CENTRE FOR
INVASIVE SPECIES SOLUTIONS

TILAPIA BIOCONTROL: PROSPECTING AND EVALUATION, STAGE 1

FINAL REPORT FOR PROJECT P01-B-003

AUTHORS

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We acknowledge the Traditional Custodians of the lands on which we meet and work and pay our respects to Elders — past, present and emerging.

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TILAPIA BIOCONTROL: PROSPECTING AND EVALUATION, STAGE 1

FINAL PROJECT REPORT FOR P01-B-003

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Abbreviations

ACDP	Australian Centre for Disease Preparedness
APVMA	Australian Pesticide and Veterinary Medicines Authority
BCA	Biological control agents
BIV	Bohle iridovirus
CBA	Cost-benefit analysis
CCCG	Carp Control Coordinating Group
CISS	Centre for Invasive Species Solution
CPE	Cytopathic effect
CRC	Cooperative Research Centres
CRRDC	Council of Rural Research and Development Corporations
Ct	Cycle threshold
EDTA	Ethylenediaminetetraacetic acid
EIC	Environment and Invasives Committee
EMAI	Elizabeth Macarthur Agricultural Institute
EUS	Epizootic Ulcerative Syndrome
FBS	Fetal bovine serum
FPLV	Feline panleukopenia virus

FRDC	Fisheries Research and Development Corporation
GDP	Gross Domestic Product
GIFT	Genetically Improved Farmed Tilapia
GMO	Genetically modified organism
GoC	Gulf of Carpentaria
ICLARM	International Center for Living Aquatic Resources Management
ICTV	International Committee on Taxonomy of Viruses
IPNV	Infectious pancreatic necrosis virus
ISKNV	Infectious spleen and kidney necrosis virus
ISSG	Invasive Species Specialist Group
IUCN	International Union for Conservation of Nature
JCU	James Cook University
KHV	Koi herpesvirus
LCDV	Lymphocystis disease virus
MDB	Murray–Darling Basin
MU	Macquarie University
MYXV	Myxoma virus
NCCP	National Carp Control Plan
NNV	Nervous necrosis virus
NTS	Non-target species
OIE	Office International des Epizooties (now: World Organisation for Animal Health)
PCR	Polymerase chain reaction
RD&E	Research, Development, and Extension
RHDV	Rabbit haemorrhagic disease virus
RNA	Ribonucleic acid
SSC	Species Survival Commission
SSIMS	Self-stocking incompatible male system
SVCV	Spring viremia of carp virus
TiLV	Tilapia lake virus
TiPV	Tilapia parvovirus virus
TLEV	Tilapia larvae encephalitis virus
ToR	Terms of reference
VER	Viral encephalopathy and retinopathy
VNN	Viral nervous necrosis
WOAH	World Organization for Animal Health

EXECUTIVE SUMMARY

Mozambique tilapia (*Oreochromis mossambicus*) is listed in the top 100 of the world's worst invasive alien species and has been documented to have severe impacts on freshwater ecosystems, primarily through displacement of native species and habitat alteration. In Australia, both *O. mossambicus* and the lesser-known *Pelmatolapia mariae* (formerly *Tilapia mariae*), commonly referred to as spotted tilapia or black mangrove cichlid, have established significant populations within Queensland waters. Recent incursions into the Burnett catchment are of particular concern since this catchment is only two kilometres from the Murray–Darling Basin (MDB) watershed. Further, the threat of the species to the MDB is serious, with 50% of the basin considered to be able to support tilapia (Hutchison, Sarac, and Norris 2011).

Currently, there is no single overall option for the control of tilapia. Eradication attempts using a combination of electrofishing and poison are rarely successful in open waterways, and, given their invasive nature, there is a lack of demonstrated broadscale effective control mechanisms for tilapia. Biological control (biocontrol), where it is feasible, can be a cost-effective, safe (species-specific) and practical solution to managing invasive species because it does not require reapplication of chemicals and, once established, should be self-sustaining. Based on the development of previous viral biocontrol strategies for rabbits and carp, we used a robust assessment framework for bioprospecting of biological control agents (BCAs) for invasive tilapia in Australia. The in-depth, international review of potential tilapia-specific BCAs covered a wide range of pathogens. Tilapia pathogens fall into the general categories of viruses, bacteria, parasites, and fungi. The review identified three tilapia viruses that were considered species-specific and were categorised as tentatively worthwhile for further investigation. The three viruses were: Tilapia lake virus (TiLV), Tilapia parvovirus (TiPV), and Tilapia larvae encephalitis virus (TLEV). TiLV was considered the most promising potential BCA and was categorised as 'worthwhile for active further investigation'. These findings have been presented at national and international conferences, and published in *Biological Control* journal (Sunarto et al. 2022).

