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FINAL REPORT

Assessing the biosecurity risk of imported uncooked, whole and head-on eviscerated, barramundi and non-salmonid finfish in relation to exotic viruses

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Foreword

The Australian barramundi farming industry is a relatively new industry, which is expanding rapidly. Over the past five years, farmed barramundi production has been growing at an average rate of 14% p.a. The Australian Barramundi Farmers Association (ABFA) forecasts industry value will be $108 M in 2020-21 and anticipates that industry value will reach $200 M by 2025. For the Australian barramundi farming industry to reach its growth potential, it is essential that optimal health of farmed stock is maintained, and significant disease impacts are minimised. The competitive advantage of being free from the impacts of many internationally significant diseases also must not be jeopardised.

The most effective mechanism used globally in animal production systems to minimise the risk of external disease incursion is the maintenance of a high level of biosecurity. Importantly and uniquely, within the aquatic environment, once a disease is introduced it can be exceedingly difficult to subsequently eliminate or control it's spread.

ABFA and its members are extremely concerned that current import controls measures are not providing efficacious front-line protection for the industry.

There are currently NO mandatory requirements to decontaminate (e.g., cook) imported barramundi (and other species) potentially carrying exotic pathogens of known concern, or processing wastes (gills, guts, or frames).

There is currently NO routine post-border testing performed on imported uncooked whole and head-on eviscerated barramundi commodities, so the presence and prevalence of exotic pathogens of concern in these products are unknown.

There is currently NO routine assessment of imported uncooked head-on eviscerated barramundi relative to BICON import requirements, so the compliance of imported barramundi products relative to import conditions is not known.

There are currently NO measures that prevent further processing of imported uncooked whole and head-on eviscerated barramundi.

There are NO functional controls on imported uncooked barramundi processing waste to prevent it being discarded or released into natural waterways as bait, berley, or cheap disposal.

There are currently NO methods in use to categorically determine the country of origin or differentiate farmed and wild-caught whole and eviscerated barramundi. Thus, there remains an avenue for product substitution that can avoid some import control measures on aquaculture product.

This project has confirmed that these concerns of the barramundi farming industry members are tangible and justified, highlighting areas of control failure and biosecurity non-compliance.

The recent Auditor General report "Responding to Non-Compliance with Biosecurity Requirements" (https://www.anao.gov.au/work/performance-audit/responding-to-non-compliance-biosecurity-requirements), was strident in highlighting that Australia was placing at risk its production sectors. Numerous areas of function were deemed "inappropriate" and thus the department was unable to demonstrate that it was able to be effective at managing biosecurity risks. The Auditor General made eight recommendations which were agreed for adoption by Department of Agriculture, Water and the Environment. The findings of this research are of material interest as they interface tightly with these new recommendations.

Jo-Anne Ruscoe

CEO, Australian Barramundi Farmers Association

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Abbreviations

AFDL – Australian Centre for Disease Preparedness (ACDP) Fish Diseases Laboratory

ALOP - Appropriate Level of Protection

ANZSDP - Australian and New Zealand standard diagnostic procedures

DAWE - Department of Agriculture, Water, and the Environment

FAH IDL – The University of Sydney Farm Animal Health Infectious Disease Laboratory

FFVS – Future Fisheries Veterinary Service Pty Ltd

FRDC – Fisheries Research and Development Corporation

GGS – Gilled, gutted, scaled

ISKNV - Infectious spleen and kidney necrosis virus

MCV – Megalocytivirus

NACA - Network of Aquaculture Centres in Asia-Pacific

RSIV - Red sea bream iridovirus

SDDV - Scale drop disease virus

SGIV - Singapore grouper iridovirus

TRBIV - Turbot reddish body iridovirus

Executive Summary

Background

The Australian Barramundi farming industry is expanding rapidly with very significant flows of Government and private sector investment. Over the past five years, farmed barramundi production has been growing at an average rate of 14% p.a. The Australian Barramundi Farmers Association (ABFA) forecasts industry value will be $108 M by 2020-21 and anticipates that industry value will reach $200 M by 2025. Presently, the barramundi farming industry enjoys freedom from numerous internationally significant diseases including all from the Megalocytivirus genus of iridoviruses. These diseases are known to be causing severe impacts on farmed barramundi and other species in southeast Asia and elsewhere, globally. Australia's biosecurity system is the primary barrier to keep such disease risks offshore. The importation of barramundi and other finfish commodities for human consumption from countries where these diseases have been reported had not been fully assessed as a risk pathway for disease incursion prior to this project.

Australia currently allows the importation of uncooked farmed and wild caught non-salmonid finfish products for human consumption from several countries, as whole and head-on eviscerated product. These include key Australian native species, such as barramundi (*Lates calcarifer*) and grouper (*Epinephelus* spp.). The annual volume of uncooked whole and eviscerated barramundi product imported into Australia between 2016 and 2019 is estimated to be greater than 1,000 tonnes (DAWE, pers. Comms. 2021). It was hypothesized that importation of whole and head-on eviscerated uncooked non-salmonid fish products may contain significant exotic viruses of concern, such as red sea bream iridovirus (RSIV), infectious spleen and kidney necrosis virus (ISKNV), scale drop disease virus (SDDV), Singapore grouper iridovirus (SGIV), or turbot reddish body iridovirus (TRBIV). These viruses are considered exotic to Australia and could present a significant biosecurity and disease threat, due to their highly contagious and pathogenic nature, if infection were to occur within an aquaculture or naïve wild fishery setting. It was also hypothesized that imported whole and head-on eviscerated uncooked non-salmonid fish products may contain a substantial volume of high-risk processing wastes, which could make their way into waterways, therefore facilitating an incursion pathway for exotic diseases.

The Australian Department of Agriculture, Water, and the Environment (DAWE) does not currently consider there to be sufficient evidence to suggest that current import conditions for uncooked non-salmonid finfish do not effectively manage the risk of pests and diseases of concern arriving in Australia. Until further evidence is generated to demonstrate that importation of uncooked non-salmonid finfish products carries a risk that exceeds Australia's Appropriate Level of Protection (ALOP), then regulatory actions to mitigate these perceived biosecurity risks are not likely to be implemented.

Objectives

1. Determine for the first time in Australia, the presence of RSIV, ISKNV, SDDV, SGIV, and TRBIV in imported high risk (whole and head-on eviscerated), uncooked, barramundi and grouper finfish commodities that were destined for human consumption, collected from seafood wholesale and retail outlets throughout Australia, using qPCR.
2. Review of imported whole and head-on eviscerated commodity types in relation to current import risk assessment and peer-reviewed published literature to assemble knowledge of the status of these exotic viruses as risks to the Australian farmed barramundi and grouper industries.

Methodology

Imported uncooked finfish commodities were sampled by Future Fisheries Veterinary Service (FFVS) from seafood markets, seafood retailers and supermarkets across Australia based on availability of imported high risk (whole and head-on eviscerated) barramundi and other imported non-salmonid finfish being identifiable at the point of sale. Samples were collected maintaining an appropriate chain of custody and with an approved biosecurity permit from NSW DPI to handle prohibited matter in NSW. This targeted survey involved a minimum sample size of 101 to provide a design prevalence of 5% for an unknown large (>10,000) population size with a required population sensitivity of 99%, using an assumed unit (test) sensitivity of 90% and specificity of 100%, achieved through confirmatory re-testing of suspect positive samples. Tissue sub-sampled for diagnostic testing included gill, liver, and haematopoietic organs (spleen and kidney) on whole fish, and remnant tissue from above organs, from head-on eviscerated fish products. Samples were tested for the virus species SDDV, ISKNV (including the genotypes ISKNV, RSIV and TRBIV) and SGIV using qPCR. Any samples identified as positive for ISKNV by qPCR were to be distinguished for genotype identity based on partial sequence of the major capsid protein gene using conventional PCR and or nested PCR assays and sequence analysis. Confirmatory re-testing was performed on any samples that tested positive or inconclusive using the original nucleic acid extract and an additional nucleic acid sample prepared from the tissue homogenate by AFDL. Data was collected to better understand the type and volume of imported whole and head-on eviscerated barramundi and grouper commodities available to the public at retail outlets and a literature review of current import conditions and peer-reviewed literature relevant to the biosecurity measures for imported whole and head-on eviscerated barramundi and other non-salmonid finfish products intended for human consumption.

Results and Discussion

The final sample set included 119 imported, uncooked, non-salmonid finfish, labelled with 3 common names: Barramundi (n=78), Golden Pomfret (n=40) and Silver Pomfret (n=1). Finfish commodities were sampled from 13 seafood retailers and 4 supermarkets, across four states and territories, between June and October 2020.

The testing detected the presence of ISKNV viral DNA by qPCR in 7 of the 119 imported finfish product samples. Confirmatory testing, performed at AFDL, confirmed presence of ISKNV viral DNA was in 2 samples and an indeterminate result was reported for 3 of these samples. The quantity of ISKNV viral DNA detected was low, reflected by high Ct values, so further testing to distinguished for genotype identity (RSIV, ISKNV, TRBIV) was not able to be performed. SGIV viral DNA was detected by qPCR in 4 of the 119 imported fish product samples. Confirmatory testing was not available for SGIV. SDDV viral DNA was not detected in any of the 119 samples tested.

Exotic ISKNV-like and SGIV viral DNA was detected in uncooked whole and head-on eviscerated non salmonid finfish commodities imported into Australia. The positive survey result indicated that the prevalence of ISKNV-like and SGIV qPCR positive fish was >5% based on the sampling design and assumed test performance characteristics.

ISKNV-like and SGIV are known to cause high mortality internationally in fish species, such as barramundi and grouper, and are currently exotic to Australia. This is a significant biosecurity risk to Australian barramundi farming industry, wild catch industry and native barramundi populations, which contribute to significant food production and tourism sectors in Australia.

All head-on eviscerated barramundi commodities sampled appeared to be non-compliant to current BICON import conditions in relation to their product form, as all products were not completely de-gilled and did not have all internal organs completely removed from inside of the body of the fish. Organ tissue from the kidney was readily detectable in 100% of samples, while the heart tissue and various other internal organs such as the liver, spleen, and various mixed organ remnants, occasionally including sections of intestine, were frequently identified in sampled products. This finding highlighted an increased risk for the generation of high risk processing waste in these non-compliant commodities, which is unregulated in terms of disposal and could enter local waterways, which is a limitation of the current import conditions to safeguard Australia's aquaculture industry from exotic disease risk.

Recommendations

1. Project data be considered by DAWE to be sufficient to trigger a review of import risk analysis of non-salmonid (specifically barramundi) commodities to consider the full suite of rapidly emerging global disease threats and appropriately adjust Australia's ALOP to align with current scientific literature and to promote safe trade.
2. DAWE to review compliance of imported non-salmonid commodities to BICON requirements.
3. Barramundi farms review their on-farm biosecurity measures considering the disease risks highlighted by this project.

4. Develop educational materials for commercial crab/lobster and recreational fishing industries, as well as seafood retailers and wholesalers, to ensure that no processing waste from imported non-salmonid fish (destined for human consumption) is used as, or mixed with, bait.
5. DAWE to consider revising BICON to only allow importation of barramundi (and other aquaculture derived) product types, which are in a consumer ready form and unlikely to result in the generation of any processing waste (e.g., fillets-only). This mitigation measure would assist in addressing the increased biosecurity risk associated with imported barramundi and other non-salmonid finfish products, and the increased scale of consequence (due to the growth of the barramundi farming industry), should the exotic pathogens elude the current biosecurity border controls.

Keywords

Barramundi (*Lates calcarifer*); Grouper (*Epinephelus* spp.); Pomfret (*Xenobrama* spp., *Pampus* spp., other unknown); Megalocytivirus; Iridovirus; Red sea bream iridovirus (RSIV), Infectious spleen and kidney necrosis virus (ISKNV), Scale drop disease virus (SDDV), Singapore Grouper Iridovirus (SGIV), Turbot reddish body iridovirus (TRBIV); Import policy; Import risk assessment (IRA); Biosecurity; Virus; qPCR; Compliance.

Introduction

Background

Australia currently allows the importation of uncooked non-salmonid finfish products for human consumption from several countries (Department of Agriculture, 2019). This includes some uncooked farmed and wild caught finfish, as whole or head-on eviscerated product type, depending on the country of origin and finfish species. Key Australian native species, such as barramundi (*Lates calcarifer*) and grouper (*Epinephelus* sp.), are reportedly among the fish species imported into Australia in whole and/or head-on eviscerated product forms. A recent FRDC funded risk assessment on exotic disease introduction and spread among Australian barramundi farms highlighted the ongoing risk from importation of uncooked barramundi products into Australia (Hernandez-jover et al., 2017). This risk assessment highlighted red sea bream iridovirus (RSIV), infectious spleen and kidney necrosis virus (ISKNV) and scale drop disease virus (SDDV) as moderate risks, based on current import practices in Australia. Singapore grouper iridovirus (SGIV) and turbot reddish body iridovirus (TRBIV) have been more recently linked to international exotic disease outbreaks in barramundi and grouper (Teng et al., 2008; Tsai et al., 2020), and have not specifically been assessed in terms of risk, based on current import practices in Australia.

RSIV, ISKNV, and SGIV are significant disease threats and are targeted on Australia's list of aquatic diseases of concern, while SDDV and TRBIV may soon also be specifically added to this list, as they are considered a significant concern internationally. RSIV and ISKNV are listed by the OIE as globally significant disease threats. All five of these viruses are considered exotic to Australia, and present a significant biosecurity and disease threat, due to their highly contagious and pathogenic nature, should infection occur within an aquaculture or potentially within naïve wild fishery setting (Hick et al., 2016; OIE, 2019a). These viruses are reported to infect a wide range of species across many countries, including countries that export uncooked whole and head-on eviscerated finfish products into Australia (Hick et al., 2016; OIE, 2019a).

Seafood intended for human consumption cannot be imported for use as bait (AQIS, 1999). However, it is possible that such product, or processing waste from the product is used for this purpose. Such use, whilst discouraged, is not illegal throughout Australia. This situation was identified with imported uncooked prawn products that are implicated in the introduction of white spot syndrome virus (WSSV) (Landos, 2017), and anecdotally with finfish product. There remains a considerable potential for the use of imported uncooked barramundi and non-salmonid finfish processing waste (e.g. heads, frames, viscera) to be used as crab/lobster bait, or for fishing berley, which if infected, could pose a risk for

direct exposure of Barramundi and other susceptible fish in tropical estuaries (Hernandez-jover et al., 2017). This poses a risk to not only the wild Barramundi populations, but particularly to farmed barramundi operations which draw their culture water from such locations. Additional risks exist with household wastes and processing facility wastes, which if not treated, may feed directly into the aquatic environment through the domestic sewerage system (Hernandez-jover et al., 2017).

In 2017/18 the Australian farmed barramundi sector harvested a gross volume of 7,980 tonnes at a value of $79.8 million AUD, over the past five years the industry has grown at an average rate of 14% p.a., with a forecast value of $108 million AUD in 2020-21 and anticipated industry value of $200 million AUD by 2025 (ABFA pers. Comms. 2021). The commercial wild-catch sector harvested a gross volume of 1,132 tonnes in 2017-18 with a value of $10.9 million AUD (Steven et al., 2020). Barramundi is also an important native species for indigenous livelihoods, commercial and recreational fishing, and a key species within freshwater and coastal ecosystems across northern Australia. These sectors are all at risk of being exposed to exotic pathogens that could be inadvertently introduced via imported barramundi and other finfish products, which could pose significant economic and social consequences.

The Australian Department of Agriculture, Water, and the Environment (DAWE) does not currently consider there to be sufficient evidence to suggest that current import conditions for uncooked barramundi and non-salmonid finfish do not effectively manage the risk of pests and diseases of concern arriving in Australia. Until further evidence is generated to demonstrate that importation of uncooked barramundi and non-salmonid finfish products carry a risk that exceeds Australia's Appropriate Level of Protection (ALOP), then regulatory actions to mitigate these perceived biosecurity risks are not likely to be implemented.

Risk mitigation requirements for importation of whole farmed barramundi include that the exporting country must, among other things, declare it has in place health surveillance and monitoring and that the fish were not derived from a population slaughtered as an official disease control measure. However, this self-declaration is not supported by further post-border testing to ensure compliance. There are currently, and have not been any, protocols in place to test imported whole and head-on eviscerated barramundi and non-salmonid finfish for the exotic iridoviruses, so the efficacy of the existing import controls has not been subject to assessment at point of sale. The objective of the present study was to assess the efficacy of current import conditions and compliance of product commodities to import requirements and to determine the presence or absence of exotic viral genetic material in imported uncooked barramundi and non-salmonid fish commodities that were in wholesale and retail outlets for human consumption. Where any positive genetic material was detected then

confirmatory re-testing was performed to reduce the risk of a false positive PCR test result. Recommendations are provided in response to research findings to guide subsequent actions and research.

