Identification of muscle parasite in Yellowtail Kingfish (Seriola lalandi) and Mahi Mahi (Coryphaena hippurus), and determination as to the efficacy of non-invasive screening technology for the purpose of identifying infected fish in a commercial fish processing environment

Progress Report preceding termination of the project

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FRDC Project No **2017-020**

FRDC 2017-020 Progress Report

Progress towards proof of concept of using small animal ultra sound imaging for the purpose of identifying muscle parasites in Yellowtail kingfish and Mahi mahi.

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Introduction

Yellowtail kingfish (*Seriola lalandi*) (YTK) and Mahi mahi (*Coryphaena hippurus*) (MM) are commercial fish species landed along the coast of New South Wales (NSW) by line fishers of the inshore fisheries. The vast majority of fish are landed by "day fishers" operating between Coffs Harbour in the north, and Wallis Lakes in the south. Conducting a day fishing operation permits the transport of fish on a daily basis to Sydney to be on the market floor within 24 hours of landing.

The fishery is primarily a winter fishery, operating between May and November, when waters cool sufficiently for YTK to move from waters further south. Fishers are mostly multi-licence operators who move between YTK and other species like bonito. Fishing is almost exclusively line fishing by hand or drop lining. Many of the fishers choose to operate alone.

The research described in this progress report has been conducted with the aim of addressing the four objectives set out with the first Milestone. The objectives are;

1. Proof of concept as to the efficacy of ultrasound imaging for parasite identification
2. Establish the rate of incidence of claims for parasite infection for Yellowtail kingfish and Mahi mahi.

Initial field trip (August 2018)

The PI travelled to Coffs Harbour on 30th August 2018 to discuss fishing activities and receive briefings from local fishers of YTK. The PI met with Coffs Harbour Co-Op staff to discuss fishing and processing practices. The PI also met with local fishers to discuss their own experiences. Several local fishers will target YTK during the peak season (winter and spring) while others only land YTK as by-catch from targeting other species. MM are a bycatch within this fishery, as they are rarely landed in numbers large enough to target specifically.

The 30th of August 2018 was a poor fishing day for YTK as a seal was sighted in the fishing grounds early that morning, resulting in only one YTK being landed that day by any of the local fishers. No MM were landed that day either. Local fishers reported that on a good day's fishing they could land up to 200kg of YTK in a mornings fishing.

The PI had kept in contact with Fishers from the Coffs region during the course of the project. However, to date there have been no reports of any claims for mushiness in YTK or MM that have been made back to the fishers.

Sourcing parasitised fish

The vast majority of YTK and MM landed from members of the Professional Fishers Association (PFA) are sent to Sydney Fish Market (SFM) the day of landing, and sold on the floor of SFM the next business day. The PI adopted several methods of contact with key stakeholders who may have had access to parasitised YTK and MM during the course of the research.

Direct communication with the fishers was made by way of the PFA weekly newsletter issued every Friday. The newsletter provided stakeholders with information as to the objectives of the project, and asked for any interested fisher to contact the PI to be involved in future work. The PI received contact from two interested fishers.

Key personnel from the SFM were consulted as to the process of claims made from wholesalers through the SFM. Arrangements were made to report any claims to the PI and attempt to obtain the infected material for further analysis.

The PI met with SFM staff in December of 2018 in an attempt to identify any additional pathways for the research team to obtain parasitised fish. As a result of this meeting Quality Assurance (QA) staff from the SFM made contact directly with qualified buyers of YTK in an attempt to disseminate the request for access to any rejected YTK product due to mushiness.

Since the project commenced, until the time of writing this report, there has not been a single claim made to the SFM with regard to mushiness in YTK or MM. If mushiness is presenting to customers and wholesalers, the loss is being absorbed within those businesses rather than making a claim back to the SFM.

The PI made contact with four of the Co-Op general managers within the PFA in May of 2019 to discuss the issue. Knowledge of the issue and any recent reporting varied greatly. The most detailed response came from Wallis Lake Fish Co-Op. In the previous 12 months the Co-Op had made five shipments direct to wholesalers, three of which resulted in claims back to the Co-Op for mushiness in YTK. As a result Wallis Lake has chosen to send future shipments to the SFM floor. However, they have not been any further claims since coming back through the SFM.