CSIRO imported TiLV isolates from Israel into Australia's high-containment laboratory and developed the capability to work with this exotic virus. Wild fish of each species, *O. mossambicus* and *P. mariae,* were caught in Queensland and spawned in captivity at James Cook University (JCU). The offspring were raised for six months before being transported to the NSW Department of Primary Industry's Elizabeth Macarthur Agricultural Institute (EMAI) for virus challenge. The aim of this experiment was to assess the susceptibility of two species of tilapia present in Australian waterways–*O. mossambicus* and *P. mariae*–to infection with TiLV. The incidence of infection and disease was 100% in juvenile *O. mossambicus* and *P. mariae* when challenged with the virus by intraperitoneal injection. Disease was characterised by inappetence, reduced activity and colour changes that progressed rapidly to death in many cases. After TiLV injection, mortality was 100% for *O. mossambicus* at day 9 and 63% for *P. mariae* at day 14. Fish challenged by cohabitation to reflect a more natural route of transmission also resulted in 100% infection, disease, and mortality for *O. mossambicus*. Cohabitation resulted in mortality of 46% for *P. mariae*. Immersion for one hour resulted in 100% disease and mortality of *O. mossambicus,* but the same immersion challenges did not cause any TiLV disease in *P. mariae*. High loads of TiLV were detected by RT-qPCR in all fish that died or were euthanised due to clinical signs of disease. Lesions observed on histopathology were consistent with disease caused by TiLV, including liver necrosis, syncytia, cytoplasmic inclusions, prominent nucleoli, and perivascular leukocytes. Taken together, this study provided a proof-of-principle demonstration of the susceptibility of Australian genotypes of *O. mossambicus* and *P. mariae* to TiLV as an initial step in assessing the suitability of the virus for biocontrol. Further assessment of the safety and efficacy of BCAs for potential use in Australia requires detailed assessment, which would require significant, potentially long-term, investment.

The successful identification of TiLV as the most promising BCA for invasive tilapia and the susceptibility of two tilapia species present in Australian waterways to TiLV were used to inform a business case, including an ex-ante cost-benefit analysis (CBA), to advance the selection of new tilapia BCAs for future management of invasive tilapia in Australia. This business case was developed

by AgTrans Research and proposed a six-stage research, development and extension (RD&E) program to advance the selection and testing of new tilapia BCAs in Australia. The six stages would include: Stage 1: Bioprospecting and evaluation (Project P01-B-003); Stage 2A: Efficacy testing; Stage 2B: Complementary tilapia control methods, such as genetic biocontrol; Stage 3: Safety testing; Stage 4: Planning and modelling optimal release; Stage 5: Other assessments and regulatory approvals; and Stage 6: Release and clean-up strategies.

The ex-ante CBA was conducted to assess whether the proposed investment would be out- weighted by the estimated potential benefits of proposed BCAs. Publicly available information on TiLV as the selected BCA was used in the analysis. The primary benefit of the proposed tilapia biocontrol investment is expected to be a net reduction in the total annual impact costs of tilapia to the Australian community and economy. This primary benefit would be driven by the release of a new tilapia BCA, leading to a reduction in tilapia biomass and associated negative impacts. The total RD&E investment required was estimated at $18.69 million. The projected net benefit from the investment was $52.53 million. This gave a net present value of $33.84 million, a benefit-cost ratio of 2.81 to 1, an internal rate of return of 9.3%, and a modified internal rate of return of 7.1%.