Clarification of taxonomy and nomenclature of iridoviruses of fish

It is recognised that there can be some confusion with terminology of iridoviruses that cause disease in fish. Family *Iridoviridae* includes viruses with a large double stranded DNA genome and enveloped virion. The family contains three genera within subfamily *Alphairidovirinae* which infect vertebrate hosts, including fish i.e. *Ranavirus*, *Megalocytivirus* and *Lymphocystivirus* (Chinchar et al., 2017). There are multiple species of virus within these genera that are relevant to fish health including pathogens that are exotic to Australia and listed by the World Organization for Animal Health (OIE, 2019b) and/or on the Australia's National List of Reportable Diseases of Aquatic Animals (Animal Health Committee, 2020). The genus *Megalocytivirus* contains 2 relevant virus species: *infectious spleen and kidney necrosis virus* (ISKNV) and *scale drop disease virus* (SDDV). There are 3 genotypes of the ISKNV species: *red sea bream iridovirus* (RSIV), *infectious spleen and kidney necrosis virus* (genotype ISKNV) and *urbot reddish body iridovirus* (TRBIV). There are many names used for isolates of iridoviruses based on host fish species from which they were derived. However the host range is broad and these isolates can be classified within the species and genotype delineations provided by the International Committee on Taxonomy of Viruses and this nomenclature ensures clarity (ICTV, 2021). The OIE lists red sea bream iridovirus disease (RSID) as a notifiable disease with a definition that includes infection with either the RSIV or ISKNV genotypes of the ISKNV species (OIE, 2019b). Australia's national list of reportable diseases lists both red sea bream iridoviral disease and ISKNV-like viruses and megalocytiviruses are additionally considered as a higher biosecurity threat to Australia's environmental biosecurity (ABARES, 2019). TRBIV is not listed by the OIE, however, this may change with recent reports of disease in *Lates calcarifer* in Taiwan, expanding the range for this genotype previously seen in flatfishes from China and South Korea and ornamental fish (Tsai et al., 2020; Koda et al., 2018). The ANZSDP for 'megalocytiviruses' considers the species ISKNV and not the other megalocytivirus species, SDDV (Crane et al., 2018). These 2 pathogens species require different diagnostic tests for detection. The real-time PCR (qPCR) used for surveillance screening can detect all 3 genotypes of ISKNV (Peter Mohr pers.com. 2020). Positive results can be distinguished for genotype identity based on partial sequence of the major capsid protein gene using conventional PCR and or nested PCR assays described by (Kurita et al., 2012; Rimmer et al., 2012).

Singapore grouper iridovirus (SGIV) is a species within the genus *Ranavirus*, which also contains virus species that infect amphibians and freshwater fish (Whittington et al., 2010). This species is the cause

of grouper iridovirus disease listed on Australia's National List of Reportable Diseases of Aquatic Animals and is exotic to Australia (Animal Health Committee, 2020).

Objectives

1. To determine the presence of infectious spleen and kidney necrosis virus (ISKNV) including the genotypes red sea bream iridovirus (RSIV), turbot reddish body iridovirus (TRBIV)and the ISKNV genotype, scale drop disease virus (SDDV), and Singapore grouper iridovirus (SGIV) in imported whole and head-on eviscerated, uncooked, barramundi and non-salmonid finfish that were destined for human consumption, collected from seafood wholesale and retail outlets throughout Australia, using qPCR.
2. Review of imported whole and head-on eviscerated commodity types in relation to current import risk assessment and peer-reviewed published literature to assemble knowledge of the status of these exotic viruses as risks to the Australian farmed barramundi and grouper industries.

Method

Retail Finfish Commodity Surveillance

Sample selection

Imported uncooked finfish commodities were sampled from seafood markets, seafood wholesalers and retailers and supermarkets across Australia based on availability of whole and head-on eviscerated barramundi and other non-salmonid finfish product being identifiable at the point of sale as imported. Sampling targeted major city locations where imported uncooked barramundi and non-salmonid finfish product have been previously identified. At each location samples were targeted in relation to the perceived level of risk consisting of (from highest to lowest pathogen risk): Whole fresh imported (not frozen) vacupak; whole frozen; head-on eviscerated and gilled frozen with scales; head-on eviscerated and gilled frozen with scales removed. Sampling did not target specific countries of origin, fish size, or production system type. The minimum number of samples of imported non-salmonid finfish to be collected was 101 based on modelling performed using Epitools (www.epitools.ausvet.com.au) to test for freedom from infection with a design prevalence of 5% for an unknown (>10,000) population size with a required population sensitivity of 99%. Given the peer reviewed screening tests used in the project have yet to be validated for their test performance (diagnostic sensitivity or specificity) we used a conservative assumed unit (test) sensitivity of 90%. For this study design, specificity was assumed to be 100%, based on confirmatory re-testing of suspect positive tissue at a second accredited laboratory (Elizabeth Macarthur Agricultural Institute (EMAI)and/or AFDL). Whole and head-on eviscerated (also referred to as GGS - gilled, gutted, scaled) barramundi and non-salmonid finfish samples were purchased directly from point of sale from each outlet. Individual samples were kept as individual products within their retail packaging. Each bag was labelled with the specimen number and details of the address of purchase, date of purchase, and purchaser were kept. Samples purchased in 10Kg boxes remained un-opened until freighted or delivered directly to the University of Sydney, Farm Animal Health Infectious Diseases Laboratory (FAH IDL). All samples were stored separately in an icebox during the days of collection. Samples were labelled with signed tape seals to ensure chain of custody, copies of purchase receipts and digital images, before being stored in -20°C freezer until freighted for testing at the FAH IDL. Sub-sampling was not performed in the field, all sub-sampling of fish occurred within the FAH IDL. At the point of retail, sample selection targeted a maximum of 10 commodities per seafood retailer, per sampling event, and 3 similar batch commodities. Decontamination of sampler occurred between locations and handling of individual commodities using basic sanitary practice (ethanol-based hand sanitiser) and new disposable gloves worn when handling each sample. Where product was purchased in large sealed

10Kg boxes a random number generator was used to select individual fish to be tested from the fish within the box. Testing performed on whole fish targeted gill, liver, and haematopoietic organs (spleen and kidney), due to the reported tropism of the target viruses for these organs, thus increasing the likelihood of detection and sensitivity of the test assay (OIE, 2016). Testing performed on head-on eviscerated fish targeted remnant tissue of the above organs, where identified. All available purchase information, sampling information, test results was entered into an excel spreadsheet.

Laboratory tests

The laboratory testing was conducted under Prohibited Matter Permit BN20/4392 (NSW Department of Primary Industries). The FAH IDL was accredited according to ISO 17025(2017) by the National Association of Testing Authorities, Australia (NATA) for multiple aquatic pathogens including the ISKNV qPCR assay described by Rimmer et al., (2012). The tests described in this report were not within the scope of accreditation. The FAH IDL operates at PC2 as certified by the OGTR and undertook the project according to the prohibited matter permit (BN20/4392). As a further measure of quality control inter-laboratory comparability panels for megalocytivirus and SDDV provided by the ACDP Fish Diseases Laboratory (AFDL; Panel identification: 20-03374 and 20-03376) were tested with satisfactory results to demonstrate proficiency for these qPCR assays prior to testing the experimental samples.

Tissue dissection and homogenisation

Fish were thawed at 4°C and inspected for the presence of the most relevant tissues for detection of the target viruses. Tissue homogenates were prepared from pooled subsamples of kidney, liver, spleen, and gill (all tissues, or any combination of any of these tissues that were available from the individual fish). Tissues were weighed and prepared as a 1/10 w/v tissue homogenate consistent with the ANZSDP for megalocytivirus infections of finfish (Crane et al., 2018).

Sub-samples of tissue pools were added to 2.0ml screw-cap tubes with 0.1 mm Zirconium/silica beads, and 3mm glass beads. The calculated 9X volume of sterile homogenising medium (phosphate buffered saline) was added to each sample tube. Homogenates were prepared by processing on the Tissuelyser bead mill for 2 x 1min cycles at 30Hz before centrifuging at 1000 x *g* for 10 min. Nucleic acids were purified from a 50 µl aliquot of the tissue homogenate supernatant using the MagMax™-96 Viral RNA Isolation Kit (Ambion, Thermofisher) with a MagMAX™ Express-96 Magnetic Particle Processor (Applied Biosystems, Thermofisher) or BioSprint® 96-Workstation (Qiagen) according to the manufacturer's instructions. Purified nucleic acids were stored at -80°C.

Real-time PCR assays for detection of viral DNA

Scale drop disease virus (SDDV).

Tests were conducted using the assay described by de Groof et al., (2015) with probe-based detection chemistry according to the modifications kindly provided by AFDL. Survey samples were tested in duplicate 25µl reactions prepared with the AgPath-ID One-step RT-PCR Reagents (Thermofisher) with 2 µl of purified nucleic acid as template. Each plate included a negative control (homogenizing medium prepared at the time of the experimental samples), no template control and 2 dilutions of positive control (SDDV de Groof modified qPCR positive control plasmid, AFDL, Batch: 1908-21-1004) that was provided at 2 concentrations for expected Ct values of 25 and 32 (i.e. close to the limit of detection of the assay). Thermocycling was conducted for 45 cycles using an Mx3000 qPCR machine (Stratagene). The fluorescence signal was normalized, and baseline corrected before assigning a threshold according to the amplification of the positive controls using a proprietary algorithm (Stratagene). The amplification curves of all samples were examined individually, and a positive result was assigned if there was a logarithmic increase and a Ct value was assigned as the fractional cycle number when fluorescence signal exceeded the threshold. The result was considered negative when a Ct value was not assigned.

Infections Spleen and Kidney Necrosis virus (ISKNV).

The assay was used according to the method described by Mohr et al., (2015), as specified by the ANZSDP. Briefly, survey samples were tested in duplicate reactions prepared in 25 µl volumes with the AgPath-ID One-step RT-PCR Reagents, 2 µl of purified nucleic acids template, 900 nM of each primer RSIV RT F (5’ TGA CCA GCG AGT TCC TTG ACT T 3’), RSIV RT R (5’ CAT AGT CTG ACC GTT GGT GAT ACC 3’) and 250 nM RSIV Probe (5’ 6FAM AAC GCC TGC ATG ATG CCT GGC TAMRA 3’). Control samples included on each qPCR plate were as previously described with the positive control (CSIRO Megalocytivirus qPCR positive control plasmid, AFDL, Batch: 1708-10-1222) used at 2 concentrations as described for SDDV. Thermocycling was conducted with an Mx3000 qPCR machine according to the program: 95°C for 10 minutes followed by 45 cycles of 95°C for 15s and 60°C for 60s. The fluorescence signal and interpretation of the results were as described for the SDDV assay.

Singapore Grouper Iridovirus (SGIV).

In the absence of a standard procedure or published test we used the assay described by Li et al., (2016). This assay uses a primer pair with a 213 bp amplification target in the major capsid protein gene with SYBR Green detection chemistry. Survey samples were tested in duplicate 25 µl reactions prepared with the QuantiTect SYBR Green PCR Kit (Qiagen). Each reaction contained 500 nM each of the forward (5’ GCACGCTTCTCTCACCTTCA 3’) and reverse primer (5’ AACGGCAACGGGAGCACTA 3’) and 2 µl of purified nucleic acid template. Negative control samples were tested on each qPCR plate as previously described. The positive control for this assay was a gBlock dsDNA gene fragment designed

with the MCP sequence of the SGIV reference sequence (GenBank NC_006549.1) reported by Song et al., (2004) and sourced from Integrated DNA Technologies. This was used at 3 x 10-fold dilutions with the final dilution close to the limit of detection of the assay (Ct value >37). Thermocycling was performed on an Mx3000P qPCR machine according to the program: 95°C for 15 min then 45 cycles at 95°C x 30s, 60°C x 30s and 72°C x 30s. The SYBR fluorescence signal was acquired during the extension phase and a dissociation curve was determined at the completion of cycling to assess the melting temperature (Tm) of any amplification products. A threshold was assigned for the baseline corrected ROX normalized SYBR fluorescence signal for the positive control samples. The amplification curves for survey samples were examined individually and a Ct value was assigned if they exceed the threshold with logarithmic increase. A sample was considered positive if a Ct value was assigned in both replicate wells and the Tm was the same as the positive control (83.85 +/- 0.5°C). A sample was negative if there was no Ct value or if the Tm of the amplicon was not consistent with the positive control. In the absence of validation data for this assay, a conservative approach to interpretation of positive results was used, that is, a sample that tested positive in duplicate reactions, the result was repeated for an additional nucleic acid sample prepared from the tissue homogenate and the average Ct value was <40 for each extract.

Turbot Reddish body Iridovirus (TRBIV) and Red Sea bream Iridovirus (RSIV).

The ISKNV assay used according to the method described by Mohr et al., (2015) is suitable for detection of the RSIV genotype (megalocytivirus ANZSDP, Crane 2018). Further, this assay was expected to detect the TRBIV genotype (Peter Mohr pers.com. 2020). AFDL were unable to provide reference material for TRBIV clade 1 or 2, so a gBlock for TRBIV Clade 2 partial MCP gene was run as an additional positive control in ISKNV qPCR. AFDL informed the project team that a recent collaborative study performed with the OIE refence expert for RSIV, Dr. Yasuhiko Kawato, clearly demonstrated that the Mohr et al., (2015) real-time PCR has a similar limit of detection for RSIV, ISKNV and TRBIV viruses (Currently in-review, Peter Mohr pers.com.). Therefore, the Mohr et al., (2015) qPCR was used in this project should already be fit for the purpose of TRBIV detection (given the evidence accumulated with plasmids in the absence of infected material).

Confirmatory testing

Any samples that tested positive or inconclusive were retested using the original nucleic acid extract and an additional nucleic acid sample prepared from the tissue homogenate with the appropriate assay. Regardless of the outcome of retesting, an aliquot of the nucleic acids and tissue homogenate for each of these samples was submitted to the NSW DPI Elizabeth Macarthur Agriculture Institute,

Virology Laboratory and forwarded to AFDL for confirmatory testing. The method for conventional and nested PCR for sequence analysis was conducted by AFDL on confirmed positive ISKNV samples according to the method described in the ANZSDP for megalocytiviruses (ISKNV). Neither of these laboratories provided a test for SGIV and a well characterized confirmatory or alternative test method was not described in the literature.

Review of import protocol and commodity types

Data was collected to better understand the type and volume of imported non-salmonid whole and eviscerated fish commodities available to the public at retail and wholesale outlets. This included country of origin, species, description, weight, length, internal organs present and packaging details. A review of current import conditions of whole and head-on eviscerated non-salmonid retail products was performed. A further review of data on the quantity of imported whole barramundi product was sought from DAWE. Alternate methods of risk mitigation were identified by considering recent published literature and project findings.

Project variations

The targeted imported fish species of the project were initially restricted to barramundi and grouper. However, following difficulties in finding imported whole and head-on eviscerated grouper commodities at retail and wholesale outlets at the time of sampling, the target species inclusion criteria was extended to also include up to two other marine non-salmonid finfish species, which have been found to be susceptible to the target exotic iridoviruses. Golden pomfret and silver pomfret were included. This variation was implemented to ensure minimum sample number was able to be met. It is likely that COVID-19 impact on global seafood commodity movement, types and volume may have altered the types of products available in Australian seafood retailers, as imported grouper product was anecdotally reported prior to the project commencement but unable to be located at the time of sample collections.

Results

Retail Finfish Commodity Surveillance

Sample location and type

The final sample set included 119 imported, uncooked, non-salmonid finfish, labelled with 3 common names: Barramundi (n=78), Golden Pomfret (n=40) and Silver Pomfret (n=1). Fish products labelled as barramundi were consistent with the appearance of *Lates calcarifer*, whereas fish commodity labelled as golden pomfret and silver pomfret samples were not able to be accurately identified to species level, as this information was not consistently available at the point of sale. Many of the golden pomfret fish product from China in boxes were labelled as *Xenobrama microlepsis*, however, the project did not have scope to perform DNA bar coding to confirm the genus and species of each product labelled as pomfret. At retail outlets, common names only were available. Therefore, common fish names, as per their primary labelling at the point of sale, were used throughout the project.

No imported 'grouper' species fish were identified or sampled during the project sampling period, despite previous anecdotal reports of this commodity type being observed for sale in retail outlets. It was possible that COVID-19 disruptions to product supply may altered the type and volume of imported finfish product available at retail. Numerous wholesale and retail venues were checked on multiple occasions for grouper unsuccessfully.

No whole fresh imported (not frozen) vacupak fish were identified or sampled and head-on eviscerated finfish products were not differentiated based on presence or absence of scales in the project.

