communication

Intellectual Property

MWDC has confidentially agreements in place in relation to the data and knowledge from the first trial and this second trial that requires agreement of all parties to be released. This area is negotiable depending in type of information gathered.

APPENDIX 2. YTK TRIAL 2 STEERING GROUP – TERMS OF REFERENCE

1. Purpose and Role

The YTK Trial 2 Steering Group (YTK2SG) will provide strategic direction to the YTK Trial 2 project and oversight of the activities of the working group.

The Steering Group's key roles include

- Governance of project
- Overall Project Advice for Management
- Overall Responsibility for Project outcomes
- Management of Contract MWDC/IOFA and obligations

The YTK2SG will also provide a forum for research, government and industry leaders to discuss key issues facing the sector in Western Australia and provide feedback to government as appropriate.

2. Members

The membership of the YT2SG will include:

- CEO of the Mid West Development Commission (MWDC), Gavin Treasure
- MWDC Board Member - Todd West
- Director of Indian Ocean Fresh Australia, Erica Starling
- Director of the Australian Centre for Applied Aquaculture Research, Greg Jenkins, (also a member of Marine Fishfarmers Association)
- Commercial Member –Mike Grant – Independent
- Other parties that from time to time may be invited to meet with the group as required and approved by the Chair – OBSERVER STATUS

Proxies or replacements of members at meetings in the event that a member cannot attend will be considered by the Chair with advance notice

3. Chair

The Chair of the YTK2SG will be the CEO of the MWDC

The Chair in conjunction with the Director of Indian Ocean Fresh Australia (ES) will set the agenda for each YTK2SG meeting. The Chair will be responsible for ensuring meetings run efficiently and effectively. Erica Starling will lead the meeting through the Agenda.

4. **Secretarial Support**

   IOFA is responsible for providing secretarial support to the YTK2SG meetings, and to the group with assistance as required from the MWDC time to time.

5. Meetings

- The YTK2SG will meet quarterly or as required by the MWDC.
- If required, additional meetings may take place to discuss pertinent issues.
- Additional meetings may be suggested by members and approved by the YTK2SG Chair.
- There is no requirement for a quorum at YTK2SG meetings.
- Meetings are held at the MWDC (or other premises as required) and teleconference facilities can be made available for those who are unable to attend.

6. Minutes

- Agenda items will be called for and an Agenda forwarded to YT2SG members 5 working days PRIOR to the date of the scheduled meeting.
- IOFA is responsible for organising meetings, taking minutes and for disseminating correspondence with regard to YTK2SG issues.
- Minutes are disseminated within five working days after the meeting.

7. Reporting

- Reports may be prepared and presented as approved by the YTK2SG members and Chair from time to time.

8. Remuneration

- There are no sitting remuneration associated with meetings unless approved by the Project Steering Group.
- Remuneration of travelling expenses will be considered by the Committee on a case by case basis.

Appendix 3. YTF TRIAL 2 RESEARCH REFERENCE GROUP

TERMS OF REFERENCE

1. Purpose and Role

The YTK Trial 2 Research Reference Group (YTK2RRG) will provide detailed scientific advice into research planning for the YTK Trial 2. Assisted by the Research Co-ordinator the group will formulate a comprehensive research plan, including operational trials and laboratory based research for the duration of the project. The plan is to be approved by the Project Steering Group.

This plan will guide the activities of the Research Coordinator who will implement and manage the research plan in conjunction with the working group for the duration of the project

The YTK2RRG will oversee the collation of research data, results and outcomes by the Research Coordinator and ensure the Project Steering Group is informed as required
The group will oversee the reporting and communication and extension activities as required by the CRC /FRDC on behalf of the MFA

2. Members

The membership of the YTK2RRG will include:

- CRC /FRDC Research Coordinator
- Dr Fran Stephens (DoFWA)
- Dr Gavin Partridge (ACAAR)
- Justine Arnold (IOFA Technical Officer)
- Mike Kendall (MWDC)
- Erica Starling (IOFA)
- Steve Duda (Local vet)

There will be no proxies or replacements of members at meetings in the event that a member cannot attend.