The positive investment criteria suggest that the initial investment would be worthwhile given the estimates made of the current and future potential impact and control costs of tilapia in Australia, likely pathways to impact for proposed new BCAs, the RD&E investment and associated timelines required, and the risks involved. Further, the proposed investment can be staged conditionally (stop/go points) so that, as the investment proceeds along a particular pathway, the direction of the RD&E could be adjusted according to successful milestones and new information arising, avoiding lost investment potential, and maximising the value and impact of outcomes delivered. The business case, including an ex-ante CBA, has been published by the Centre for Invasive Species Solutions (Hardaker and Chudleigh 2021).

The successful breeding of two species of invasive tilapia at JCU has not only provided supplies of healthy fish for the current Project P01-B-003, but also created opportunities for future genetic biocontrol of tilapia (Stage 2B). Macquarie University (MU), through Project P01-B-005: Proof-of-concept for genetic biocontrol in a vertebrate, has validated components of self-stocking incompatible male system (SSIMS) technology in the zebra fish model. Built on findings from this project, CSIRO is currently supporting a Masters student from Deakin University who is characterising genes associated with SSIMS technology in tilapia. This support is to ensure continuity of the project workflow from proof-of-concept of genetic biocontrol in the zebra fish model to translation to tilapia as a target species. Our engagement with both MU and JCU has created opportunities for translation of SSIMS technology from the zebra fish model to tilapia as a target species. The project has also engaged with Dr Nick Whiterod, the Nature Glenelg Trust, and Professor Claus Wedekind of University of Lausanne, Switzerland, who is assessing a Trojan Y-chromosome biocontrol approach for tilapia.

This project has delivered successful identification of TiLV as a highly promising BCA for invasive tilapia, with strong evidence in the two tilapia species present in Australian waterways. The positive CBA and the business case provide compelling data to engage key stakeholders at state and federal levels. The establishment of a unique capability in tilapia breeding and a novel genetic biocontrol strategy that could amplify the control achieved, with the ultimate aim of eradication warrants further discussion. Coordinated investment in these tools would provide a powerful integrated strategy for the effective biocontrol of invasive tilapia in Australia.

GENERAL INTRODUCTION

Mozambique tilapia (*O. mossambicus*) is listed in the top 100 of the world's worst invasive alien species and has been documented to have severe impacts on freshwater ecosystems primarily through displacement of native species and habitat alteration. In Australia, both *O. mossambicus* and the lesser-known *P. mariae*, commonly referred to as spotted tilapia or black mangrove cichlid, have established significant populations within Queensland waters. Recent incursions into the Burnett catchment are of particular concern since this catchment is only two kilometres from the Murray–Darling Basin (MDB) watershed. Further, the threat of the species to the MDB is very serious, with 50% of the basin considered able to support tilapia (Hutchison, Sarac, and Norris 2011).

While there is now an effective environmental DNA (eDNA) surveillance tool (Noble et al. 2015) for early detection and mapping of the distribution of tilapia, current management mechanisms are inadequate to control tilapia once invasion has occurred. Indeed, it is now clear that current control programs are failing to stop tilapia spread, and options for management post-incursion are extremely limited. Eradication is routinely attempted by using a combination of electrofishing and poison, but is rarely successful in open waterways because of the invasive nature of tilapia. Eradication was thought to be achieved for one incursion of *P. mariae* in a restricted length of Eureka Creek (Mitchell River Catchment) (Pearce et al. 2009). However, the detection of *P. mariae* in the same section of Eureka Creek again in 2019 cast doubt over the success of the original attempts.

Eradication of infestations in other systems (e.g. Fitzroy River Catchment) has not been possible. Indeed, there is a lack of demonstrated effective control mechanisms for tilapia and there is a critical need to develop and evaluate potential biological control agents (BCAs) for tilapia. Where feasible, biocontrol can be a cost-effective, safe, and practical solution to manage invasive species at the landscape scale because it does not require reapplication of chemicals or poisons, it is species-specific, self-amplifying and, once established, may be self-sustaining.

The objectives of the project were: (1) to conduct bioprospecting for BCAs for invasive tilapia in Australia; 2) to develop capability to work with potential BCAs for tilapia in Australia; 3) to determine the susceptibility of two tilapias species present in Australian waterways (*O. mossambicus* and *P. mariae*) to tilapia lake virus (TiLV) and demonstrate the efficacy of TiLV as a potential BCA for tilapia in Australia; 4) to undertake a cost-benefit analysis (CBA) of tilapia biocontrol; and 5) to develop a business case for investing in the necessary research (e.g. target susceptibility and specificity) to gain approval for the release of an agent. The expected outcomes of the project are: 1) biocontrol tools and strategies in place for long-term invasive tilapia management and 2) sustainable reduction of the impacts of established tilapia.