Finfish commodities were sampled from 13 seafood wholesaler/retailers and 4 supermarkets, across four states and territories, between June and October 2020 (Table 1). All fish products were delivered intact and in person to the FAH IDL for identification, labelling and storage at -20°C. Further details on product type and sample location can be found in Table 1, and all individual commodity sample details in Appendix 1.

qPCR detection of exotic notifiable viruses

ISKNV viral DNA was detected by qPCR in seven imported fish product samples during testing at FAH IDL using the qPCR assay that targeted the MCP gene (See Table 2). The quantity of viral DNA was low, reflected by high Ct values. These seven samples were sent to AFDL for confirmatory testing. SGIV viral DNA was detected by qPCR in four imported fish product samples during testing at FAH IDL (See Table 2). The quantity of viral DNA was low, reflected by high Ct values. SDDV viral DNA was not detected in any of the 119 samples tested. Complete qPCR results for all 119 samples are presented in Appendix 1.

Table 1. Summary of the common name used for labelling, product type, sample location and reported country of origin of imported non-salmonid fish commodities sampled in the project.

		Barramundi	Golden pomfret	Silver pomfret	Total
Product type	Whole	21	37	1	59
	Head-on eviscerated	57	3	0	60
Sample location	QLD	16	6	0	22
	NSW	54	34	1	89
	ACT	5	0	0	5
	SA	3	0	0	3
Country of origin	Taiwan	54	3	0	57
	Malaysia	16	18	1	35
	Thailand	5	0	0	5
	China	0	19	0	19
	n/a (Imported)	3	0	0	3
Total		*78*	*40*	*1*	*119*

Confirmatory testing

Ten tissue homogenate (75 µL) and 10 fish tissue nucleic acid extract (20 µL) samples were referred to AFDL in Geelong, via NSW DPI, for confirmatory testing (Table 2, Appendix 1). There were 2 samples that were positive for ISKNV by qPCR at both laboratories (FAH IDL and AFDL). These were considered to have confirmed evidence of exotic ISKNV viral DNA present in the sample, thereby remaining suspect rather than confirmed positive detections. An additional 3 samples that were positive in the ISKNV qPCR at the FAH IDL were considered indeterminant when tested at AFDL and 2 positive results were not confirmed. Whereas samples that detected exotic viral DNA via qPCR at only one laboratory (FAH IDL or AFDL) were considered to be indeterminant for the presence of ISKNV DNA, most likely indicating a low concentration of exotic viral DNA was present in the sample. It was not possible to determine the sequence and genotype identity of the ISKNV in these samples as negative results were obtained using conventional nested PCR. This is consistent with the lower analytical sensitivity of the conventional PCR assay and the low concentration of ISKNV DNA in the samples. A confirmatory test for SGIV was not available at AFDL.

Table 2. Confirmatory testing results performed at AFDL, from imported non-salmonid fish commodities sampled in the project, which tested positive to target viral DNA through initial qPCR testing at FAH IDL. Gilled, gutted and scaled product type also referred to as head-on eviscerated in the project.

Sample ID	Laboratory reference	Date collected	Species (label)	Country of origin (reported)	Product type	Organs sampled	FAH IDL qPCR result		AFDL qPCR result		Interpretation
							SGIV	ISKNV	ISKNV (tissue)	ISKNV (nucleic acid)	
19	20/115-1	23/07/20	Barramundi	Taiwan	Gilled, gutted and scaled	K	N	**P**	**I**	**I**	ISKNV likely present at low concentration
23	20/116-2	23/07/20	Golden pomfret	Malaysia	Whole	K, L, G, S	**P**	N	NT	NT	SGIV possibly present at low concentration
24	20/117-1	23/07/20	Silver pomfret	Malaysia	Whole	K, L, G, S	N	**P**	**I**	**P**	ISKNV detected
34	20/120-1	8/07/20	Barramundi	Taiwan	Whole	K, L, G, S	N	**P**	**I**	N	Possible ISKNV presence at low concentration
60	20/141-5	18/08/20	Barramundi	Taiwan	Whole	K, L, G, S	**P**	N	N	N	SGIV possibly present at low concentration
62	20/141-6	18/08/20	Golden pomfret	China	Whole	K, L, G, S	**P**	N	N	N	SGIV possibly present at low concentration
63	20/142-2	18/08/20	Golden pomfret	China	Whole	K, L, G, S	**P**	N	N	N	SGIV possibly present at low concentration
87	20/198-3	21/10/20	Barramundi	Taiwan	Whole	K, L, G, S	N	**P**	I	N	ISKNV likely present at low concentration
88	20/200-1	21/10/20	Barramundi	Taiwan	Gilled, gutted and scaled	K, L	N	**P**	N	N	Possible ISKNV presence at low concentration
89	20/200-2	21/10/20	Barramundi	Taiwan	Gilled, gutted and scaled	K, L, G, S	N	**P**	N	N	Possible ISKNV presence at low concentration
90	20/200-3	21/10/20	Barramundi	Taiwan	Gilled, gutted and scaled	K, L, S	N	**P**	**P**	N	ISKNV detected

Abbreviations; K, Kidney; L, Liver; S, Spleen; NT, Not tested; I, Indeterminate; N, Negative; P, Positive; ISKNV, Infectious spleen and kidney necrosis virus; SGIV, Singapore grouper iridovirus; FAH IDL, The University of Sydney Farm Animal Health Infectious Diseases Laboratory; AFDL, ACDP Fish Diseases Laboratory.

Interpretation

Exotic ISKNV-like viral DNA was detected using the ANZSDP for "megalocytiviruses" and confirmed at a second laboratory in uncooked whole and head-on eviscerated non-salmonid finfish (barramundi and pomfret) commodities imported into Australia. The survey was designed to detect the target viruses at a prevalence greater than 5% within the targeted commodity types. Exotic SGIV viral DNA was also detected in uncooked whole and head-on eviscerated non-salmonid (barramundi and pomfret) finfish commodities imported into Australia, at a prevalence greater than 5%. The SGIV result was not able to be verified at AFDL as confirmatory testing was not available. Exotic SDDV viral DNA was not detected in uncooked whole and head-on eviscerated non-salmonid (barramundi and pomfret) finfish commodities imported into Australia, at a prevalence greater than 5%.

Review of Import Protocol and Commodity Types

Commodity Type and Product Compliance

Seafood product labelling at the point of purchase in retailers was found to be variable. Product information such as, 'Species', 'farmed or wild caught', 'for human consumption only', 'not to be used as bait or feed for aquatic animals', were not consistently displayed or available at all points of purchase. Country of origin was not displayed or available on all sampled products in retail outlets. There was substantially more product information on consumer-ready pre-packaged, and wholesale 10Kg boxed commodities, compared to fish purchased from retail displays. It is likely the 10Kg boxes of imported finfish were unpacked for display in retail. At most specialty seafood retail outlets, upon selection of a whole or head on eviscerated product to purchase, the shops would offer to fillet the product. This enquiry did not appear to be exclusive to domestic whole fish products but was also observed when purchasing imported whole fish products during sample collection. The fate of processing wastes (head, frame, remnants of viscera) from these imported non-salmonid fish was not tracked in the project.

Imported whole and head-on eviscerated barramundi and pomfret finfish commodities were found to range in weight from 480 grams to 2260 grams and length of 230mm to 545mm (See Table 3). Fish of 2260 gram weight are clearly larger than plate sized fish and are thus less likely to be cooked whole. Such large product is therefore more likely to generate a processing waste stream. Individual product weights and lengths can be found in Appendix 1.

All imported whole barramundi commodities sampled appeared to be compliant to current Australian Biosecurity Import Conditions (BICON) in relation to their country of origin, as they appeared to originate from Taiwan (See

Table 4), which is a country approved to import whole farmed barramundi into Australia (along with the Philippines) (BICON, verified 25/03/21).

All imported golden and silver pomfret commodities appeared to be compliant to current BICON in relation to their country of origin and product form, as these species were not found to be listed in the 'High Risk species (Specified species)' or the 'Risk species (Approved Specified species) list'), therefore are considered 'low-risk species (non-specific species)' (BICON, verified 25/03/21). However, we were not able to assess compliance to current BICON in relation to the requirement for low-risk species (non-specific species) to not be grown or harvested in an aquaculture system (farmed). Golden pomfret (also commonly referred to as golden pompano) are commonly farmed. Sampled products were not consistently labelled as 'wild-caught' or 'farmed', and we did not have an analytical method for differentiating farmed and wild-caught fish product.

All head-on eviscerated barramundi commodities sampled appeared to be non-compliant to current BICON in relation to their product form. Three samples did not provide country of origin (Table 4) and all products were not completely de-gilled and did not have all internal organs completely removed from inside of the body of the fish, as is a current requirement (BICON, verified 25/03/21). A considerable quantity of internal organ remnants was found remaining in many of the imported head-on eviscerated barramundi commodities (See

Table 5; Appendix 3;

Figure 1). Organ tissue from the kidney was readily found in 100% of samples, while the heart tissue and various other internal organs such as the liver, spleen, and various mixed organ remnants, occasionally including sections of intestine, were often identified in sampled products. In two instances the complete viscera were present as the barramundi apparently entered the machine upside down leading to removal of the dorsal fin and accompanying musculature (See Figure 2).

Similarly, to the golden and silver pomfret, we were not able to assess compliance to current BICON in relation to whether imported head-on eviscerated barramundi was wild-caught or farmed, as this information was often not present with the product in retail outlets, and we did not have an analytical method for differentiating farmed and wild-caught fish.

There is currently no routine compliance checking performed on whole or head-on eviscerated non-salmonid finish commodities that are imported into Australia to confirm the imported commodity meets current import conditions or aligns with the description of the product label (DAWE per comm. 16/03/21). Full details for each imported commodity sampled can be found in Appendix 1.; images displaying the type of information present at point of purchase for sampled imported products can

be found in Appendix 2.; and images of internal organ tissue present in head-on eviscerated barramundi can be found in Appendix 3.

Figure 1. Imported uncooked head-on eviscerated barramundi commodity sampled during the project showing a range of internal organs remaining in the product. Sample ID 52,78-79, SVC ID – 20/139

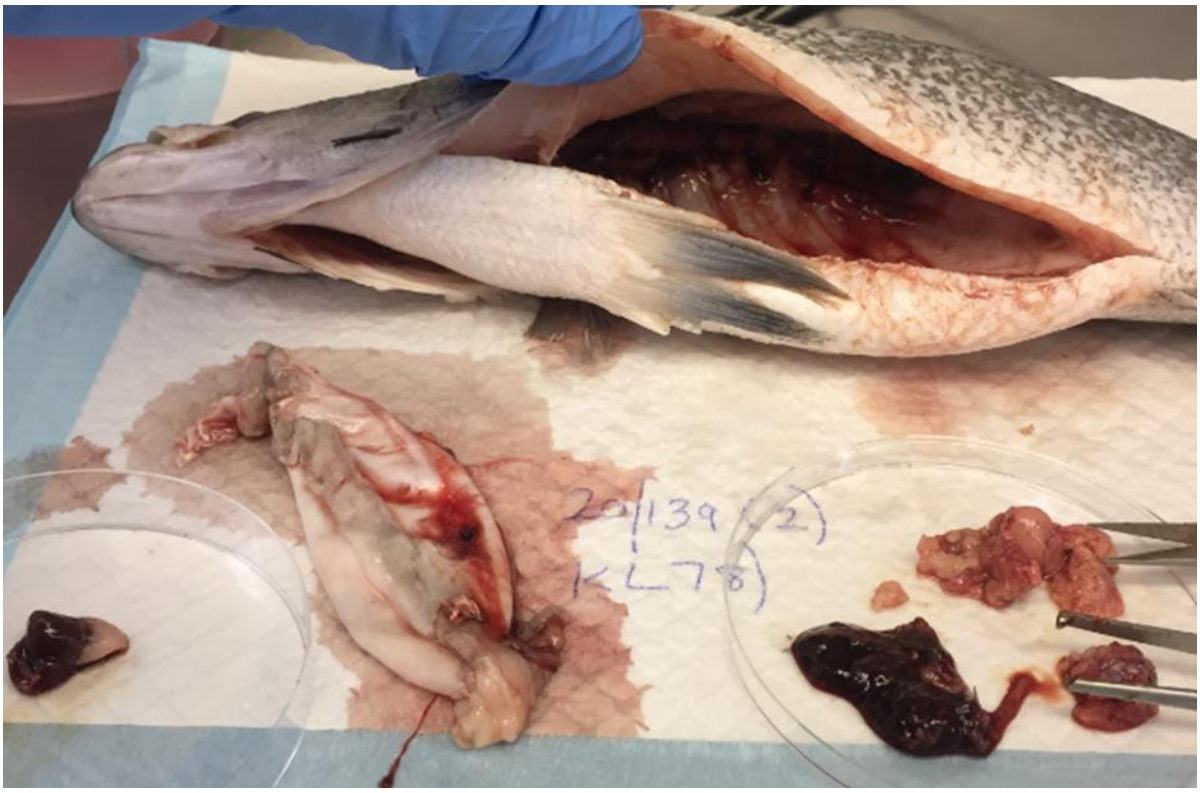


Figure 2. Imported uncooked head-on eviscerated barramundi commodity sampled during the project showing unusual gutting approach. Sample ID 64-66, SVC ID – 20/143

Table 3. Product weight and length of imported non-salmonid fish commodities sampled in the project.

	Barramundi	Golden pomfret	Silver pomfret	All species

	n	78	40	1	119
Product weight (g)	Average	758	813	1530	783
	Minimum	480	520	-	480
	Maximum	2260	1300	-	2260
Product length (mm)	Average	368	294	340	343
	Minimum	240	230	-	230
	Maximum	545	450	-	545

Table 4. Country of origin of imported non-salmonid fish commodities sampled in the project.

Country of origin (reported)	**n (All species)**	**Barramundi**		**Golden pomfret**		**Silver pomfret**	
		Whole	Head-on eviscerated	Whole	Head-on eviscerated	Whole	Head-on eviscerated
Taiwan	**57**	21	33	3	0	0	0
Malaysia	**35**	0	16	18	0	1	0
Thailand	**5**	0	5	0	0	0	0
China	**19**	0	0	16	3	0	0
Not available	**3**	0	3	0	0	0	0

Table 5. Percentage of organ identified for all or some tissues present in the imported head-on eviscerated barramundi commodities sampled in the project (n=57).

Organ	**Tissues present**	
	n	(%)
Eye	57	100
Brain	57	100
Kidney	57	100
Heart	26	46
Gill	9	16
Liver	18	32
Spleen	6	11
Mixed, unidentified (including gastrointestinal)	26	46

Discussion

Retail Finfish Commodity Surveillance

Infectious spleen and kidney necrosis virus (ISKNV)

We detected exotic ISKNV-like viral DNA present in uncooked whole and eviscerated non-salmonid finfish (barramundi and pomfret) commodities imported into Australia, at a prevalence greater than 5%. This finding demonstrated that the current biosecurity regulations are not preventing entry of exotic fish viruses into Australia.

Infection with ISKNV is listed by the OIE (as part of the definition of red sea bream iridovirus disease) because of the ability to cause disease with significant production losses at a national or multinational (zonal or regional) level. This pathogen is considered exotic to Australia and is both a nationally notifiable pathogen and identified on Australia's Environmental Pest List due to the potential for disease it can cause in barramundi and a large number of other fish species of commercial, social and cultural value (DAWE 2020). Due to the lower analytical sensitivity of the conventional and nested PCR assays for ISKNV, it was not possible to determine the sequence and genotype identity of the ISKNV-like viral DNA in these samples, nor to speculate on the potential infectivity of the virus in these samples with low viral load. We were not able to distinguish between ISKNV, RSIV and TRBIV genotypes, which would require conventional PCR and confirmation by sequence analysis, due to the low Ct value test results in the positive commodities.

The ISKNV species is a broad group within the genus *Megalocytivirus* which are emerging as pathogens in an increasing number of host fish species and global locations (Tsai et al., 2020). TRBIV is a genotype within the species that has more recently been reported to cause similar disease in barramundi in addition to flatfish and ornamental species. With further consideration it may be included alongside ISKNV and RSIV as an aetiological agent of OIE listed red sea bream iridoviral disease (OIE, 2019a). Regardless, TRBIV is listed as an exotic and reportable pathogen in Australia as a causative agent of disease from infection with ISKNV-like viruses (DAWE, 2020).

Infection with ISKNV and RSIV are well known cause clinical disease in barramundi with high mortality (>80%) following natural and experimental infections (Zhu et al., 2021; Ni et al., 2021). The prevalence of ISKNV-like virus is reportedly increasingly in farmed barramundi and pomfret populations in China and Southeast Asian countries (Zhu et al., 2021; Ni et al., 2021; OIE, 2019a). The prevalence and global economic impact associated with ISKNV-like viruses on aquaculture fish species is so severe that considerable effort by multiple research institutes is being diverted to attempt to understand immune

responses (Wu et al., 2021) and cellular mechanisms (Guo et al., 2012) associated with infection and to develop preventative measures such as vaccine production (Dong et al., 2013; Kawato et al., 2016). It appears that cross protection is incomplete for the 3 different genotypes within ISKNV indicating that vaccines need to be developed and used for each of ISKNV, RSIV and TRBIV genotypes, where relevant.