From time to time other parties may be invited to meet with the group as required – OBSERVER STATUS

Dr Janet Howieson (Curtin)

Dr Mike Snow (DoFWA- Fish Health)

Dr Cecile Dang (DoFWA – Fish Health)

And others as required

3. Chair

The Chair of the YTK2RRG will be Erica Starling. The Chair will set the agenda for each YTK2RRG meeting in conjunction with the Research Coordinator. The Chair will be responsible for ensuring meetings run efficiently and effectively and the Research Coordinator will lead the meeting.

4. **Secretarial Support**

South Metropolitan TAFE is responsible for providing secretarial support to the YTK2RRG.

5. Meetings

- The YTK2RRG will meet monthly either in person or by teleconference.
- If required, additional meetings may take place to discuss pertinent issues.
- There is no requirement for a quorum at YT2RRG meetings.
- The location of meetings may vary between Perth and Geraldton and teleconference facilities can be made available for those who are unable to attend.

6. Minutes

- Agenda items will be called for and an Agenda forwarded to YTK2RRG members 5 working days PRIOR to the date of the scheduled meeting.
- The Research Coordinator in consultation with the Chair is responsible for organising meetings and for disseminating correspondence with regard to YTK2RRG issues.
- Minutes are disseminated within five working days after the meeting.