These objectives were addressed, and are reported in three chapters:

Chapter 1: Bioprospecting for biological control agents for invasive tilapia in Australia. This chapter addresses objective 1 and consists of an in-depth review of tilapia pathogens and an assessment of their potential as BCAs, a process known as bioprospecting, with emphasis on assessing biocontrol options for invasive tilapia. This chapter has been published in the *Biological Control* journal[1] (Appendix 1).

Chapter 2: Susceptibility of Australian-origin Mozambique tilapia (*O. mossambicus*) and spotted tilapia (*P. mariae*) to TiLV. This chapter addresses objectives 2 and 3 and provides proof-of-principle demonstration of the susceptibility of two tilapia species presents in Australian waterways to TiLV. A manuscript for publication in peer-reviewed journal has been drafted.

Chapter 3: Business case to advance the selection and testing of new tilapia biocontrol agents in Australia. This chapter addresses objectives 4 and 5. The business case and ex-ante CBA were developed by AgTrans Research and proposed a six-stage research, development, and extension

[1] https://doi.org/10.1016/j.biocontrol.2022.105020

program to advance the tilapia biocontrol research in Australia. The report has been published by the Centre for Invasive Species Solutions (Hardaker and Chudleigh 2021).[2]

The ‘General discussion and conclusion’ section discusses and summarises the key findings, the outcomes, and the next steps of tilapia biocontrol research.

[2] The report is available at https://invasives.com.au/wp-content/uploads/2023/02/PO1-B-003-R1_Tilapia-Biocontrol-RDE-Business-Case-contents.pdf

CHAPTER 1: BIOPROSPECTING FOR BIOLOGICAL CONTROL AGENTS FOR INVASIVE TILAPIA IN AUSTRALIA

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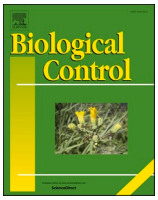

Bioprospecting for biological control agents for invasive tilapia in Australia

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HIGHLIGHTS

- Mozambique tilapia is listed in the top 100 of the world's worst invasive species.
- We used a robust framework to assess the safety and efficacy of viral biocontrol.
- Tilapia lake virus has been considered as the most promising biocontrol agent.
- We have identified the essential information required for biocontrol of tilapia.

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Tilapia parvovirus (TiPV)
Tilapia
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ABSTRACT

Originating in Africa, tilapia (Pisces, *Cichlidae*) now have a worldwide distribution and are both a prime model system for evolutionary biology and an important aquaculture species in over 135 countries. In contrast, Mozambique tilapia (*Oreochromis mossambicus*) is also listed in the top 100 of the world's worst invasive alien species and has been documented to have severe impacts on freshwater ecosystems primarily through displacement of native species and habitat alteration. In Australia, both *O. mossambicus* and the lesser-known spotted tilapia (*Tilapia mariae*) have established significant populations within Queensland waters, and recent incursions into northern New South Wales are of great concern. Eradication attempts using a combination of electrofishing and piscicide (poison) are rarely successful in open waterways, and given their invasive nature, there is a lack of demonstrated broad-scale effective control mechanisms for tilapia. Biological control (biocontrol), where it is feasible can be a cost-effective, a safe (species specific) and practical solution to managing invasive species because it does not require reapplication of chemicals or poisons, and once established should be self-sustaining. Based on the development of previous viral biocontrol strategies for rabbits and carp, we used a robust assessment framework for bioprospecting of biocontrol agents and found that tilapia lake virus (TiLV), and possibly tilapia parvovirus (TiPV), may offer the potential for biocontrol for invasive tilapia in Australia. TiLV causes high mortality in wild and cultured tilapia, but not in other species, and spreads through a waterborne route - an important transmission pathway for a successful viral biocontrol of fish. However, safety and efficacy, two major concerns for a successful biocontrol virus, need to be taken into consideration before the use of any exotic biocontrol virus is considered. Herein, we describe a systematic approach to assess known pathogens for their suitability as potential agents for biological control of tilapia and outline the possible next steps to further investigate the top candidates.