This small snapshot study generated evidence to demonstrate that exotic megalocytiviruses are entering Australia via imported uncooked barramundi and golden pomfret. This previously considered hypothetical entry risk pathway, can now be re-considered as a demonstrated risk pathway. Whilst the levels of virus found in this study were low, the propensity for emergency harvested fish, with higher viral load to end up in international seafood supply chains is well recognised. The 2016 white spot syndrome virus (WSSV) outbreak investigation in black tiger prawn (*Penaeus monodon*) farms in South-eastern Queensland demonstrated that the detection rates of imported uncooked prawns do not stay the same over time, in seafood supply chains from international locations to Australia. As the Auditor General pointed out the rates of non-compliance are also not static. The apparent biosecurity risk associated with OIE listed, locally reportable, exotic, ISKNV-like virus, in imported uncooked non salmonid finfish commodities warrants reconsideration of the Import Risk Analysis of Non-Salmonid fish and immediate implementation of enhanced border security measures to protect the Australian farmed barramundi industry.

It should be recognised that there is little value in attempting to implement such measures after the virus escapes into wild fish populations in Australia. Again, the 2016 South-eastern Queensland WSSV outbreak demonstrates that when exotic aquatic animal viruses enter Australia, the chances of achieving eradication are remote. Prevention from entry is essential to underpinning the considerable historical, current, and future investment in aquaculture development in Australia.

Scale drop disease virus (SDDV)

Scale drop disease virus was characterised recently as an emerging pathogen and new species within the genus *Megalocytvirus* that causes severe disease in barramundi in Singapore, Malaysia, Indonesia, and Thailand (Senapin et al., 2019; Nurliyana et al., 2020; Kerddee et al., 2020; de Groof et al., 2015; Gibson-Kueh et al., 2012). The present survey did not detect exotic SDDV viral DNA present in uncooked whole and eviscerated non-salmonid finfish commodities imported into Australia. This survey was powered to detect a prevalence greater than 5% in the target commodities with 99% confidence. Disease caused by SDDV is not yet internationally listed by the OIE as globally significant aquatic animal disease. However, SDDV is listed as exotic and reportable in Australia as a causative agent of disease from infection with ISKNV-like viruses (DAWE, 2020).

This small snapshot study did not identify an apparent biosecurity risk associated with exotic SDDV virus in tested imported uncooked non-salmonid finfish commodities. However, this may only provide minimal and transient confidence in the absence of SDDV in imported uncooked whole and eviscerated non-salmonid finfish commodities imported into Australia, due to the small sample size and brief period of sampling in this project, relative to the volume of commodity imported. The study has identified a biosecurity risk in an entry pathway and plausible release pathway based on the presence of visceral tissues in imported uncooked barramundi products. The prevalence and virus load in barramundi are likely to have brief, high peaks at times when acute disease outbreaks occur in regions of export. Therefore, a point in time survey is not ideal for quantifying the risk associated with this pathogen.

Singapore grouper iridovirus (SGIV)

Singapore grouper iridovirus belongs to the genus *Ranavirus* and is the cause of a severe disease of grouper in China and several Southeast Asian countries (Qin et al., 2003; Wei et al., 2019). We detected possible exotic SGIV viral DNA present in uncooked whole and eviscerated non-salmonid finfish commodities imported into Australia, at a prevalence greater than 5%. This result was not able to be confirmed until confirmatory testing becomes available. Extensive validation of the diagnostic test is required to determine an appropriate cut-off for defining a positive result. In the absence of a test with validation data, these samples were interpreted as test positive for SGIV based on the arbitrary definition: amplicons with the same melting temperature as the positive control were generated with a Ct value <40 from two different nucleic acid purifications from the tissues. The positive predictive value of the laboratory test results could not be determined without an estimate of the diagnostic sensitivity and specificity of the assay. Therefore, it was concluded possible that small amounts of SGIV viral DNA were present in these fish samples.

Disease caused by SGIV is not yet internationally listed by the OIE as globally significant aquatic animal disease. However, SGIV is listed as exotic and reportable in Australia as a causative agent of grouper iridoviral disease (DAWE, 2020). Infection with SGIV has been associated with disease causing high mortality in grouper (*Epinephelus* spp.) from 1998 (Qin et al., 2003; (Song et al., 2004). We could not find any reported cases of SGIV associated with disease in barramundi or pomfret populations. However, Qin et al., (2003) found SGIV can grow can cause rapid cytopathic effects and produce high viral titres in the cell lines of barramundi, therefore it may be possible.

This small snapshot study identified an apparent biosecurity risk associated with a locally notifiable and exotic virus, SGIV, in imported uncooked non-salmonid finfish commodities.

Import species

Megalocytiviruses have been shown to infect more than 50 fish species (Girisha et al., 2021), including barramundi (*Lates calcarifer*) (Crane et al., 2018). We detected exotic viral DNA (ISKNV-like or SGIV) in all tested species (barramundi, silver pomfret, golden pomfret) of imported uncooked finfish tested in the project, indicating a prevalence greater than 5% in the targeted commodities. It is therefore considered that importation of uncooked barramundi, silver pomfret, and golden pomfret finfish products pose a biosecurity risk, in absence of efficacious risk mitigation measures.

Import commodity type

Imported whole and head-on eviscerated uncooked or frozen fish are considered to carry a substantially higher pathogen load and represent an increased biosecurity risk (Oidtmann et al., 2017). This observation was reflected in this project where these commodity types (whole and head-on eviscerated) were found to have exotic viral DNA (ISKNV-like and SGIV), at a prevalence greater than 5%. The tissue tropism of these iridoviruses means that much greater pathogen quantities of virus are likely to be present in visceral tissues, within an infected animal. The commodity type therefore impacts the biosecurity risk two-fold as this tissue also generates a plausible release pathway through a waste stream with crab/lobster pots and burley. It is therefore considered that importation of whole and head-on eviscerated finfish products pose a biosecurity risk, in absence of efficacious risk mitigation measures.

Country of origin

We detected exotic ISKNV-like viral DNA in uncooked finfish products imported from Malaysia and Taiwan, and exotic SGIV viral DNA in uncooked finfish products imported from Malaysia, Taiwan, and China. We did not detect any exotic viral DNA in samples from Thailand, however, only 5 samples were collected from Thailand, so this result may not reflect the true scenario. It should be noted there is no certainty that samples actually came from Thailand, as products were labelled at point of retail where the least stringency to labelling requirements was observed.

The Network of Aquaculture Centres in Asia-Pacific (NACA) regularly reports on significant aquatic disease emergence and surveillance in member countries. These reports include many countries from which imported uncooked finfish were sampled in the project (Malaysia, Taiwan, and China). Taiwan does not appear to report aquatic animal disease emergence and surveillance in the NACA Quarterly Aquatic Animal Disease reports. Unfortunately, the project was not designed to assess prevalence of exotic viruses from each country of origin, but pooled ‘imported’ product. NACA and peer-reviewed

literature have reported SGIV and ISKNV-like viruses to occur in China, Malaysia and Taiwan (NACA, 2021; Tsai et al., 2020).

In Taiwan, Iridoviral disease outbreaks caused by megalocitivirus infections in cultured fish have occurred numerous times since 2005 (Tsai et al., 2020). Megalocitivirus isolates from Taiwan have been further identified to a range of different genetic isolates. In a study by Wang et al., (2009) PCR amplification and sequence analysis of the major capsid protein gene found isolated collected between 2005 and 2008 were most similar to RSIV sharing 97% identity. According to the GenBank database and the phylogenetic tree from Dong et al., (2017) (H. T. Dong et al., 2017) there are phylogenetic differences between isolates of RSIV group megalocitivirus detected from diseased barramundi (*Lates calcarifer*) in Taiwan (Wen et al., 2016). Australia BICON currently does not consider the importation of whole farmed barramundi from Taiwan as an appreciable biosecurity risk for RSIV and ISKNV-like viruses, whereby imported commodities would be required to be eviscerated and/or from wild capture origins and/or further processed (e.g., filleted). This project and scientific literature would suggest this trade may carry an appreciable biosecurity risk for RSIV and ISKNV-like viruses.

Megalocytiviruses have been detected in a wide range of countries (Girisha et al., 2021), with distribution and impact appearing to increase, globally (Zhu et al., 2021; Thanasaksiri et al., 2021; Lopez-Porras et al., 2018). Given the expanding global distribution of the exotic viruses which were the targets of this project, little confidence can be gained that imported uncooked finfish commodities originating from countries where the viruses has been previously detected, will be free of the virus. The absence of border testing and other compliance measures, to demonstrate freedom from these viruses further reduces the confidence that a robust biosecurity system is in place.

Virus viability

The project did not include any cell culture, *in vitro* or transmissibility trials to determine whether the exotic viral DNA that was detected was associated with infectious virus capable of transmitting to susceptible host species. This type of research should be explored in a subsequent project, but it was considered that the high Ct value of tested samples may reflect low viral load and limit the ability to perform this research within this project.

It is considered plausible that megalocytiviruses might remain infectious in seafood products given iridoviruses are reasonably stable to freezing (Plumb et al., 2011). Nakajima et al., (1994) found that RSIV infectivity was not significantly affected by two cycles of freezing (-80°C) and thawing (20°C) or by ultra-sonification at 45kHz for 1 minute. Similarly, Yuan et al., (2016) found that SGIV was not negatively affected by three cycles of freezing/thawing. ISKNV was relatively resistant to heat and

changes in pH and remained infectious in aquarium water for >2 days without fish being present (C. Fusianto et al., 2019).

The OIE report that RSIV is stable in tissue at –80°C for up to a few years (OIE, 2019a). Therefore, it seems plausible that exotic viral DNA detected in uncooked frozen fish tissue imported into Australia could reflect viable and infectious virus. The New Zealand Ministry of Agriculture and Forestry also reached a similar conclusion in relation to the likelihood of RSIV to be viable in frozen imported fish tissue imported into New Zealand (MAF Biosecurity New Zealand, 2008). Matsuura et al., (2021) used frozen RSIV inoculum isolated from kidney and spleen tissue of Pacific bluefin tuna (PBT, *Thunnus orientalis*) with RSIVD to validate the oral transmission infection pathway in PBT and Greater amberjack (*Seriola dumerili*).

Diagnostic limitations

None of the qPCR assays have comprehensive validation data available so the diagnostic characteristics such as sensitivity and specificity were not known, preventing the calculation of predictive values for the test results. Considering the performance limitations of diagnostic tests available this project was designed to detect the presence of target exotic virus DNA, rather than to determine the possible prevalence of each target exotic virus in imported commodity or determine than abundance of virus in each tested commodity. The use of repeated qPCR testing of the same sample was performed to increase diagnostic specificity and reduce the likelihood of false positive test results, while it was acknowledging that imperfect test sensitivity may result in some false negative test results.

Review of Import Protocol and Commodity Types

Commodity type and product compliance

We detected a substantial quantity and diversity of internal organ tissues and gills remaining in uncooked imported head-on eviscerated barramundi products (See Table 5, and Appendix 3). This finding did not align with current Australian Biosecurity Import Conditions (BICON) for importation of these products, where "t*he product must be at least de-gilled and eviscerated (gutted) prior to importation and must be accompanied by a valid import permit." (See Appendix 4).* Target exotic viruses, such as ISKNV, are known to be abundant across many organ tissues in infected fish, such as the spleen, kidney, liver, stomach, and gills (Zhu et al., 2021; Ni et al., 2021). RSIV has been detected in most fish tissues including gills, liver, kidney, spleen, heart, stomach, intestine, muscle, eye, brain, leucocytes (Kyung Choi et al., 2006) other exotic iridoviruses such as SDDV have also been shown to have a wide organ distribution (liver, kidney, spleen, gill, brain, eye, muscle, mucous, fin, blood (Charoenwai et al., 2020). The organ tissues identified in sampled uncooked imported finfish commodities in this project may reflect an elevated biosecurity risk. This potential risk is recognised

within the import conditions, which require these organs to be removed prior to importation as a risk mitigation measure. However, given there appears to be very poor compliance to these conditions in observed products and negligible compliance action by Australia's border control, the biosecurity mitigation measure of evisceration and de-gilling of imported farmed barramundi appears ineffective. Given this lack of risk mitigation, the trade may then exceed Australia's ALOP.

Routine testing for exotic viral pathogens at the border

We did not find any evidence that routine testing for exotic viral pathogens in imported uncooked whole and eviscerated non-salmonid finfish being performed in Australia. It was confirmed by DAWE (pers. Comm.) that this activity is not performed on imported whole and head-on eviscerated non-salmonid finfish products. Therefore, it is unknown what the true prevalence of exotic viral DNA is within the trade product, and whether the risk profile to Australia barramundi farms has shifted over time, relative to prevalence, viral load or volume of this commodity entering Australia. However, it is clear that the risk profile will increase when related to the growing value of the Australian barramundi farming industry.

Potential impact from an exotic viral disease incursion

Disease outbreaks associated with megalocytiviruses are associated by high mortality in numerous species of fish that are important commercially, culturally and environmentally in Australia (Fusianto et al., 2021; Ramírez-Paredes et al., 2020; Girisha et al., 2020). It is reasonable to consider the impact of incursion of these viruses into Australian barramundi farms could be exceedingly high. Australian barramundi farms are presently free from these viruses, hence fish are likely to have naïve immune systems and low levels of protection.

The value of the Australian aquaculture industry is rapidly increasing and projected to grow further. In 1999, when the 'Import Risk Analysis on Non-viable Salmonids and Non-salmonid Marine Finfish' was performed, the Australian barramundi aquaculture sector contributed an estimated annual gross production volume of less than 500 tonnes at a gross value less than $5 million (Department of Primary Industries and Fisheries, 2008). Currently, the Australia barramundi aquaculture sector contributes an estimated annual gross production volume of 9,044 tonnes at a gross value approximating $100 million AUD (ABFA, pers. Comm. 2021), alongside approximately 150 FTEs of labour (Cobcroft et al., 2020). It is has been recently predicted that Australia barramundi aquaculture sector volume and value contribution may increase by up to 5 times by 2030 (Cobcroft et al., 2020) with recent investment announcements such as, http://www.mainstreamaquaculture.com/2021/05/31/mainstream-aquaculture-announces-the-aquisition-of-port-douglas-aquaculture-site/ and

https://naif.gov.au/media-releases/naif-announces-stage-2-of-humpty-doo-barramundi-funding/ demonstrating further significant production expansion is imminent.

In comparison to the non-native introduced terrestrial production animal species (such as cattle, pigs and chickens), many of Australia's non-salmonid finfish species are native to Australia and contribute significant value to tourism, wild capture, cultural and social identity. Deloitte Access Economics (2013) estimated the total economic contribution of recreational activity and commercial fishing in the World Heritage Great Barrier Reef at $5.68 billion. The total value revenue from all commercial fishing (including aquaculture) in the World Heritage Great Barrier Reef area in 2010-11 was estimated to be around $193 million and generated employment equivalent to 975 full-time jobs (Deloitte Access Economics, 2013). Total recreational expenditure (fishing, boating, sailing, and visiting an island) was estimated to be $332.4 million, with fishing generating the most economic activity, largely due to its popularity, with over 3.4 million fishing trips estimated to have taken place in 2012 (Deloitte Access Economics, 2013)

It is the combined value of all potentially impacted industries that should be considered when setting Australia's Acceptable Level of Protection (ALOP) for biosecurity risk mitigation for aquatic exotic viruses. Significant learning opportunities to improve Australia's biosecurity against incursion of aquatic pathogens are available, after the failures associated with the WSSV have been now reviewed in detail by parliamentary enquiries, through the Inspector General of Biosecurity and most recently in the Auditor General's report on Biosecurity non-compliance.