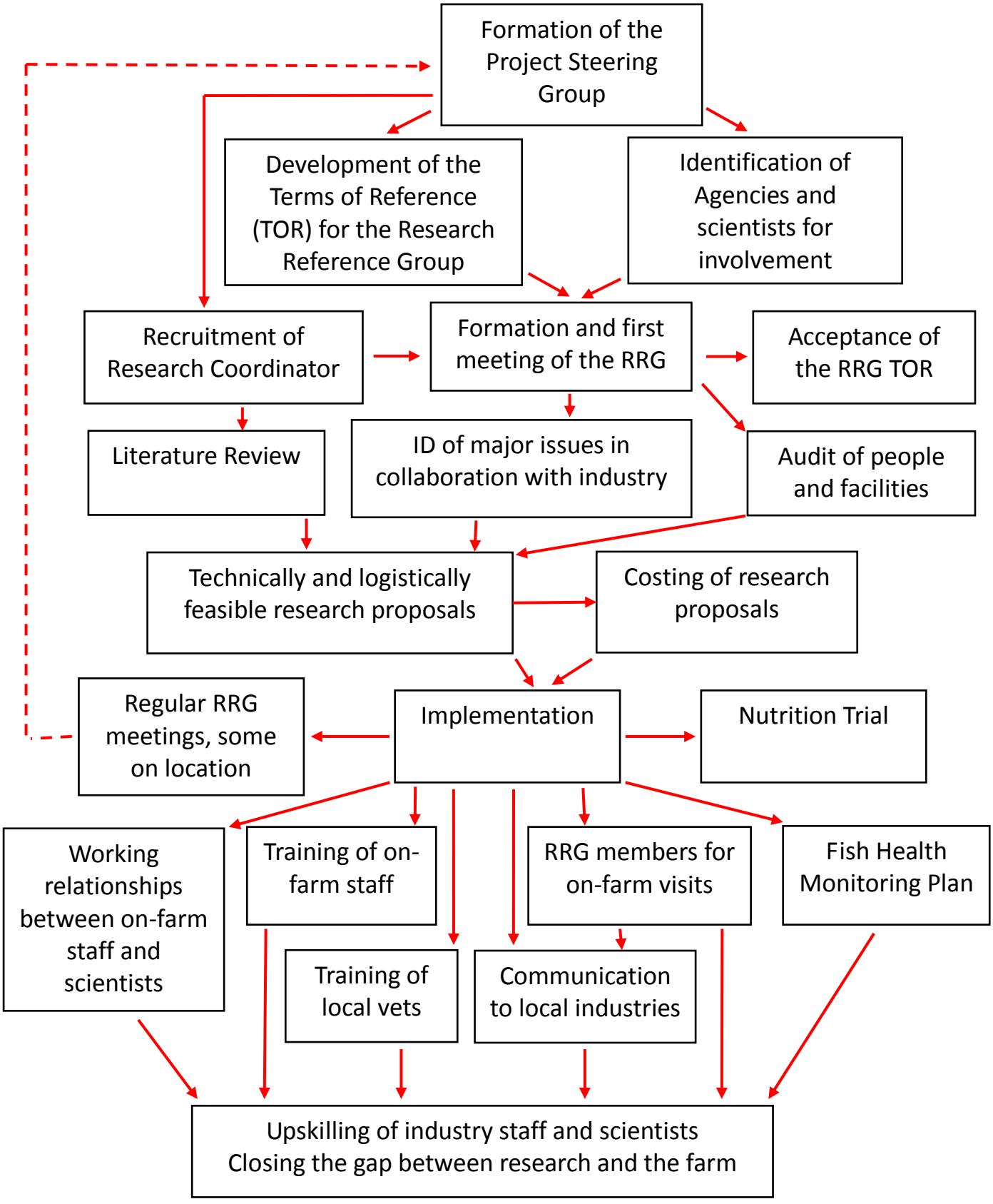
7. Reporting

- Reports may be prepared and presented as approved by the YTK2RRG members and Chair from time to time.
- The YTK2RRG will formally report to the Project Steering Group as required, notice will be given, in the form of a written report and possibly a presentation by a member if required.
- Formal quarterly reports of research activities will be required to meet the overall Project Milestones in March, June, Sept and Dec 2015.
- All reporting and communication and extension activities as required by the CRC /FRDC

8. Remuneration

- There are no sitting remuneration associated with meetings unless agreed by the Project Steering Group.
- Remuneration of travelling expenses will be considered by the Committee on a case by case basis.

Appendix 4. RESEARCH PLAN DEVELOPMENT FLOW DIAGRAM



APPENDIX 5. RESEARCH PLAN FOR THE YELLOWTAIL KINGFISH AQUACULTURE INDUSTRY WITHIN WESTERN AUSTRALIA

Key areas for R&D for the YTK2 trial are:

1. Nutrition
2. Fish Health and Parasites
3. Environment
4. Harvest and Post-Harvest

Background and supply of juveniles

The intention was to provide the best quality juveniles possible for stocking into the Indian Ocean Fresh Australia (IOFA) seacage for the YTK 2 Project, funded by the MWDC and the WA Royalties for Regions program.

As ACAAR has had some yet to be identified water/fish health issues at their facility, the YTK broodstock they hold on site did not spawn at the period when larvae were needed for stocking. The delayed larval rearing pushed back the start of the project by almost a year and in order to prevent further delays, alternative sources of YTK eggs were found. Clean Seas Tuna Limited (CST) agreed to ship newly spawned YTK eggs from their hatchery in South Australia to ACAAR in Perth. The Batavia Coast Marine Institute (BCMI) in Geraldton also hold YTK broodstock which they were able to spawn, and also shipped YTK eggs to ACAAR.

The juvenile YTK were produced at the ACAAR hatchery and shipped to the cage site in Geraldton, WA during late October and early November 2015.Due to multiple shipments of eggs, numerous cohorts of YTK juveniles from South Australia and Western Australia were reared and stocked into both the sea cage site and the nutrition trial (see Nutrition section).

All current data and knowledge attained to date was utilised for YTK production.

During early investigations into the potential reasons for the poor performance of the ACAAR YTK broodstock and resultant eggs, scientists at the Port Stephens Fisheries Institute, NSW Department of Primary Industries (NSW Fisheries) were most helpful and provided YTK eggs from their broodstock for a larval rearing experiment at ACAAR. NSW

Fisheries also sampled eggs and larvae grown out at their facility to compare heavy metals, specifically chromium, between the sites.

There was no formal collaboration on the larval rearing of YTK in conjunction with CST, however they were very helpful and willing to provide eggs to WA under very reasonable conditions and this was much appreciated.