The inability to source wild harvest YTK that have presented with mushiness created a major problem with regard to trialling the ultrasound (US) technology. Without infected fish the technology could simply not be tested.

Trial of ultrasound imaging technology using commercially sourced YTK proof of concept assessment was eventually made possible by the supply of parasitised YTK. The fish were sourced by Dr Gaven Partridge of the Australian Centre for Applied Aquaculture Research (Fremantle, W A). Parasitised fish were sourced from a commercial producer. The fish had been previously identified as “heavily infected” and had been kept frozen as whole fish for several months. Infected fish were approximately 2kg in weight each. Sex of fish was not determined.

Control fish (not infected) were sourced by fish spawned at the Fremantle Aquaculture Centre. These fish were also provided by Dr Partridge and his team. Control fish were approximately 1kg in weight, and sex was not determined prior to assessment.

Assessment methodology

Diagnostic imaging by ultrasound on both control and infected YTK were conducted by Dr Jennifer Richardson of The Animal Hospital at Murdoch University (School of Veterinary and Biomedical Science, South Street Murdoch, WA). The assessment was conducted on 27th December 2018. Imaging was conducted using a Toshiba Aplio 500 ultrasound machine (Toshiba Corporation, Minato-ku, Tokyo, Japan) and the transducer used was a Toshiba 18L7 linear transducer.

Three fish, two known infected fish and one known non infected fish were evaluated. The fish were scanned for lateral recumbency on both right and left sides using the linear transducer. The infected fish were identified as “Sample2” and “Sample4”. The non-infected fish was identified as “Control”. The report in full and Images of the ultrasound imaging can be found in Appendix 1.

Results

There was a difference in the size of the fish and the muscle mass with the control being much smaller. The hyperechoic bands, consistent with the appearance of fat within the muscle, were thinner and spread further apart in the control fish (thinner and smaller) than the other fish. The muscle mass between was anechoic with specular echoes throughout. No parasitic pseudocysts or spores were able to be identified on any of the fish.

One infected fish (Sample2) and the control fish were also scanned using computed tomography (CT). The size difference was mostly the thickness of the muscle mass. The larger “Sample2” also

contained a much larger number and thickness to the fat banding (grey bands) through the muscle when compared to the smaller control fish. No parasitic pseudocysts or spores were able to be identified on any of the fish.

Conclusions

The infected YTK used in the assessment were provided as examples of heavily infected with the unicapsular muscle parasite. If the technology was going to be at all capable of identifying the pseudocysts of this parasite, then it should have been capable of doing so with these fish.

Neither the ultrasound nor the CT imaging was capable of identifying pseudocysts or spores of parasitic material in the infected fish. The number of samples used for this assessment is very small and without genuine statistical significance. However, in light of the heavy levels of infection reported to be present in these fish, it would appear unlikely that this technology will prove useful as a non-invasive diagnostic tool for unicapsular in YTK.

Recommendations

The key recommendation is that the project activate the STOP/GO clause in the project proposal to end the proposal at this Progress Report. This recommendation is based on the following;

Inconclusive results from Ultrasound technology

The use of ultrasound was not tested to the level repetition that would produce statistically sound results. This is primarily due to the lack of access to parasitised fish to enable completion of the work.

However, a small trial was conducted on YTK in WA. These fish were identified as heavily parasitised. The use of both ultrasound and CT diagnostics failed to identify any key features that would enable differentiation of heavily parasitised YTK from YTK spawned and grown out in a parasite free environment.

If the technology was ever going to have a chance at identifying parasite caused anomalies in the muscle tissue, then this would be the best opportunity available. Failure to achieve so during this preliminary testing suggests that further work in this area of research is not warranted at this time.

Lack of clarity as to the scale of the problem

During the course of this work, the research team have not been successful in obtaining a single parasitised fish or portion of parasitised fish that had been sold though the SFM. The research team had measures in place to ensure any claims made by buyers were collated (anonymously) during the course of the research. This period of time is approximately 15 months. Considering almost all YTK landed in NSW are sold through the SFM, this suggests two factors at play;

1. The rate of incidence is in all likelihood, much lower than has been reported by industry stakeholders.
2. Any losses incurred after sale though the SFM are most likely being absorbed by end users and distributors rather than making a claim to the SFM.