End-use of imported uncooked fish products

Further processing (de-heading and filleting) of imported non-salmonid finfish was an observed behaviour during sample collection. The generation of uncooked processing waste is a certainty, rather than a hypothetical possibility. It was not clear how seafood retailers were disposing of the waste by-product (e.g., head, frame, internal organs) from further processing of these products. Some retailers had fish heads and frames for sale separately, however their provenance was not labelled. We have anecdotal reports of these by-products being sold and/or used by recreational and commercial fishers in crab pots, lobster pots and as berley. The reports were not able to be verified, however, given alternative disposal methods such as municipal waste are often associated with a cost, it appears a plausible scenario. It is not possible to guarantee that further gutting, cleaning, filleting, freezing, thawing, cooking, unpacking, packing, or repackaging does not occur on imported whole and head-on eviscerated fish commodity within Australia. Most fish consumption in Australia is of highly processed products (e.g. fillets), with organ and offal meats having a low frequency of consumption on a population level in Australia (Sui et al., 2016). Therefore, it appears certain that importation of uncooked whole and eviscerated fish products could generate a processing waste stream in Australia,

which is largely unregulated in terms of disposal. Presently there appears no measures enacted and enforced to control and prevent this waste stream entering the environment and/or being used for other purposes such as bait and berley. There appears to be negligible social benefit, cultural or economic benefit (with ample other safer replacement options) gained from the importation of barramundi (*Lates calcarifer*) head, gills, frames, and viscera, while presenting a significant biosecurity risk.

Seafood Labelling.

There were inconsistent and insufficient labelling present in retailers alongside imported finfish commodities to ensure consumers were aware that imported fish and waste by-products were not permitted to enter natural waterways (See Table 2). Information such as fish species, farmed or wild caught origin, and country of origin were often not readily available for consumers. It was often difficult to determine the actual genus and species of many finfish products available at point of sale. This could provide an avenue for product substitution within a species category where the identification of the imported finfish commodity is not verified.

Minimum viral dose to produce disease

Disease outbreaks from ISKNV have been capable of producing very high viral load (up to 3.0 x 10^8 copies of the genome per mg tissue) (Ramírez-Paredes et al. 2020;Putra et al., 2020), and very high prevalence in infected populations (Fusianto et al., 2021). These features of disease may facilitate spread.

We could not find any information on the minimum concentration of the project target exotic viruses to cause disease. However, the notion that there is a minimum infective dose which can be enumerated from laboratory trials and applied to field circumstance is flawed. Fusianto et al., (2019) found that ISKNV inoculum diluted below the limit of quantification of the qPCR assay was still infectious to Murray cod (*Maccullochella peelii*) following intraperitoneal inoculation, producing similar Ct values to the current project findings. Such a notion also fails to consider the roles of host immunity and environmental contributions to infection and disease expression and transmission. Stress was found to trigger ISKNV mortality outbreaks in a study by Ramírez-Paredes et al., (2020), with the author suggesting ISKNV was widespread and persistence in the environment or a capable of a latent infection. The influence of stress on viral disease manifestation is well known in fish (Tort, 2011; Mauri et al., 2011). He et al., (2002) found that experimental infection of mandarin fish (*Siniperca chuatsi*) with ISKNV produced clinical disease only at elevated water temperatures and at lower water temperatures clinical disease and mortality did not occur. This mortality pattern reportedly aligned with natural outbreaks of ISKNV in mandarin fish. He et al., (2002) also demonstrated horizontal ISKNV

transmission and clinical disease via cohabitation, bath inoculation and oral inoculation. The limitations of such studies also need to be appreciated. The absolute water temperature at which the disease may express, is very likely to vary with other associated stressors such as myriad types of pollutants and fish husbandry.

Identification of sub-clinical Infections

The reliance on the visual appearance of disease in finfish products being exported to Australia maintains a risk of disease incursion, where pathogens do not reliably produce clinical disease or visual changes in the host fish that could be detected prior to harvest or at border inspection. The lack of consistent pathognomonic changes to the external appearance of a diseased to fish is expected to carry a low diagnostic sensitivity (or high false negative rate), when this method of disease detection, is used alone.

Megalocytiviruses are known to exhibit prolonged sub-clinical infections with disease manifesting under conditions involving various combinations of host and environmental risk factors (Putra et al., 2020). Putra et al., (2020) found no significant association between gross abnormalities and infection with ISKNV. A similar finding was concluded by Fusianto et al., (2021), with no differences in the prevalence and quantity of megalocytivus in tissues from fish with or without clinical signs of disease; and Girisha et al., (2021) who found multiple species of fish positive for ISNKV despite appearing apparently health during sampling. Despite a lack of visually obvious disease Putra et al., (2020) found ISKNV in 5 of 7 farms at an apparent prevalence, as detected by qPCR, in juvenile grouper (*Epinephelus* spp.) in Indonesian nurseries between 0-13%, with viral quantity considered moderate to high (up to 3.0×10^8 copies of the genome per mg tissue). This suggests that detection of disease through visual inspection of finfish products, before or after importation, will not provide a reliable method to identify exotic megalocytiviruses, even where the disease prevalence and viral load are high.

De-contamination of high-risk by-products

We did not find any evidence that waste by-products (*e.g.,* head, frame, internal organs) from further processing of imported non-salmonid finfish, which were not intended to be consumed, were decontaminated by retailers to minimise risk of these by-products entering natural waterways. Imported fish were commonly presented side-by-side with other fish on ice in speciality retailer outlets. No functional biosecurity measures were observed to be in place to prevent surface cross-contamination between fish in such settings. Fusianto et al., (2019) assessed the stability of ISKNV and susceptibility to physical and chemical disinfectants. A range of methods to provide efficacious disinfection of ISKNV including heating (65°C for 20 minutes), sodium hypochlorite (1000 mg/L for 30 minutes), pH (≤3 or 11≥ for 30 minutes), Virkon™ (1% or 30 minutes), benzalkonium chloride (650 mg/L

for 10 minutes). Implementation of these methods could achieve a meaningful risk reduction for virus entry into natural waterways from contaminated surfaces and/or waste by-products, should high risk product continue to be imported.

Differentiating farmed vs wild-caught commodities.

The current Australian Biosecurity Import Conditions (BICON) for importation of uncooked whole and head-on eviscerated non-salmonid finfish requires importers to confirm the product is wild caught and not grown or harvested in an aquaculture system at any stage, except for barramundi from Taiwan and the Philippines (See Appendix 4). The project did not have scope to forensically determine the rearing origin of imported finfish commodities or differentiate between product sourced from wild capture fisheries and aquaculture. Only a small proportion (not calculated) of commodity types that were sampled had information at point of sale on packaging that detailed whether the product was farmed or wild caught.

Emerging global disease threats

The disease status of fish from many regions of the world is poorly defined and in many cases is unknown. The frequency and severity of mass mortality events in fish populations known to be changing and increasing, globally, over time (Fey et al., 2015). There are other emerging viruses of barramundi that are yet to be fully classified but could pose a biosecurity threat and be relevant to Australia's biosecurity that were not included in the previous import risk assessment (IRA) (AQIS, 1999), for example *Lates calcarifer* Birnavirus (LcBV) (Chen et al., 2019) and *Lates calcarifer* Herpesvirus (Meemetta et al., 2020).

The 2016 south east Queensland WSSV outbreak, which decimated the Australian prawn industry in Moreton Bay and Logan River areas, illustrates how importation of uncooked seafood products pose significant risks to Australian aquaculture (Landos, 2017). A range of factors led to a substantial quantity of high risk WSSV positiive commodity entering Australia, some of which entered waterways adjacent to commercial prawn farms prior to and during the disease outbreak (Landos, 2017). Subsequent testing of imported commodities revealed ~90% of imported product was qPCR positive for the WSSV, thereby demonstrating the extent of the risk this trade posed to farms and wild capture fisheries (Landos, 2017). It is hypothesised a similar disease transmission pathway could occur in relation to exotic megalocytiviruses or other new and emerging finfish viral diseases, where significant volumes of uncooked whole and eviscerated non-salmonid finfish products are imported into Australia each year thereby generating significant uncontrolled processing waste streams that may release the virus into Australian waters.

This project has generated evidence that adds to the global body of literature supporting the claim that emerging aquatic animal diseases are being distributed regionally and globally through trade in fish and aquaculture products. Diseases caused by megalocitiviruses have not been found in barramundi (*Lates calcarifer*) in Australia with considerable passive surveillance material passing through diagnostic laboratories annually for over more than 20 years. Girisha et al. (2021) suggests that improvements are needed to the existing monitoring and surveillance programmes for transboundary diseases, such as ISKNV, and to strengthen the quarantine measures to block exotic pathogens entering India, which may have a devastating effect on the native aquatic biodiversity. The same advice could be applied to Australia in light of the further evidence generated in this project.

Conclusion

The Australian barramundi farming industry has been fortunate to have not yet encountered a significant exotic disease incursion, such as the incursion of WSSV which adversely impacted the Australian prawn farming industry first in 2016 and again in 2020. For the barramundi industry to reach its full growth potential, it is essential that optimal health of farmed stock is maintained, and significant disease impacts are minimised to avoid the destruction of considerable Government and private sector investment capital. The most effective mechanism used globally in animal production systems to reduce the risk of external disease incursion is through the maintenance of a high level of biosecurity. This must occur across all levels, from the country border to the individual tank or pond. It is of utmost importance for the Australian barramundi farming industry, wild catch industry and native barramundi populations in Australia that a high level of protection from exotic disease risks is provided.

This project detected exotic ISKNV-like and SGIV viral DNA present in uncooked whole and eviscerated non-salmonid finfish commodities imported into Australia, indicating a prevalence greater than 5%. This outcome reflects a potential biosecurity risk to Australian barramundi farming industry, wild catch industry and native barramundi populations. This project provides supportive evidence that an exotic virus entry, release and establishment pathway exists due to presence of exotic virus in imported uncooked non-salmonid seafood commodities and non-compliance of visceral cleaning of whole and head-on eviscerated non-salmonid finfish. The outcomes of virus entry into naïve wild fish populations can be severe as was observed with the pilchard herpesvirus outbreaks across southern Australia in 1995 and 1998 where an estimate of 70% of the pilchard biomass was lost. It was not possible to determine the sequence and genotype identity of the ISKNV-like viral DNA in the positive samples, nor to speculate on the potential infectivity of the virus, due to the high Ct value test (low viral load) results. However, each of the 3 genotypes represent a serious disease threat to a broad number of fish species and would require different disease prevention measures in the event of an incursion. All uncooked head-on eviscerated barramundi finfish commodities sampled in the project were non-compliant to import conditions due to the remnants of high-risk internal organs. It is considered that there is potential for substantial waste by-product (e.g., head, frame, internal organs) from further processing of imported non-salmonid fin-fish commodities. Should this product enter natural waterways (e.g., for crab/lobster bait or berley) there is a plausible risk for seeding exotic virus into exposed naïve fish populations with many potentially susceptible species enabling propagation of infection and disease. The entry of susceptible species such as grouper species into crab pots to eat baits is common. Should disease transmission occur the impacts could be catastrophic impact.

We propose the DAWE review of import risk analysis of non-salmonid (specifically barramundi) commodities to consider the full suite of rapidly emerging global disease threats and appropriately adjust Australia's ALOP to align with current scientific literature and the current and future significance of the Australian barramundi farming industry. This process offers an opportunity to protect Australia's aquaculture, recreation and commercial fishing sectors whilst continuing to support safe trade. The review should be informed by the growing body of scientific literature and recent reviews of Australia's biosecurity performance.

Implications

This project detected exotic ISKNV-like and SGIV viral DNA present in uncooked whole and head-on eviscerated non-salmonid finfish commodities imported into Australia and sampled from seafood wholesalers and retailers, at a prevalence greater than 5%. This data provided proof that the previously hypothetical risk was present within imported non-salmonid finfish (barramundi and pomfret). This outcome has highlighted a potential biosecurity risk to Australian barramundi farming industry, wild catch industry and native barramundi populations in addition to other susceptible species. The project has demonstrated that the present biosecurity measures do not prevent entry of megalocytiviruses into Australia. The project results and review provide an assembly of information suitable to inform a review of the Import Risk Analysis of Non-salmonid commodities.

Incursion of aquatic animal exotic viruses could have catastrophic impacts to the Australian aquaculture industry, and native fish populations that contribute to significant food production and tourism sectors in Australia. The project has identified non-compliance of imported uncooked head-on eviscerated barramundi commodity to meet BICON import conditions, and highlighted limitations in the import conditions to safeguard Australia's aquaculture industry from exotic disease incursion. The entry, release and establishment pathways are all plausible for a high impact exotic pathogen. These data provide opportunities to improve Australia's biosecurity operations.

Recommendations

It is recommended that:

1. Project data be considered by DAWE to be sufficient to trigger a review of import risk analysis of non-salmonid (specifically barramundi) commodities to consider the full suite of rapidly emerging global disease threats and appropriately adjust Australia's ALOP to align with current scientific literature and to promote safe trade.
2. DAWE to review compliance of imported non-salmonid commodities to BICON requirements.
3. Barramundi farms review their on-farm biosecurity measures considering the disease risks highlighted by this project.
4. Develop educational materials for commercial crab/lobster and recreational fishing industries, as well as seafood retailers and wholesalers, to ensure that no processing waste from imported non-salmonid fish (destined for human consumption) is used as, or mixed with, bait.
5. DAWE to consider revising BICON to only allow importation of barramundi (and other aquaculture derived) product types, which are in a consumer ready form and unlikely to result in the generation of any processing waste (e.g. fillets-only). This mitigation measure would assist in addressing the increased biosecurity risk associated with imported barramundi and other non-salmonid finfish products, and the increased scale of consequence (due to the growth of the barramundi farming industry), should the exotic pathogens elude the current biosecurity border controls.

Further development

It was not possible to determine the sequence and genotype identity of the ISKNV-like viral DNA detected in these qPCR positive samples, nor to speculate on the potential infectivity of the virus in these collected samples due to the high Ct value (low viral load) test results. Should further imported uncooked whole and head-on eviscerated finfish commodity with an apparently higher viral load become available further diagnostic testing and research would be possible and allow for further data to be generated. There are other emerging diseases in aquaculture that present a risk to Australia. Diagnostic capacity and monitoring the biosecurity risk pathways should include horizon scanning for these threats. For example, barramundi herpesvirus and barramundi aquabirnavirus.

Extension and Adoption

This project was developed in collaboration with ABFA, DAWE and NSW chief Veterinary Officer. The findings have been communicated to assist maintenance of Australia's aquatic biosecurity policy and ALOP. The full report findings and project methodology will hopefully be of further benefit to DAWE in relation to assessment of overseas surveillance of aquatic diseases and/or local testing of exotic viruses as part of a verification or disease surveillance programs. Diagnostic testing was performed under NSW DPI prohibited matter permit for infectious spleen and kidney necrosis virus-like viruses (ISKNV) and grouper iridoviral disease (such as Singapore grouper iridovirus) (BN20/4392), with confirmatory testing performed through NSW DPI andAFDL and findings communicated with the NSW CVO prior to release. Diagnostic testing methodology was aligned with protocols optimised at AFDL and inter-laboratory compatibility testing performed between FAH IDL and AFDL to ensure consistent and accurate results were obtained and reported.

The project findings, once approved for release, will be disseminated through the Australian Barramundi Farmers Association (ABFA), and the extension work of Future Fisheries veterinary Service Pty Ltd (FFVS), to encourage farms and businesses to adjust their farm biosecurity practices in response to the apparent altered biosecurity risk in relation to exotic viruses.

The project findings can be used to guide further research and to guide communication to the public about preventing waste from fish destined for human consumption from entering natural waterways.

Appendix 1.