Nutrition

In previous grow out trials for YTK in WA, shortfalls were identified in the dietary formulations of the commercial feeds used on farm. This resulted in nutritional deficiencies and health issues, such as skeletal myopathy and secondary bacterial infections of the intestines, pancreas and livers. Therefore it was decided that during the current production run the fish in the seacage will be fed on the best possible diets based on previous experiences with commercial diets and analyses of currently available diets.

Subsamples of all batches of diets were analysed for proximate composition and other key factors known to influence YTK health including but not limited to:

1. Taurine
2. Vitamin E
3. Selenium
4. Histidine
5. Fatty acid profile for detection of the lipids used in the diet.

Without multiple cages, replicated nutrition trials are unable to be conducted at sea and nutrition trials were therefore conducted in land-based tanks at the ACAAR hatchery in Fremantle, Western Australia. The main focus of these trials is to compare the performance of commercial grower diets on growth, FCR and health of fish over commercially relevant time scales, *i.e*. from stocking to harvest size. The tank trials incorporated the diet fed to the seacage fish into the experimental design. All of the trial fish were pit-tagged in the left shoulder dorsal muscle. The tanks were stocked at a low density at the start of the trial and were maintained at the approximate density of the cage trial as they grew, with some of the excess fish being utilised for routine sampling. Routine health monitoring was completed every month to determine the effect of diets on the overall health of the fish. This trial also allows for the effects of cage versus tank condition, and at different temperatures. The trial is utilising twelve replicate 10 m^3 tanks at ACAAR

and will run over a 12 month period. This long term grow out study at ACAAR is being supported by the FRDC, ACAAR and IOFA.

The 'size to market' is a further aspect that has been identified as a high priority by the industry, and will be investigated within the nutrition trial once the fish reach 1.5 kg. The individual tagged fish will allow the scientists to check for any growth or health differences between fish cohorts and treatments.

Fish Health and Parasite Management

A Fish Health Monitoring Plan has been developed with input from the Fish Pathologists at the DoFWA Fish Health Unit (See Appendix 6).

Fish will be routinely sampled from the seacage for a range of parameters to monitor their health including:

1. Organ sampling for tissue histopathology
2. Blood parameters – including biochemistry profiling, whole blood smears
3. Bacteriology including screening of potential pathogens observed in the previous trial including *Vibrio harveyi* and *Photobacterium damsellae*
4. Routine fluke counts

Archived samples will also be taken routinely and collected for posterity, which may be used for any further research that may be highlighted in the future.

New Technologies

ACAAR is also undertaking an FRDC – supported project (2014 -729) for Praziquantel (PZQ) research. The focus of the PZQ project is new technologies which may not have any immediate benefit to the current pilot scale trial. We would therefore include other PZQ work that may have more immediate benefits, i.e. further palatability and efficacy trials using a garlic extract compound. CST have expressed an interested in the FRDC PZQ project and there may be collaboration with them on efficacy trials if palatability and bioavailability trials under this FRDC project prove encouraging. NSW Fisheries has said they are not interested in investigating PZQ for fluke control. Further short term trials will investigate the use of PZQ, specifically improvements in palatability and bioavailability.

There is consideration to utilising the potential of flow cytometry in fish immunology. Dr Cecile Dang at WA Fisheries has experience with flow cytometry used in the oyster industry. Flow cytometry is a relatively new technology in fish immunology, including basic characterisation and functional studies of fish immune cells and the reaction of cell biochemistry in relation to infection and immunostimulation. Flow cytometry is expensive and extraction of fish cells can be a time consuming and arduous task as cells need to be kept sterile and the incubation in the various biomarkers and fluorescent stains are time sensitive. This will be a challenge as sampling will need to be done at the cage site on the boat as soon as the fish is killed. This study will only determine if cell extraction may be logistically possible and whether preserved viable cells could be transported to the flow cytometry lab for analyses. On site trials will be carried out to determine if organs can be successfully preserved in culture medium and processed into single cell suspensions.