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1. Introduction

Tilapia refers to a group of subtropical to tropical tilapiine fish of the family *Cichlidae*, one of the most species-rich families of vertebrates (Kocher, 2004). Tilapia are grouped into three genera according to parental care patterns: *Oreochromis* (maternal mouthbrooders), *Sarotherodon* (paternal or biparental mouthbrooders), and *Tilapia* (substrate-spawners) (Trewavas, 1982a, Trewavas, 1982b). The rapid radiation of cichlid fish in their origin, the East Africa Great Lakes, resulted in the evolution of almost 2000 unique species in the past 10 million years, making the African cichlids an ideal model system for studying the mechanism of vertebrate evolution and speciation (Kocher, 2004, Seehausen, 2006, Trewavas, 1947). The adaptive nature of cichlids also contributed to the successful spread of tilapia worldwide. They have been introduced into five continents (Asia, North and South America, Europe and Australia) for reasons including biological control of aquatic weeds and insects, as ornamental species, to augment capture fisheries, and as an aquaculture commodity (Canonico et al., 2005, De Silva et al., 2004). They are now the second most important aquaculture commodity after carp (FAO, 2019), despite also being listed in the Global Invasive Species Database among the top 100 of the world's worst invasive alien species (GISD, 2006, Lowe et al., 2000).

Tilapia culture has expanded worldwide, initially with Mozambique tilapia, *Oreochromis mossambicus*, and then the more productive Nile tilapia, *O. niloticus*. Currently, tilapia are farmed in over 135 countries with global production estimated at 4.5 Mt and valued at US$7.5 billion (FAO, 2019). Tilapia, also known as the 'aquatic chicken' because they offer affordable and high-yield source of protein, exhibit high value aquaculture traits including high fecundity, rapid growth rate, tolerance to adverse water quality, and relative resistance to disease and other stressors (De Silva et al., 2004). Because they can be raised in a wide range of production systems – from subsistence backyard ponds to high intensity farms - they have made a significant contribution to food production, poverty alleviation and livelihood support in Asia and the Pacific nations (De Silva et al., 2004).

Mozambique tilapia are maternal mouthbrooders (Trewavas, 1982a, Trewavas, 1982b). They can grow up to 40 cm long and 1.1 kg, and are considered a "model invader" because they are aggressive, have extraordinary environmental adaptability, phenotypic plasticity, high hybridization capacity and rapid reproduction (Pérez et al., 2006). They are considered an invasive species in Australia, and also in the Bahamas, Dominican Republic, Mexico and the United States of America (USA) (GISD, 2006). In Australia, tilapia have caused severe damage to the natural environment primarily through displacement of native species, habitat alteration, predation, and as a vector of diseases and non-native parasite transmission (Hutchison et al., 2011, IA-CRC, 2012a, Russell et al., 2012b, Russell et al., 2010, Wilson et al., 2019). A study conducted in Queensland (Greiner and Gregg, 2008) suggested that the current economic impact costs of tilapia may lie between A$1.2 million and A $13.6 million per annum (2020/21 dollar terms). If targeted efforts to control tilapia are not undertaken, the economic costs of tilapia in Queensland could increase to over A$35.4 million per annum (Hardaker and Chudleigh, 2021). Further, it is likely that, on a national scale, the impact costs could be significantly higher if tilapia are allowed to spread into other key Australian waterways, in particular to the Murray-Darling Basin (MDB).

Despite the importation of live tilapia into Australia being prohibited since 1963, the ornamental *O. mossambicus* from either Singapore or Indonesia were released by a Brisbane aquarist in 1977 (Bluhdorn and Arthington, 1989, McKay, 1977, McKay, 1978). Since then, the species has been reported to establish in many eastern catchments in Queensland, from Brisbane to Cairns (Fig. 1). The population in the Burnett catchment is of particular concern since this catchment is only two kilometres from the MDB watershed. Another area at high risk of invasion is the Gulf of Carpentaria (GoC) (IA-CRC, 2012b), in which both *T. mariae* and *O. mossambicus* recently established in the Walsh River catchment in 2017 and 2019, respectively (B. Holmes, *unpubl. data*). In addition, the species has also been established in Western Australia in Geraldton in 1978 and later in the Gascoyne, Chapman, Minilya and Lyndon Rivers, all of which constitute part of the Pilbara Drainage (Morgan et al., 2004).