Table 6. Product Key list from all samples

Sample ID	SVC No. Sample ID	Product location	Date collected	Species	Country of origin	Product weight (g)	Product length (mm)	Product type	Organs sampled	Other organs present	FAH IDL qPCR SGIV	FAH IDL qPCR SDDV	FAH IDL qPCR ISKNV	AFDL qPCR ISKNV (tissue)	AFDL qPCR ISKNV (nucleic acid)
1	20/110-1	St Peters, NSW, 2044	23/07/20	GP	China	780	280	WHL	K, L, G, S	All	N	N	N	-	-
2	20/110-2	St Peters, NSW, 2044	23/07/20	GP	China	720	280	WHL	K, L, G, S	All	N	N	N	-	-
3	20/110-3	St Peters, NSW, 2044	23/07/20	GP	China	760	265	WHL	K, L, G, S	All	N	N	N	-	-
4	20/110-1	St Peters, NSW, 2044	23/07/20	Barra	Taiwan	560	380	GGS	K	E, B, H	N	N	N	-	-
5	20/110-2	St Peters, NSW, 2044	23/07/20	Barra	Taiwan	690	400	GGS	K, L	E, B, H	N	N	N	-	-
6	20/110-3	St Peters, NSW, 2044	23/07/20	Barra	Taiwan	530	380	GGS	K, L	E, B, H	N	N	N	-	-
7	20/111-1	Marrickville, NSW, 2204	23/07/20	GP	Malaysia	740	265	WHL	K, L, G, S	All	N	N	N	-	-
8	20/111-2	Marrickville, NSW, 2204	23/07/20	GP	Malaysia	640	270	WHL	K, L, G, S	All	N	N	N	-	-
9	20/111-3	Marrickville, NSW, 2204	23/07/20	GP	Malaysia	720	300	WHL	K, L, G, S	All	N	N	N	-	-
10	20/112-1	St Peters, NSW, 2044	23/07/20	Barra	Taiwan	700	385	GGS	K, L	E, B, H	N	N	N	-	-
11	20/112-2	Hurstville, NSW, 2220	23/07/20	Barra	Malaysia	530	340	GGS	K	E, B	N	N	N	-	-
12	20/112-3	Hurstville, NSW, 2220	23/07/20	Barra	Malaysia	550	345	GGS	K	E, B	N	N	N	-	-
13	20/113-1	Hurstville, NSW, 2220	23/07/20	Barra	Malaysia	540	325	GGS	K	E, B	N	N	N	-	-
14	20/113-2	Kogarah, NSW, 2217	23/07/20	Barra	Taiwan	1240	355	WHL	K, L, G, S	All	N	N	N	-	-
15	20/113-3	Kogarah, NSW, 2217	23/07/20	Barra	Taiwan	1020	350	WHL	K, L, G, S	All	N	N	N	-	-
16	20/114-1	Kogarah, NSW, 2217	23/07/20	Barra	Taiwan	800	305	WHL	K, L, G, S	All	N	N	N	-	-
17	20/114-2	Kogarah, NSW, 2217	23/07/20	GP	China	780	270	WHL	K, L, G, S	All	N	N	N	-	-
18	20/114-3	Kogarah, NSW, 2217	23/07/20	GP	China	1200	370	WHL	K, L, G, S	All	N	N	N	-	-
19	20/114-4	Haymarket, NSW, 2000	23/07/20	Barra	Taiwan	650	350	GGS	K	E, B, H	N	N	P	I	I
20	20/114-5	Haymarket, NSW, 2000	23/07/20	Barra	Taiwan	630	350	GGS	K	E, B	N	N	N	-	-
21	20/115-1	Haymarket, NSW, 2000	23/07/20	Barra	Taiwan	600	330	GGS	K, G	E, B	N	N	N	-	-
22	20/115-2	Hurstville, NSW, 2220	23/07/20	GP	Malaysia	790	290	WHL	K, L, G, S	All	N	N	N	-	-
23	20/115-3	Hurstville, NSW, 2220	23/07/20	GP	Malaysia	830	290	WHL	K, L, G, S	All	P	N	N	-	-
24	20/116-1	Casula, NSW, 2170	23/07/20	SP	Malaysia	1530	340	WHL	K, L, G, S	All	N	N	P	I	N
25	20/116-2	Casula, NSW, 2170	23/07/20	GP	Malaysia	1300	350	WHL	K, L, G, S	All	N	N	N	-	-
26	20/117-1	Eight Mile Plains, QLD, 4113	7/07/20	Barra	Malaysia	550	350	GGS	K, L	E, B, M	N	N	N	-	-
27	20/117-2	Eight Mile Plains, QLD, 4113	7/07/20	Barra	Malaysia	520	310	GGS	K	E, B, M	N	N	N	-	-
28	20/118-1	Eight Mile Plains, QLD, 4113	7/07/20	Barra	Malaysia	560	360	GGS	K	E, B, M	N	N	N	-	-
29	20/118-2	Eight Mile Plains, QLD, 4113	7/07/20	Barra	Malaysia	540	345	GGS	K	E, B, M	N	N	N	-	-
30	20/118-3	Eight Mile Plains, QLD, 4113	7/07/20	Barra	Malaysia	480	340	GGS	K	E, B, M	N	N	N	-	-
31	20/118-4	Eight Mile Plains, QLD, 4113	7/07/20	Barra	Malaysia	500	350	GGS	K, G	E, B	N	N	N	-	-
32	20/118-5	Eight Mile Plains, QLD, 4113	7/07/20	Barra	Malaysia	520	345	GGS	K	E, B	N	N	N	-	-
33	20/118-6	Morningside, QLD, 4170	8/07/20	Barra	Taiwan	640	370	GGS	K	E, B, M	N	N	N	-	-
34	20/118-7	Capalaba, QLD, 4157	8/07/20	Barra	Taiwan	2080	545	WHL	K, L, G, S	All	N	N	P	I	N

35	20/119-1	Fyshwick, ACT, 2609	6/06/20	Barra	Thailand	600	360	GGS	K, L, G, S	E, B, H	N	N	N	-	-
36	20/119-2	Fyshwick, ACT, 2609	6/06/20	Barra	Thailand	530	360	GGS	K	E, B, H	N	N	N	-	-
37	20/119-3	St Peters, NSW, 2044	18/08/20	GP	China	740	270	WHL	K, L, G, S	All	N	N	N	-	-
38	20/120-1	St Peters, NSW, 2044	18/08/20	GP	China	740	280	WHL	K, L, G, S	All	N	N	N	-	-
39	20/120-2	St Peters, NSW, 2044	18/08/20	GP	China	720	290	WHL	K, L, G, S	All	N	N	N	-	-
40	20/120-3	St Peters, NSW, 2044	18/08/20	Barra	Taiwan	660	345	WHL	K, L, G, S	All	N	N	N	-	-
41	20/121-1	St Peters, NSW, 2044	18/08/20	Barra	Taiwan	680	345	WHL	K, L, G, S	All	N	N	N	-	-
42	20/121-2	St Peters, NSW, 2044	18/08/20	Barra	Taiwan	700	340	WHL	K, L, G, S	All	N	N	N	-	-
43	20/136-1	St Peters, NSW, 2044	18/08/20	Barra	Taiwan	600	345	GGS	K	E, B	N	N	N	-	-
44	20/136-2	St Peters, NSW, 2044	18/08/20	Barra	Taiwan	540	340	GGS	K, G	E, B, H	N	N	N	-	-
45	20/136-3	St Peters, NSW, 2044	18/08/20	Barra	Taiwan	530	355	GGS	K, L	E, B, H	N	N	N	-	-
46	20/136-1	Hurstville, NSW, 2220	18/08/20	Barra	Malaysia	560	345	GGS	K	E, B, M	N	N	N	-	-
47	20/136-2	Hurstville, NSW, 2220	18/08/20	Barra	Malaysia	560	355	GGS	K	E, B	N	N	N	-	-
48	20/136-3	Hurstville, NSW, 2220	18/08/20	Barra	Malaysia	540	350	GGS	K	E, B	N	N	N	-	-
49	20/136-1	Haymarket, NSW, 2000	18/08/20	Barra	Taiwan	820	370	GGS	K, L, G	E, B, H	N	N	N	-	-
50	20/136-2	Haymarket, NSW, 2000	18/08/20	Barra	Taiwan	730	380	GGS	K, G	E, B, H	N	N	N	-	-
51	20/136-3	Haymarket, NSW, 2000	18/08/20	Barra	Taiwan	660	375	GGS	K	E, B	N	N	N	-	-
52	20/137-1	St Peters, NSW, 2044	18/08/20	Barra	Taiwan	600	360	GGS	K, L	E, B, H, M	N	N	N	-	-
53	20/137-2	Hurstville, NSW, 2220	18/08/20	GP	Malaysia	820	265	WHL	K, L, G, S	All	N	N	N	-	-
54	20/137-3	Hurstville, NSW, 2220	18/08/20	GP	Malaysia	810	270	WHL	K, L, G, S	All	N	N	N	-	-
55	20/138-1	Hurstville, NSW, 2220	18/08/20	GP	Malaysia	820	260	WHL	K, L, G, S	All	N	N	N	-	-
56	20/138-2	Kogarah, NSW, 2217	18/08/20	GP	Taiwan	1300	430	WHL	K, L, G, S	All	N	N	N	-	-
57	20/138-3	Kogarah, NSW, 2217	18/08/20	GP	Taiwan	1300	450	WHL	K, L, G, S	All	N	N	N	-	-
58	20/139-1	Kogarah, NSW, 2217	18/08/20	GP	Taiwan	1200	410	WHL	K, L, G, S	All	N	N	N	-	-
59	20/139-2	Kogarah, NSW, 2217	18/08/20	Barra	Taiwan	600	240	WHL	K, L, G, S	All	N	N	N	-	-
60	20/139-3	Kogarah, NSW, 2217	18/08/20	Barra	Taiwan	800	250	WHL	K, L, G, S	All	P	N	P	N	N
61	20/140-1	Kogarah, NSW, 2217	18/08/20	Barra	Taiwan	800	255	WHL	K, L, G, S	All	N	N	N	-	-
62	20/140-2	Hurstville, NSW, 2220	18/08/20	GP	China	520	230	WHL	K, L, G, S	All	P	N	P	N	N
63	20/140-3	Hurstville, NSW, 2220	18/08/20	GP	China	580	260	WHL	K, L, G, S	All	P	N	P	N	N
64	20/141-1	Haymarket, NSW, 2000	18/08/20	Barra	n/a	720	390	GGS	K, G	E, B	N	N	N	-	-
65	20/141-2	Haymarket, NSW, 2000	18/08/20	Barra	n/a	760	380	GGS	K	E, B	N	N	N	-	-
66	20/141-3	Haymarket, NSW, 2000	18/08/20	Barra	n/a	720	395	GGS	K	E, B	N	N	N	-	-
67	20/141-4	no sample													
68	20/141-5	Athol Park, SA, 5012	10/07/20	Barra	Taiwan	830	380	GGS	K, H	E, B, M	N	N	N	-	-
69	20/141-6	Athol Park, SA, 5012	10/07/20	Barra	Taiwan	840	400	GGS	K, G	E, B, M	N	N	N	-	-
70	20/142-1	Athol Park, SA, 5012	10/07/20	Barra	Taiwan	830	395	GGS	K	E, B, M	N	N	N	-	-
71	20/142-2	Fyshwick, ACT, 2609	21/08/20	Barra	Thailand	690	360	GGS	K	E, B, H	N	N	N	-	-
72	20/143-1	Fyshwick, ACT, 2609	21/08/20	Barra	Thailand	640	365	GGS	K	E, B, H	N	N	N	-	-
73	20/143-2	Fyshwick, ACT, 2609	21/08/20	Barra	Thailand	650	390	GGS	K	E, B, H	N	N	N	-	-
74	20/143-3	St Peters, NSW, 2044	23/07/20	Barra	Taiwan	750	390	GGS	K, L	E, B, H, M	N	N	N	-	-
75	20/144-1	St Peters, NSW, 2044	23/07/20	Barra	Taiwan	780	410	GGS	K, L	E, B, H, M	N	N	N	-	-
76	20/149-1	Morningside, QLD, 4170	8/07/2020	Barra	Taiwan	600	350	GGS	K	E, B	N	N	N	-	-
77	20/149-2	Morningside, QLD, 4170	8/07/2020	Barra	Taiwan	620	380	GGS	K	E, B	N	N	N	-	-
78	20/149-3	St Peters, NSW, 2044	18/08/20	Barra	Taiwan	800	405	GGS	K, L	E, B, H, M	N	N	N	-	-
79	20/157-1	St Peters, NSW, 2044	18/08/20	Barra	Taiwan	800	375	GGS	K	E, B, H, M	N	N	N	-	-
80	20/157-2	Capalaba, QLD, 4157	8/07/20	Barra	Taiwan	2100	500	WHL	K, L, G, S	All	N	N	N	-	-

81	20/157-3	Capalaba, QLD, 4157	8/07/20	Barra	Taiwan	2260	545	WHL	K, L, G, S	All	N	N	N	-	-
82	20/196-1	Coopers Plains, QLD, 4108	14/09/20	GP	China	740	285	GGS	K	E, B	N	N	N	-	-
83	20/196-2	Coopers Plains, QLD, 4108	14/09/2020	GP	China	720	300	GGS	K	E, B	N	N	N	-	-
84	20/196-3	Coopers Plains, QLD, 4108	14/09/2020	GP	China	800	310	GGS	K	E, B	N	N	N	-	-
85	20/196-4	Kogarah, NSW, 2217	21/10/2020	Barra	Taiwan	1670	460	WHL	K, L, G, S	All	N	N	N	-	-
86	20/196-5	Kogarah, NSW, 2217	21/10/2020	Barra	Taiwan	1040	380	WHL	K, L, G, S	All	N	N	N	-	-
87	20/196-6	Kogarah, NSW, 2217	21/10/2020	Barra	Taiwan	1660	450	WHL	K, L, G, S	All	N	N	P	I	N
88	20/197-1	Haymarket, NSW, 2000	21/10/2020	Barra	Taiwan	760	390	GGS	K, L	E, B, H, M	N	N	P	N	N
89	20/197-2	Haymarket, NSW, 2000	21/10/2020	Barra	Taiwan	800	400	GGS	K, L, G, S	E, B, H, M	N	N	P	N	N
90	20/197-3	Haymarket, NSW, 2000	21/10/2020	Barra	Taiwan	640	370	GGS	K, L, S	E, B, H	N	N	P	P	N
91	20/198-1	Oxyley, QLD, 4075	14/09/2020	Barra	Taiwan	1030	405	WHL	K, L, G, S	All	N	N	N	-	-
92	20/198-2	Oxyley, QLD, 4075	14/09/2020	Barra	Taiwan	1000	410	WHL	K, L, G, S	All	N	N	N	-	-
93	20/198-3	Oxyley, QLD, 4075	14/09/2020	Barra	Taiwan	990	410	WHL	K, L, G, S	All	N	N	N	-	-
94	20/199-7	Oxyley, QLD, 4075	14/09/2020	GP	Malaysia	840	295	WHL	K, L, G, S	All	N	N	N	-	-
95	20/199-8	Oxyley, QLD, 4075	14/09/2020	GP	Malaysia	860	295	WHL	K, L, G, S	All	N	N	N	-	-
96	20/199-9	Oxyley, QLD, 4075	14/09/2020	GP	Malaysia	850	295	WHL	K, L, G, S	All	N	N	N	-	-
97	20/199-1	Hurstville, NSW, 2220	21/10/2020	GP	Malaysia	780	280	WHL	K, L, G, S	All	N	N	N	-	-
98	20/199-2	Hurstville, NSW, 2220	21/10/2020	GP	Malaysia	740	270	WHL	K, L, G, S	All	N	N	N	-	-
99	20/199-3	Hurstville, NSW, 2220	21/10/2020	GP	Malaysia	780	300	WHL	K, L, G, S	All	N	N	N	-	-
100	20/199-4	Marrickville, NSW, 2204	21/10/2020	GP	Malaysia	800	280	WHL	K, L, G, S	All	N	N	N	-	-
101	20/199-5	Marrickville, NSW, 2204	21/10/2020	GP	Malaysia	760	275	WHL	K, L, G, S	All	N	N	N	-	-
102	20/199-6	Marrickville, NSW, 2204	21/10/2020	GP	Malaysia	640	280	WHL	K, L, G, S	All	N	N	N	-	-
103	20/200-1	Hurstville, NSW, 2220	21/10/2020	Barra	Malaysia	540	340	GGS	K	E, B, M	N	N	N	-	-
104	20/200-2	Hurstville, NSW, 2220	21/10/2020	Barra	Malaysia	500	345	GGS	K	E, B, M	N	N	N	-	-
105	20/200-3	Hurstville, NSW, 2220	21/10/2020	Barra	Malaysia	520	350	GGS	K	E, B, M	N	N	N	-	-
106	20/201-1	St Peters, NSW, 2044	21/10/2020	GP	China	720	270	WHL	K, L, G, S	All	N	N	N	-	-
107	20/201-2	St Peters, NSW, 2044	21/10/2020	GP	China	660	275	WHL	K, L, G, S	All	N	N	N	-	-
108	20/201-3	St Peters, NSW, 2044	21/10/2020	GP	China	720	275	WHL	K, L, G, S	All	N	N	N	-	-
109	20/202-1	St Peters, NSW, 2044	21/10/2020	Barra	Taiwan	620	335	WHL	K, L, G, S	All	N	N	N	-	-
110	20/202-2	St Peters, NSW, 2044	21/10/2020	Barra	Taiwan	650	355	WHL	K, L, G, S	All	N	N	N	-	-
111	20/202-3	St Peters, NSW, 2044	21/10/2020	Barra	Taiwan	780	380	WHL	K, L, G, S	All	N	N	N	-	-
112	20/203-1	St Peters, NSW, 2044	21/10/2020	Barra	Taiwan	540	355	GGS	K, L	E, B, M	N	N	N	-	-
113	20/203-2	St Peters, NSW, 2044	21/10/2020	Barra	Taiwan	590	360	GGS	K	E, B, M	N	N	N	-	-
114	20/203-3	St Peters, NSW, 2044	21/10/2020	Barra	Taiwan	580	360	GGS	K	E, B, M	N	N	N	-	-
115	20/204-1	St Peters, NSW, 2044	21/10/2020	Barra	Taiwan	620	360	GGS	K, L, S	E, B, H, M	N	N	N	-	-
116	20/204-2	St Peters, NSW, 2044	21/10/2020	Barra	Taiwan	660	365	GGS	K, L, S	E, B, H, M	N	N	N	-	-
117	20/204-3	St Peters, NSW, 2044	21/10/2020	Barra	Taiwan	620	360	GGS	K, L, S	E, B, H, M	N	N	N	-	-
118	20/204-4	St Peters, NSW, 2044	21/10/2020	GP	China	780	275	WHL	K, L, G, S	All	N	N	N	-	-
119	20/204-5	St Peters, NSW, 2044	21/10/2020	GP	China	770	275	WHL	K, L, G, S	All	N	N	N	-	-
120	20/204-6	St Peters, NSW, 2044	21/10/2020	GP	China	750	270	WHL	K, L, G, S	All	N	N	N	-	-