Environment

Continuous monitoring and logging of key water quality variables inside and outside the seacage, including dissolved oxygen and temperature will be conducted.

Water samples will be collected daily and frozen. If after 30 days no event has occurred samples will be discarded. Once a week, samples will be filtered and the filter paper frozen for PCR testing against known hazardous algal species. Filtered and frozen samples will also be archived for later pathogen PCR testing if needed.

Phytoplankton identification and counts will be completed once a month to establish baseline data. Further samples will also be stored using 70% ethanol and archived.

Harvest and Post-Harvest

Dr Fran Stephens and Mr. Rowan Kleindienst in conjunction with Centre of Excellence Science Seafood & Health (CESSH), Faculty Health Sciences, Curtin University conducted numerous trials (CRC Project 2011-754, Mr. Kleindienst Masters) to determine optimum harvest techniques to maximise flesh quality. Recommendations for future research were outlined in these studies and trials will be conducted to improve harvest methods, muscle texture at harvest and conduct post-harvest research to maximise consumer experience and shelf life.

The results of Dr Stephens' studies suggested that further work is needed to develop a method of harvesting yellowtail kingfish that will minimise struggling immediately before slaughter. Research into different holding regimes during and immediately after bleeding may also be of benefit. Water temperature at the time of harvest, such as holding fish in water at 10 °C instead of an ice slurry around 0 °C immediately post-harvest and aerating the bleeding tank water may alter the texture of the flesh and was noted as worth investigating.

A complex relationship of factors affect post-harvest quality parameters in YTK. A series of controlled harvest trials investigating flesh quality, without the influence of myxozoan parasites, will be undertaken. ACAAR serves as a well-controlled, parasite free environment and harvest trials can be run from this site, ideally before harvest trials are carried out at the seacage. The trials will compare various harvest methods.

AQUI-S® New Zealand have expressed an interest in assisting with trials in the use of their product during harvest, and this avenue will be explored. Further small scale trials could be conducted in the controlled environment of ACAAR to assess the impact of a number of other factors such as condition factor and harvest method on the rate of change of pH and lactic acid accumulation, rate of onset of rigour mortis, drip loss/water holding capacity and texture before and after cooking the flesh would provide more clarity around management strategies for harvest.

Mr Kleindienst will be undertaking a scoping report for the YTK2 Project to outline and recommend best harvest practices, identify ways to minimise the effects of myxozoan parasites, to identify gaps in research needs and to develop protocols for the proposed harvest trials at ACAAR.

Personnel skills extension

A key aspect of this project is in the capacity building and upskilling of personnel for the emerging YTK aquaculture industry within WA. The Research Coordinator collaborates with the RRG members to target the research for industry outcomes and increase the collection and transfer of knowledge and data across all participating agencies. This requires a close association to be developed between the team of researchers and industry personnel and is put into practice through collaborative research planning and participation in on-farm activities.

A major focus of the RRG members is the collection of high quality data that occurs both on-farm and at the ACAAR facility. Inconsistency across correct sampling methods was a concern in the previous YTK1 project and hindered the interpretation of the results. To better manage this for the YTK2 project, a fish health workshop was conducted in Geraldton soon after fish were stocked into the cage which was attended by the operational on-farm staff, the researchers from ACAAR, the staff at the Geraldton veterinary clinic and the pathologists from the Fisheries Fish Health Unit. The purpose of the workshop was to inform all of the researchers and farm staff about the reasons for and the requirement of the fish health sampling regime and train them in appropriate collection techniques to ensure the data was consistent across all participants.

As part of the skills extension, the staff at the veterinary clinic were trained in the correct processing of samples. The Vet and a staff member from the clinic have also attended the field training and engaged with the RRG members.

The research co-ordinator travels to Geraldton on a monthly basis to assist with the regular fish health assessment. Any additional field testing and data collection is also completed in response to the industry needs during these visits. Operational staff from the farm also travel to ACAAR for the monthly fish health assessment as part of the nutrition trial. All of these visits increase the two way transfer of knowledge and skills between researchers and on-farm personnel. They increase the probability of consistency between sampling and the accurate assessment of variations in fish growth, health and response to differing environments between the two sites.