The inability to access parasitised fish samples not only hampered the efforts to evaluate the ultrasound technology, it also prevents the determination of the true rate of incidence. Without a better understanding of the rate of incidence, there is no method to establish the quantum of the losses to the industry. And without access to the scale of the losses, any recommendations towards commensurate investment of resources into measure to alleviate these losses is near impossible.

So with a clear lack of available fish presenting with mushiness fish, and no manner in which to establish the true value of losses to the industry, we recommend that work cease on the project in its current form.

Ongoing technical support

The research team has built a national network of expertise relevant to any further research in this area. The PI is happy for the FRDC to distribute their contact details as a point of future contact for any stakeholders wanting to access some of the key research capabilities identified during the course of the project. These key personnel cover a range of expertise relevant to YTK. These capabilities include the following services.

Histology and Aquatic Veterinary Services

Roger Chong, Senior Veterinarian (Aquatic Health), Biosecurity Services Laboratory, Queensland Department of Agriculture and Fisheries. (roger.chong@daf.qld.gov.au)

Dr Terry Miller, Senior Research Scientist, Fish Heath Laboratory, Department of Primary Industries and Regional Development WA. (Terry.Miller@dpird.wa.gov.au)

Molecular Diagnostic Techniques

Dr Robert Adlard, Principal Curator (Parasitology) and Head of Marine Biodiversity, Queensland Museum. (robert.adlard@qm.qld.gov.au)

Non-invasive Veterinary Diagnostics

Dr Jennifer Richardson, Veterinary Radiologist, School of Veterinary Clinical Science, Murdoch University WA (Jen.Richardson@murdoch.edu.au)

Aquaculture of YTK and related production issues

Dr Gavin Partridge, Principal Research Scientist, Australian Centre for Applied Aquaculture Research, Department of Primary Industries and Regional Development WA (Gavin.Partridge@dpird.wa.gov.au)

Appendix 1

Diagnostic Imaging Report

MURDOCH
UNIVERSITY
PERTH, WESTERN AUSTRALIA

Department of Veterinary Clinical Science
School of Veterinary & Biomedical Sciences
South St, Murdoch
Western Australia 6150

Telephone: 1300 652 494
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The Animal Hospital at Murdoch University

DIAGNOSTIC IMAGING REPORT

Privileged information – not for unauthorized distribution

Owner: Forrest	**Date of examination:** 27/12/2018
Patient name: Fish Research	
Patient number: 210431	**Age:**
Species: Piscine	**Sex:** Unknown
Breed:	**Weight:** 0.000kg

Diagnostic Imaging: Ultrasound

Requesting Clinician: Andrew Forrest

History: Sample fish for US screening to determine presence/absence of paracytic pseudocysts or spores.

Region(s) of Interest / Study Obtained: Fish muscle.

Findings: Three fish, 2 known infected fish and 1 known non infected fish were evaluated. The fish were scanned form lateral recumbency on both right and left sides using the linear transducer.
The infected fish were identified as Sample2 and Sample4.
The non infected fish was identified as Control.
There was a difference in the size of the fish and the muscle mass with the control being much smaller. The hyperechoic bands, consistent with the appearance of fat within the muscle, were thinner and spread further apart in the control fish (thinner and smaller) than the other fish. The muscle mass between was anechoic with specular echoes throughout.
No paracytic pseudocysts or spores were able to be identified on any of the fish.

CT: One infected fiah (Sample2) and the control fish were also scanned using CT. The size difference was mostly the thickness of the muscle mass. The larger Sample2 also contained a much larger number and thickness to the fat banding (grey bands) through the muscle when compared to the smaller control fish.
No paracytic pseudocysts or spores were able to be identified on any of the fish.

Conclusion:
1. The parasite was not able to be identified by ultrasound using the frequency available or with CT.
2. The muscle thickness and fat banding within the muscle was able to be evaluated using both CT and ultrasound.

Dr Jennifer Richardson, BSc, BVMS, MVS, MACVSc, FACVSc(Radiology)

Figure 1 Image from ultrasound assessment of Sample2

Figure 2 second image from ultrasound assessment of Sample2

Figure 3 image from ultrasound assessment of Sample4

Figure 4 second image from ultrasound assessment of Sample4

Figure 5 image from ultrasound assessment of control YTK

Figure 6 second image from ultrasound assessment of control YTK