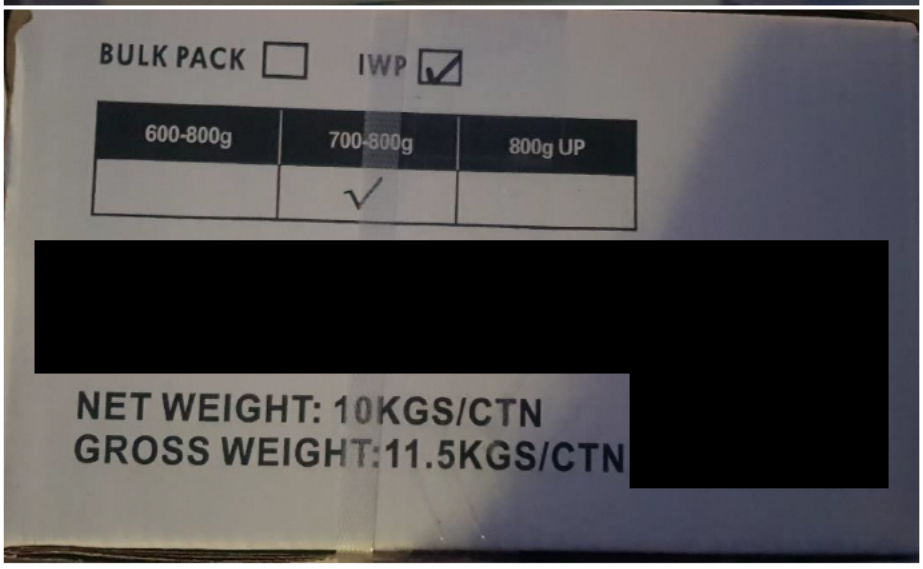
Abbreviations: Barra, Barramundi; GP, Golden Pomfret; SP, Silver Pomfret; WHL, Whole; GGS, gilled gutted and scaled; K, Kidney; L, Liver; S, Spleen; H, Heart; E, Eye; B, Brain, M, Mixed organs; NT, Not tested; I, Indeterminate; N, Negative; P, Positive; ISKNV, Infectious spleen and kidney necrosis virus; SGIV, Singapore grouper iridovirus, SDDV, Scale drop disease virus; FAH IDL, The University of Sydney Farm Animal Health Infectious Diseases Laboratory; AFDL, ACDP Fish Diseases Laboratory.

Appendix 2.

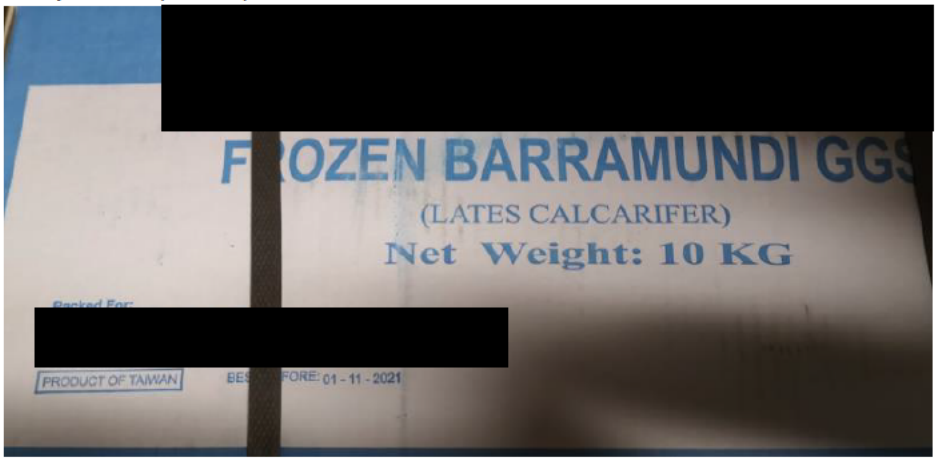
Images of sampled non-salmonid finfish commodities at point of purchase in retailers.

The image list below does not include all images captured, nor show all samples that were collected in the project. Capturing of images during sampling was at times not permissible by the retailer or practical during this aspect of the project. Where similar product packaging was encountered, only the first images were included in the list below, to avoid repetition.

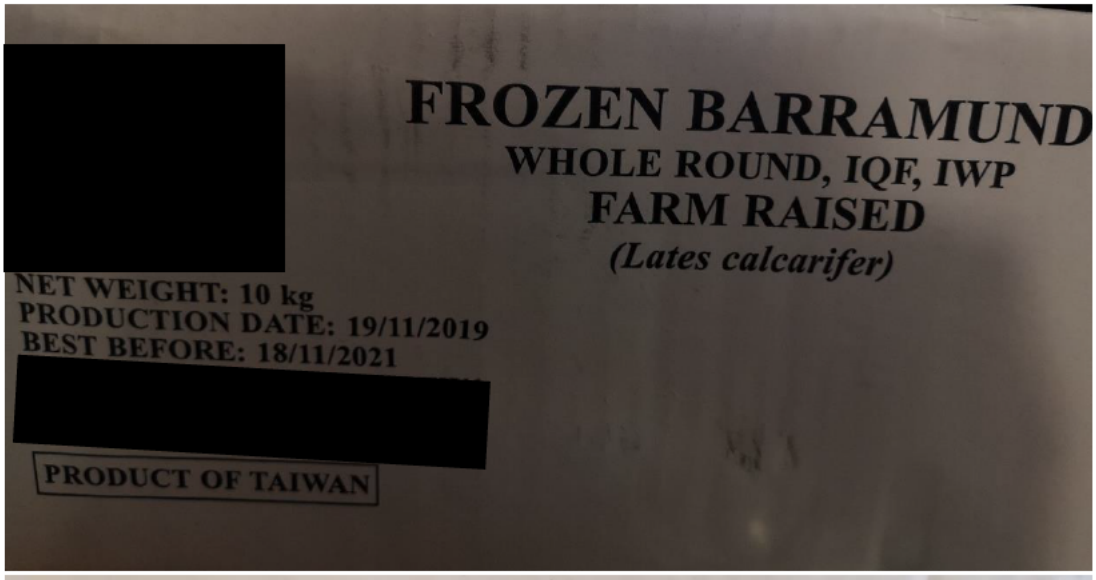
1. Sample ID 37-39, SVC ID – 20/136

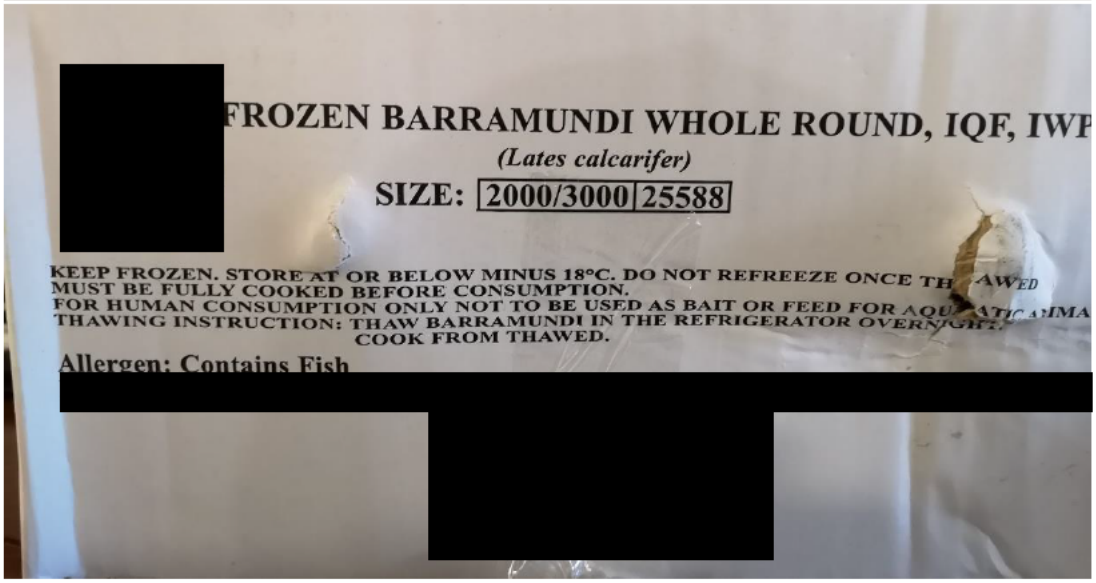


2. Sample ID 33, 76-77, SVC ID – 20/119.

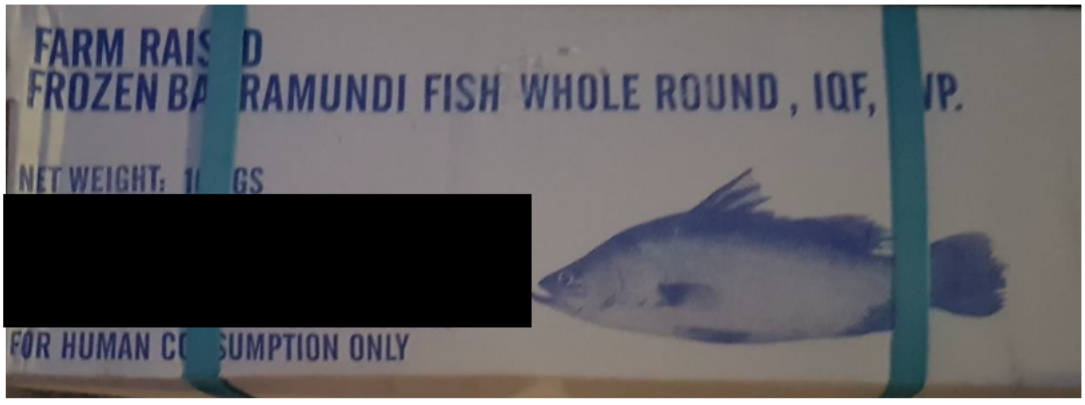


3. Sample ID 34, 80-81, SVC ID – 20/120

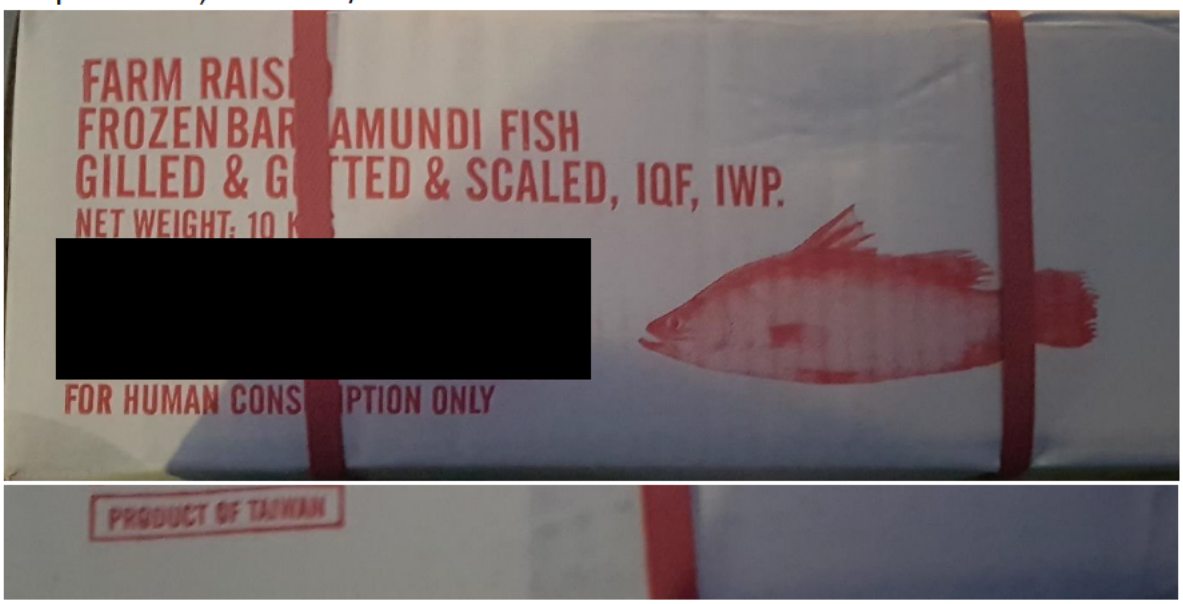




4. Sample ID 40-42, SVC ID – 20/136



5. Sample ID 43-45, SVC ID – 20/136



6. Sample ID 1-3, SVC ID – 20/110

7. Sample ID 25, SVC ID – 20/117

8. Sample ID 19-21, SVC ID – 20/115

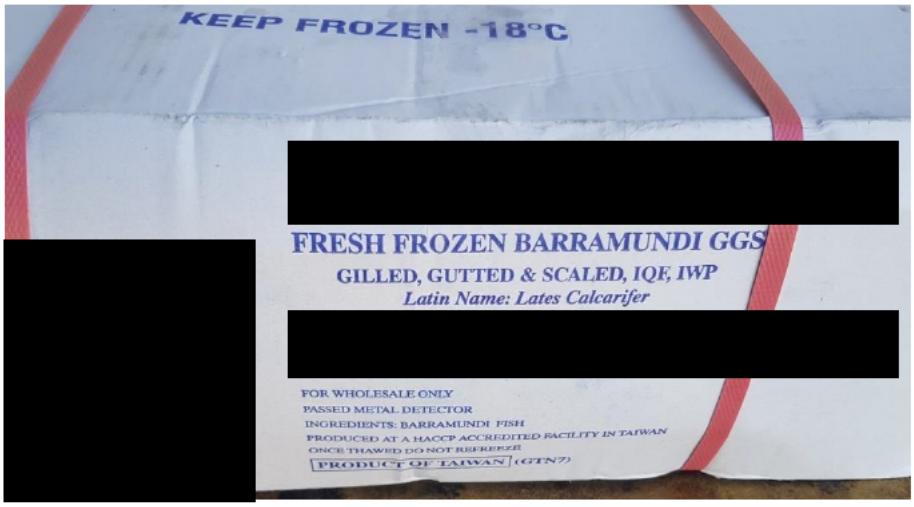
9. Sample ID 49-51, SVC ID – 20/138

10. Sample ID 35-36, SVC ID – 20/121

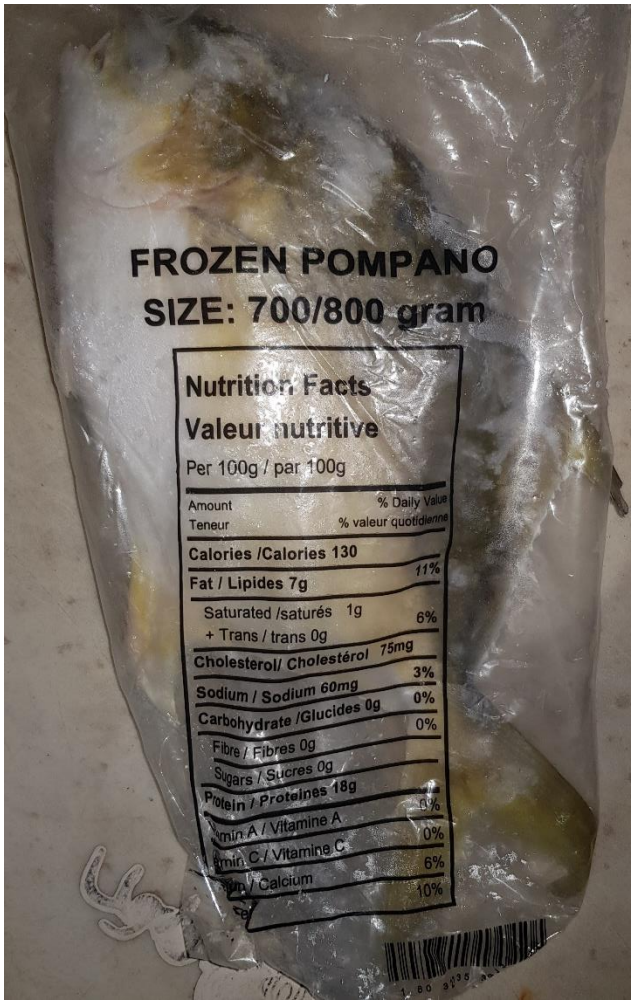
11. Sample ID 11-13, SVC ID – 20/113

12. Sample ID 14-16, SVC ID – 20/114

13. Sample ID 10, 74-75, SVC ID – 20/112



14. Sample ID 7-9, SVC ID – 20/111



15. Sample ID 64-66, SVC ID – 20/143

16. Sample ID 68-69, SVC ID – 20/157

17. Sample ID 22-23, SVC ID – 20/116

18. Sample ID 82-84, SVC ID – 20/197

Appendix 3.