The research co-ordinator also liaises with the pathologists at the Fish Health Unit, through ongoing communications and visits to their facilities to provide continual improvement in sample collection and data capture. The improvements are documented and reported to the participants in the project. These visits also provides for the ongoing transfer of histology knowledge such as interpretation of slides, photographs and analysis from Fish Health staff to the Research Coordinator. The transfer of such knowledge from the research coordinator to other participant’s during the frequent visits to the farm results in a better understanding by industry personnel of the reasons for, and interpretation of results of sampling and the accumulation of knowledge.

As well as frequent informal discussions amongst the participants, the RRG members have regular formal meetings to discuss the overall progress of the project and address any issues that require ongoing of further investigation.

APPENDIX 6. FISH HEALTH MONITORING PLAN

Daily

Observation

1. **Observe** fish behaviour and note any unusual behaviour. This could include, but not limited to, flashing, spiral swimming, increased rate of ventilation, gulping, darkened body colour, moribund fish.

Environmentals

1. **Before feeding**, record water temperature and dissolved oxygen both outside and inside sea cage at a depth of approximately 2 m and, secchi depth outside of sea cage. Also record prevailing weather (wind speed and direction).
2. **Whilst Feeding**, monitor dissolved oxygen.
3. **Stop Feeding** if dissolved oxygen is/or falls below 4.5 mg/L or 80 % Refer to 'Low DO Plan and Remedial Action Protocol'.

Algal sampling:

1. For algal samples, sample 2.5 L of water from the water column. Collect water with a flexible pipe of small diameter lowered down into the water column. Insert a stopper at the top of the pipe then lift up the base of the pipe. Remove the stopper and release the water into the bucket and mix well and take a 250mL subsample. These samples are to be **frozen daily**. After 30 days, if no event has occurred the sample can be discarded.
2. **Once a week**, a 250 mL sample is to be filtered through 1.2 µm (GF/C grade) filter paper using the vacuum pump. The filter paper is then frozen and kept for **HAB PCR testing** if an event occurs.
3. **Once a week**, a 250 mL sample is to be filtered through 1.2 µm (GF/C grade) filter paper using the vacuum pump. The filter paper is then frozen and kept for **pathogen PCR testing** if an event occurs.
4. **Once a month**, one subsample (250 mL) is to be sent to SGS Perth for baseline **phytoplankton identification and count**, no preservation is needed if the sample is couriered overnight.
5. For pathogen presence, filter 2.5 L to 1 µm and collect filtrate into sample bottle and preserve with 70 % ethanol. This sample is to be kept in archive for future analysis if necessary.

Weekly

Fluke Sampling (non-lethal sample):

1. **Collect 5 fish** weekly and anaesthetise in 5 ppm AQUI-S.
2. **Bathe** in dechlorinated freshwater for 3 min and praziquantel in seawater at 15 ppm for 10 min.
3. **Filter** bath water from each bathe with a 75 µm mesh and collect filtrate in a 70 mL container.
4. **Process** filtrate under a dissecting microscope. Count individual fluke for each species and assign a proportion of the total for each life cycle stage (recently attached, juvenile, large juvenile and adult).
5. **Preserve** flukes in 70% ethanol.

Routine Sampling Necropsy

Sampling periods:

- **10 fish per week when fish are < 100 g** [5 fish to be sent for analysis (4 fish in 10% formalin and 1 fish in 70% ethanol) and 5 fish for archiving (3 fish in 10% formalin and 2 fish in 70% ethanol)] and,
- **5 fish per fortnight when fish are > 100 g** [5 fish to be sent for analysis once a month and 5 fish preserved in 10% formalin].