T. mariae is a freshwater and estuarine cichlid native to West Africa and has become established in Australia, the United States and Russia (Courtenay and Robins, 1973, Ivoylov, 1986, Cadwallader et al., 1980). In contrast to *O. mossambicus*, which is a maternal mouthbrooder, *T. mariae* is a substrate-spawner – the females lay their eggs on hard substrate where they are fertilised by males (Russell et al., 2012a). Owing to its relatively low growth rate and fecundity, high natural mortality and

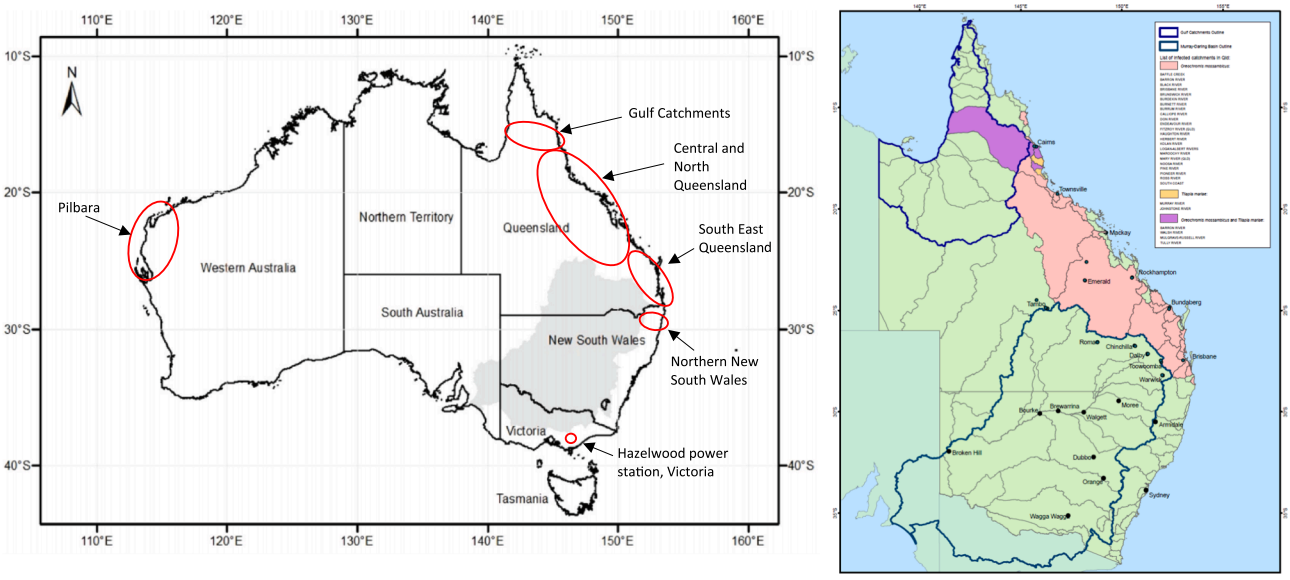

Fig. 1. Geographical distribution of tilapia in Australia. Left panel: red circles indicate approximate spread of tilapia across Australia (adapted from (Jha et al., 2013)). Right panel: *Oreochromis mossambicus* and *Tilapia mariae* distribution in Queensland (Source: Queensland Department of Agriculture and Fisheries). Blue borders indicate the Murray Darling Basin (in the south), and the Gulf of Carpentaria (in the north). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

small maximum size (32 cm long and 550 g) compared to other tilapia species, it is not extensively cultured (Bradford et al., 2011). Nevertheless, the attractively coloured *T. mariae* is a desirable ornamental fish and is most likely present in aquaria in many countries outside its natural range. When *T. mariae* was introduced to Australia is unclear (Bradford et al., 2011). The species was first found in a cooling pond of the Hazelwood power station in temperate Victoria in 1978 (Cadwallader et al., 1980). During the 1980s, the species was also detected near Cairns, North Queensland, and has since become established in surrounding river catchments and estuaries between Innisfail and Cairns (Webb, 2007). Recent spread of the species to the western-flowing Walsh River in North Queensland in 2017 has increased the risk of invasion across the GoC catchments and across to the Northern Territory in northern Australia (Fig. 1).