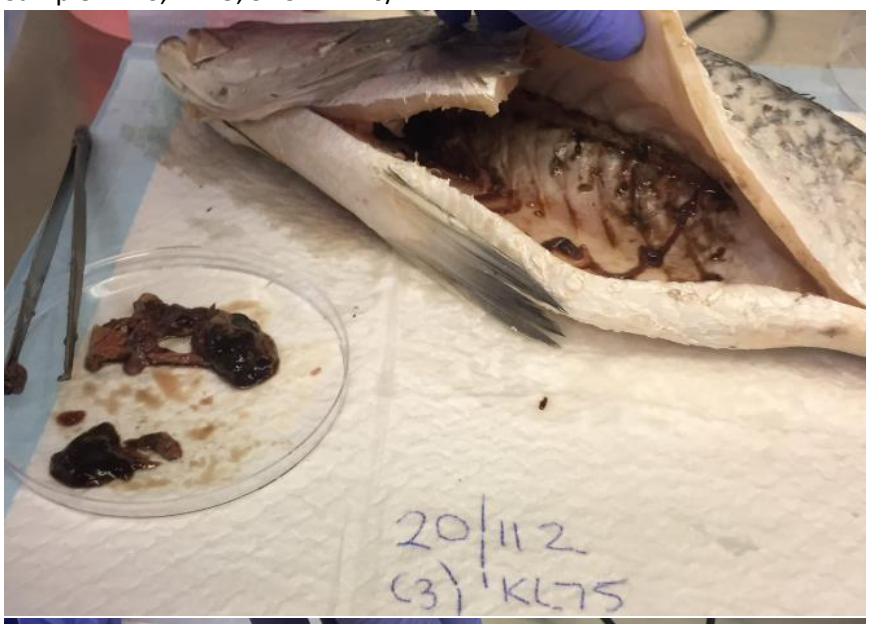
Images of head-on eviscerated barramundi commodities during dissection and tissue sub-sampling at FAH IDL.

The image list below does not include all images captured, nor show all samples that were collected in the project. Capturing of images during sub-sampling was at times not practical. Where similar images were captured, only a select few images were included in the list below, to avoid repetition. The below image list includes images of head-on eviscerated (typically labelled as - gilled, gutted and scaled) barramundi (*Lates calcarifer*) products sampled during the project, collected during tissue sub-sampling for qPCR testing.

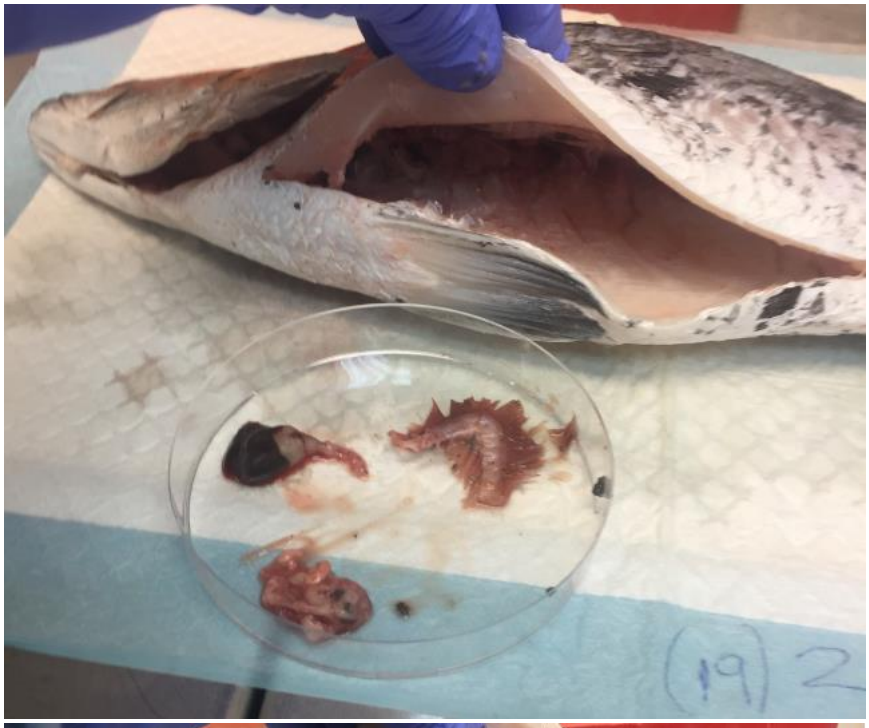
1. Sample ID 4-6, SVC ID – 20/110

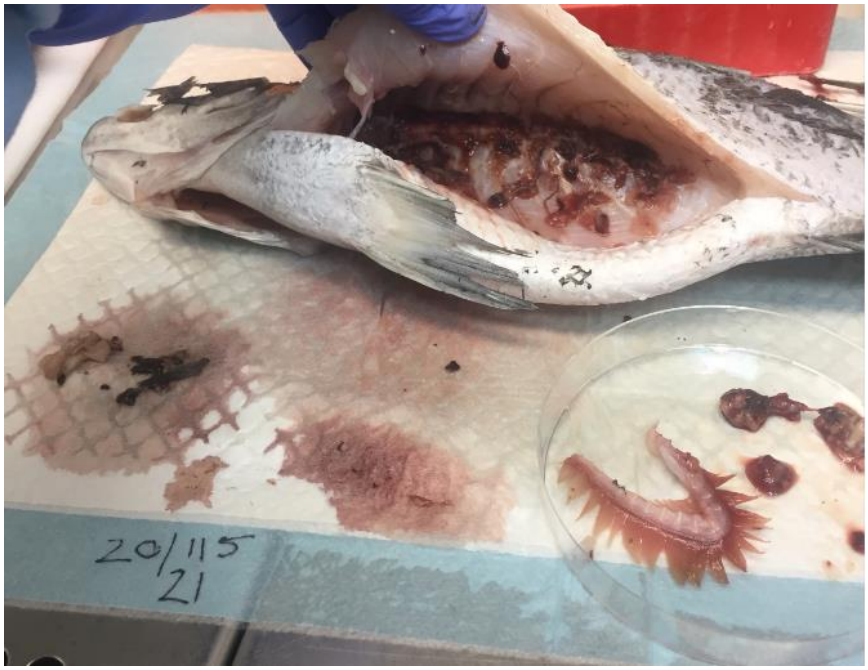
2. Sample ID 35-36, SVC ID – 20/121

3. Sample ID 10,74-75, SVC ID – 20/112

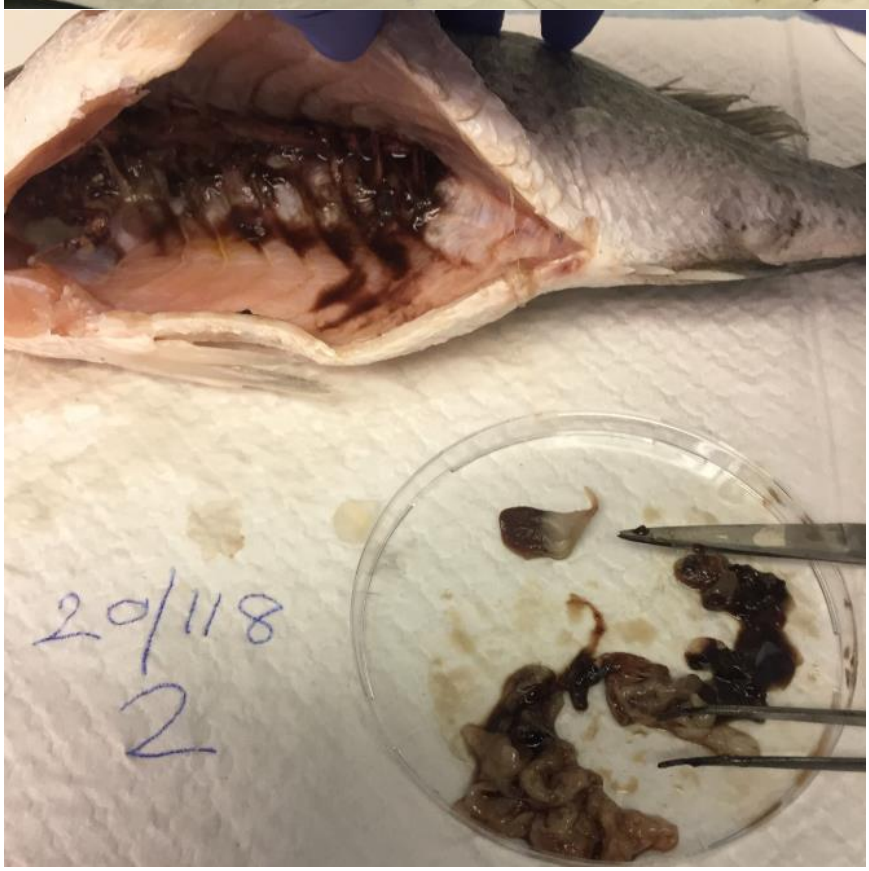


4. Sample ID 19-21, SVC ID – 20/115



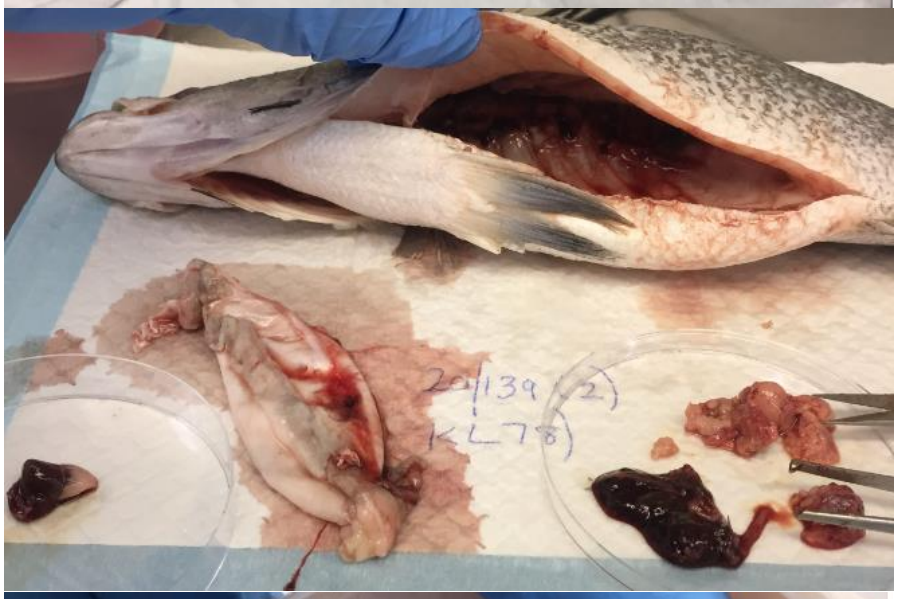


5. Sample ID 26-32, SVC ID – 20/118



6. Sample ID 43-45, SVC ID – 20/136

7. Sample ID 52,78-79, SVC ID – 20/139



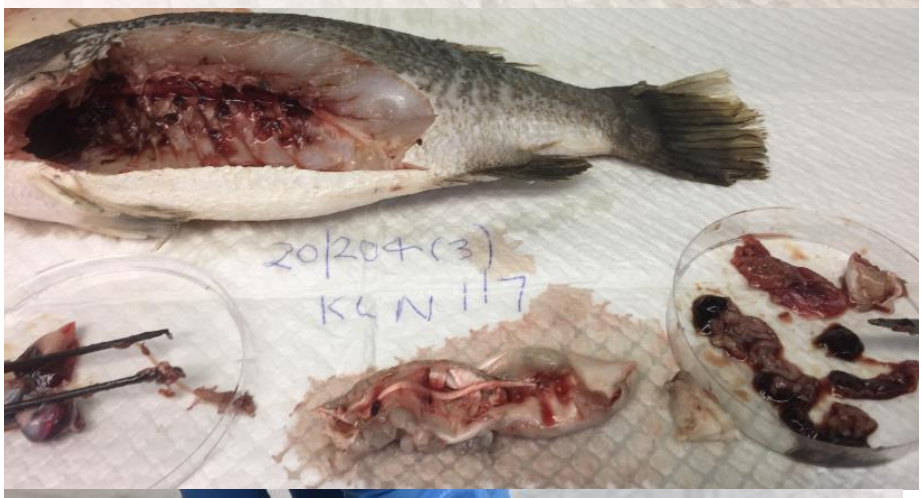
8. Sample ID 64-66, SVC ID – 20/143

9. Sample ID 71-73, SVC ID – 20/149

10. Sample ID 112-114, SVC ID – 20/199

11. Sample ID 88-90, SVC ID – 20/200

12. Sample ID 115-117, SVC ID – 20/204



Appendix 4.

BICON Australian Biosecurity Import Conditions for each sampled commodity type (Verified 29/03/2021)

Whole barramundi

Case: Finfish (excluding Salmonid) for human consumption Effective: 29 Mar 2021

Bony fish — Not in a consumer-ready form — Non-personal use — Finfish were not caught in international waters — Country of sourcing, processing and exporting with an approved competent authority — Not eviscerated — Farmed — Country of sourcing, processing and exporting is Taiwan — Barramundi, tilapia or milkfish — Not accompanied by importer and/or not less than 5kg — Standard permit conditions

To demonstrate compliance with this requirement you must present the following on an Official government certificate:

1. *A statement identifying the fish species in the consignment (i.e. scientific name, including genus and species).*
2. *A statement that that the fish were derived from an aquaculture facility subject to health surveillance and monitoring under the supervision of the Competent Authority.*
3. *A statement that the fish were not derived from a population slaughtered as an official disease control measure.*
4. *A statement that the fish were processed in a premises approved by and under the control of the Competent Authority.*
5. *A statement that the product is free from visible lesions associated with infectious disease.*
6. *A statement that the product is fit for human consumption.*

Head-on eviscerated barramundi

Case: Finfish (excluding Salmonid) for human consumption Effective: 29 Mar 2021

Bony fish — Not in a consumer-ready form — Non-personal use — Finfish were not caught in international waters — Country of sourcing, processing and exporting with an approved competent authority — Not eviscerated — Not farmed — Other than dried

Non-salmonid finfish sourced from all countries in, and islands surrounding, Asia (including Japan):

The product must not have been grown or harvested in an aquaculture system (farmed).

The product must be at least de-gilled and eviscerated (gutted) prior to importation and must be accompanied by a valid import permit.

Whole pomfret

Case: Finfish (excluding Salmonid) for human consumption Effective: 29 Mar 2021

Bony fish — Not in a consumer-ready form — Non-personal use — Finfish were not caught in international waters — Country of sourcing, processing and exporting with an approved competent authority — Not eviscerated — Not farmed — Other than dried

The low risk (non-specified) and risk (specified) fish species must meet the following import conditions.

To demonstrate compliance with this requirement you must present the following on an Official government certificate:

For the low risk fish species the following evidence has been provided:

1. *The genus and species of fish contained in the consignment.*

2. *A statement that the fish were wild caught.*
3. *A statement that the fish were not grown or harvested in an aquaculture system at any stage.*
4. *A statement that the consignment does not contain fish species other than those listed above.*
5. *A statement that the fish were processed in premises (including vessels/refrigerated warehouses) approved by and under the control of the competent authority.*
6. *A statement that the fish were inspected under the supervision of the competent authority and/or systems approved by the competent authority.*
7. *A statement that the fish are free from visible lesions associated with infectious disease.*

Head-on eviscerated pomfret

Case: Finfish (excluding Salmonid) for human consumption Effective: 23 Apr 2021

Bony fish — Not in a consumer-ready form — Non-personal use — Finfish were not caught in international waters — Country of sourcing, processing and exporting with an approved competent authority — Not eviscerated — Not farmed — Other than dried

The low risk (non-specified) and risk (specified) fish species must meet the following import conditions.

To demonstrate compliance with this requirement you must present the following on an Official government certificate:

For the low risk fish species the following evidence has been provided:

1. *The genus and species of fish contained in the consignment.*
2. *A statement that the fish were wild caught.*
3. *A statement that the fish were not grown or harvested in an aquaculture system at any stage.*
4. *A statement that the consignment does not contain fish species other than those listed above.*
5. *A statement that the fish were processed in premises (including vessels/refrigerated warehouses) approved by and under the control of the competent authority.*
6. *A statement that the fish were inspected under the supervision of the competent authority and/or systems approved by the competent authority.*
7. *A statement that the fish are free from visible lesions associated with infectious disease.*

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