Samples that need collecting are: blood (Bl), gills (G), brain (Br), muscle (M), heart (H), liver (L), spleen (S), and kidney (K) and pyloric caecae (PC). The gut needs to be dissected in three portions, the foregut (FG), midgut (MG) and hindgut (HG). All these organs need to be preserved in 10% formalin. A sample of muscle needs to be preserved in 100% ethanol for *Myxos analsyis*

4. **Collect fish** from sea cage and anesthetise in AQUI-S at 15 ppm. (If there is an incident, i.e. mortality or unusual event, sample at least 10 fish, include healthy and moribund fish during a diagnostic investigation). On the boat, fish to be lightly sedated and weighed and the blood collected, muscle pH recorded and gills removed immediately. All other organs should ideally be sampled on the boat but in instances where the weather is too severe, dissection of the remaining organs can be done on land. For fish under 100g, blood is collected and muscle pH recorded immediately after light sedation, the caudal fin, one operculum and stomach flap removed and fish preserved in 10% formalin.

5. **Blood Sampling**:

Fish used in sampling of blood for haematology and enzymology must not be stressed for long periods, this necessitates the use of light anaesthesia. Blood samples from fish can be collected from the caudal vessel, by insertion of a fine needle from the ventral midline behind the anal fin until the spine can be felt, and withdrawing the needle slightly before collection. Collect blood in lithium heparinised needles and syringes. Blood is then to be transferred into lithium heparin MiniCollect tube and immediately stored in an ice slurry. It is very important the whole blood samples are spun as soon as possible the samples need to be delivered to Chapman Animal Hospital (Attn: Steve Duda: 74 NW Coastal Highway, Geraldton).

Tests to be completed at Chapman Animal Hospital:

Blood smear (one smear air dried and one smear stained in Diff-Quik) – Smears sent to Dept. Fish Health

Haematocrit (recording red blood cell, plasma and leukocyte percentages)

The remainder of blood is then centrifuged at 10, 000 rpm for 5 minutes. Extract the top, clear layer of plasma and put into a clean Eppendorf (ideally 0.3 mL of plasma). The plasma and red blood cell pellet are then frozen and sent to Jenny Hill at VetPath (Belmont, WA) for routine biochemistry profiling and glutathione peroxidase analysis. Once frozen it is ideal if the plasma is not defrosted, so it remains frozen during transport.

Fish need to be identified with **individual tags** whilst bloods are taken. The fish can then be stored in an ice slurry if not being fully processed on the boat. Each fish sample is to be labelled with the full date and fish number and organ, i.e. 070715F1-G for the first fish caught on the 07/07/15 gill sample or 281115F4-L for the fourth fish caught on the 28/11/15 liver sample.

6. Visual Assessment

Visually inspect the external condition of each fish. If either an ulcer, reddening underneath operculum or of junction between gills and body or tissue necrosis is detected, swab and using sterile transport swab kits and send to the Ag Dept (Attn: Nicky Buller: Dept. of Fisheries Baron Hay Court, Perth). This will detect for *Vibrio* and other bacteria.

7. Morphometric Measurements of fish to include fork and total length, width, height and weight of individual fish. Use these measurements to calculate a condition index (CI). Note any malformations.

Histology and Parasitology Sampling

1. **Histology:** Dissect out each organ, record weights and visually inspect condition. Take photographs for an abnormalities observed. Routinely sample tissue for histology from gills, brain, muscle, heart, liver, spleen, kidney, pyloric caecae and gut. The gut needs to be dissected into three portions for histology, the foregut, midgut and hindgut. Cut a 1 cm piece of each section and cut the tube down the middle so it becomes flat. It is important that the same section of gut is sampled from each fish. Tissues samples to be stored in 10% formalin.

   Gut microflora analysis are to be sampled at four time points during the trial. These samples are two 1 cm pieces of gut cut down the middle and two sections of fish skin each stored in RNAlater and formalin.

2. **Parasitology:** Additional $1cm^3$ samples of muscle tissue should be kept in 70% ethanol for PCR analysis.