Nile tilapia is a highly invasive fish in more than 100 countries but is not yet established in Australia (De Silva et al., 2004, GISD, 2021, Valdez-Moreno et al., 2019, Welch, 2020). However, because of its mouthbrooding reproductive strategy and environmental adaptability, *O. niloticus* presents the same significant risk as *O. mossambicus* if found in Australia. Nile tilapia have been reported to cause severe harm to native biodiversity and ecosystems into which they are introduced (Canonico et al., 2005). These include alteration of water quality, eutrophication, and predation of eggs and young of other fish species which may lead to extinction of native fish species.

2. Management of invasive tilapia in Australia

While there is now an effective environmental DNA (eDNA) surveillance tool (Noble et al., 2015) for early detection and mapping of the distribution of tilapia, current management mechanisms are inadequate to control tilapia once establishment has occurred. Indeed, it is now clear that current education programs are failing to stop tilapia spread, and options for management post-incursion are extremely limited. Eradication is routinely attempted by using a combination of electrofishing and piscicide, but is rarely successful in open waterways because of the invasive nature of tilapia. Eradication was thought to be achieved for one incursion of *T. mariae* in a restricted length of Eureka Creek (Mitchell River Catchment) (Pearce et al., 2009). However, the detection of *T. mariae* in the same section of Eureka Creek again in 2019 cast doubt over the success of the original attempts. Eradication of infestations in other systems (e.g., Fitzroy River Catchment) has not been possible. Indeed, there is a lack of demonstrated effective control mechanisms for tilapia and thus there is a critical need to develop and evaluate other potential tilapia control agents.

Where feasible, biocontrol can be a cost-effective, safe and practical solution to manage invasive species at the landscape scale because it does not require reapplication of chemicals or poisons, and once established may be self-sustaining. Excellent examples of this methods are the use of myxoma virus (MYXV) and rabbit haemorrhagic disease virus (RHDV), which were released in 1950 and 1995, respectively, as biological control agents (BCAs) for rabbits in Australia. The sustained reductions of ~ 90 % of rabbit populations and the impacts of the two rabbit BCAs resulted in an estimated benefit of A$70 billion to Australia's agricultural industries in the 60 years between 1950 and 2010 (Cooke et al., 2013). Based on the success with the use of MYXV and RHDV, spring viremia of carp virus (SVCV; Rhabdovirus) was proposed as a potential BCA for common carp (*Cyprinus carpio*) (Stevenson, 1978), which are regarded as the most devastating invasive fish in Australia. However, subsequent research found that SVCV was not specific for carp (Family *Cyprinidae*). The virus not only infected other fish species within the Family *Cyprinidae* (for example, goldfish, tench), but also those of other families including sheatfish (*Siluridae*), guppy (*Poecilliidae*) and Northern pike (*Esocidae*) (Crane, 1995). Therefore, SVCV was inappropriate as a BCA and its investigation as a potential BCA for carp was terminated.

By 2000, Australia's *National Management Strategy for Carp Control* was adopted by the Carp Control Coordinating Group (CCCG, 2000). It recognised that the existing techniques to control carp, such as poisoning and physical removal, are often effective on a small scale but failed on a broad scale. A strategic research plan identified possible techniques for controlling carp including habitat manipulation, genetic control, and carp-specific pathogens. In the mid-2000 s, an investigation of koi herpesvirus (KHV) (Hedrick et al., 2000), taxonomically known as cyprinid herpesvirus 3 (CyHV-3) (Waltzek et al., 2005), as a potential BCA was proposed as part of an integrated carp control program (Fulton, 2006, McColl et al., 2007).