3. **Fat Analysis:** Remove and weigh fat in the body cavity of fish >100g. This is usually found around the organs e.g., pyloric caecae and hindgut. Fish livers are to be weighed used to calculate hepatosomatic indexes and then dissected for routine sampling. Ensure a 5g liver portion is retained and frozen, to be archived at IOFA for future fat analysis.

When sampling is completed you should have 12 sections in a sample jar per fish ready to send to Fish Health labs.

As Required

Diagnostic investigation

1. Initiate when there is an elevated level of mortalities, abnormal or moribund fish are observed.
2. Observation procedures
3. Environmental procedures
4. Algal Sampling procedures
5. Fluke Sampling procedures
6. Necropsy procedures on freshly dead, moribund and healthy fish is to be conducted under the direction of Fish Health labs.
7. Bacteriology: Sterilise body surface between dorsal fin and eyes and extending ventrally to pectoral fins. Using a sterile knife, cut down through backbone to reveal

head kidney. Take a swab of head kidney, using a charcoal transport swab, and send directly to Animal Health Laboratories, Dept. of Agriculture. This detects for presence of *Vibrio* and *Photobacterium*.

Weight Checks

1. **Weekly** When fish are less than 100 g
2. **Fortnightly** When fish are less than 1,000 g
3. **Harvest** Can be incorporated into the harvest packout when fish are greater than 1,000 g

Calculations

1. Condition index (CI) = body weight / total length (%)
2. Hepatosomatic Index (HIS) = liver weight / body weight (%)
3. Fat Visceral Index (FVI) = fat weight / body weight
4. Gonadosomatic Index (GSI) = gonad weight / body weight

APPENDIX 7. LIST OF RESEARCH AND PROJECT STAFF

Industry Partner – Indian Ocean Fresh Australia Pty Ltd.

Erica Starling (Director)

Justine Arnold (Technical Officer)

Stephen Murphy O'Sullivan (Technical Officer)

Lacey McDonald (Technical Officer)

Research Provider – Australian Centre for Applied Aquaculture Research

Greg Jenkins (Director)

Dr Gavin Partridge (Principal Research Scientist)

Dr Lindsey Woolley (Research Coordinator)

Robert Michael (Production Manager)

Luke Pilmer (Technical Officer / Honours Student)

Shayla Stefanetti (Honours Student)

Research Provider – Fish Health Section, Department of Fisheries Western Australia

Dr Cecile Dang (Principal Research Scientist)

Dr Fran Stephens (Senior Fish Pathologist)

Dr Jo Bannister (Fish Pathologist)

Dr Terry Miller (Parasitologist)

FRDC FINAL REPORT CHECKLIST

Project Title:	Co-ordination of the research for the Yellowtail Kingfish industry in WA		
Principal Investigators:	Greg Jenkins		
Project Number:	2014/706		
Description:	This project supported a Western Australian yellowtail kingfish growout trial by effectively coordinating and directing the research effort for the maximum benefit of the industry. It also developed a model for such coordinated research for other similar projects.		
Published Date:	May 19	**Year:**	2017
ISBN:	978-0-9871696-2-4	**ISSN:**	Not applicable
Key Words:	Yellowtail Kingfish, Research and Development Plan, aquaculture, finfish, Western Australia		

Please use this checklist to self-assess your report before submitting to FRDC. Checklist should accompany the report.

	Is it included (Y/N)	**Comments**
Foreword (optional)	N	
Acknowledgments	Y	
Abbreviations	Y	
Executive Summary		
– **What the report is about**	Y	
– **Background – why project was undertaken**	Y	
– **Aims/objectives – what you wanted to achieve at the beginning**	Y	
– **Methodology – outline how you did the project**	Y	
– **Results/key findings – this should outline what you found or key results**	Y	
– **Implications for relevant stakeholders**	Y	
– **Recommendations**	Y	
Introduction	Y	
Objectives	Y	
Methodology	Y	
Results	Y	
Discussion	N	
Conclusion	N	
Implications	Y	
Recommendations	Y	
Further development	Y	
Extension and Adoption	Y	
Project coverage	Y	

Glossary	N	
Project materials developed	Y	
Appendices	Y	