CyHV-3 was first reported in Israel and Germany in 1998 (Hedrick et al., 2000) and subsequently spread to at least 28 countries across Europe, America, Africa, and Asia (OIE, 2021b), including Indonesia, from which an isolate was transferred to Australia's high-containment laboratory, the CSIRO Australian Centre for Disease Preparedness (CSIRO ACDP). Subsequent research showed that an Indonesian isolate was highly virulent in carp sourced from Australian waters (Sunarto et al., 2011) and the virus was specific to carp (McColl et al., 2017). The results encouraged further investigations of CyHV-3 as a potential BCA as part of the National Carp Control Plan (NCCP, 2019).

Recently it has been suggested that combined viral biocontrol and genetic technologies would be a better approach for effective carp control and possible long-term eradication of carp (Thresher et al., 2014a, Thresher et al., 2014b). Based on our experience with viral biocontrol in rabbits and carp (McColl et al., 2014, McColl and Sunarto, 2020, Kerr et al., 2021, Strive and Cox, 2019), here we describe a systematic approach to assess known pathogens for their suitability as potential BCAs for tilapia, and outline the possible next steps to further investigate the top candidates.

3. Biological control agent assessment criteria

Initially, BCA assessment criteria adapted from Henzell et al. (2008) and Peacock (2015) for rabbit biocontrol in Australia were used to assess

Table 1
Biocontrol agent assessment criteria.

1.	**Appropriateness**
	Safety – the BCA should be species-specific, not infecting, let alone affecting, any non-target species in Australia (including humans). Socially acceptable – the nature and biological action of the BCA needs to be acceptable to the community. Humane – the BCA should not cause undue pain or suffering.
2.	**Effectiveness**
	Virulence – the BCA needs to cause high mortality in tilapia. Survivors are likely to seroconvert, become more resistant and may confer the resistance to their offspring through maternal immunity. This would likely lead to recovery of the tilapia populations. Impacts on all ages – ideally the BCA needs to have high impacts on both juvenile and adult tilapia. Effectiveness in wild fisheries – the BCA needs to cause high enough mortality to exceed productivity in wild tilapia populations. No unfavourable interaction with other pathogens – endemic pathogen(s) should not provide cross-protection against the BCA.
3.	**Efficiency**
	Transmission – the BCA should have the ability to transmit efficiently among tilapia and have the capacity to spread through the local, regional, and national tilapia populations (self-disseminating). Persists in the environment – the BCA should persist despite death of a high proportion of hosts and, once established, should causes repeated outbreaks. Cost for research and development – benefits should exceed the cost of testing the safety and efficacy of the BCA, risk assessment, and cost-benefit analysis. Cost for manufacture and distribution – preferably, the organism(s) could be cultured, prepared, and stored in large quantities to allow effective distribution. Public and government approval requirements – expected delay due to public and government approval processes.

Adapted from Henzell et al. (2008) and Peacock (2015)

the appropriateness, effectiveness, and efficiency of potential BCAs for tilapia in Australia (Table 1). The BCA assessment using the criteria summarised in Table 1 is a complex process and the selection criteria used in this review may not cover all aspects of the assessment. Another limitation of the assessment is that it involves subjective scoring, which affects the consistency of the results. For example, how many studies would have to be done to justify inclusion of a criterion, and then assessment of each criterion as "positive", "minor concerns", or "major concerns"?

In assessing potential BCAs for tilapia, the most important initial screening criteria were reduced to 'safety' and 'efficacy', just as had been done when assessing the potential of different viruses as potential BCAs for rabbits and carp. Species-specificity is an important determinant of the safety of a potential BCA, not only of the released BCA but also of any future generations of the agent that may arise following mutations in the field. On the other hand, it is virulence and transmission that are important in determining the efficacy of the BCA (Di Giallonardo and Holmes, 2015). Therefore, to be considered as a potential BCA candidate, an agent should, initially at least, satisfy-three key determinants – species-specificity (Table 1 criteria 1.1), high levels of virulence (criteria 2.1), and effective transmission (criteria 3.1).

3.1. Safety of the BCA

The BCA should have a narrow host range, affecting tilapia only. No other species sharing, or using, tilapia-infested waterways – be they other fish species, aquatic or terrestrial animals, or humans – should be affected (Peacock, 2015). The absence of disease in any species anywhere in the world, other than tilapia, would be the most compelling evidence for the specificity of the selected BCA. Nevertheless, given the unique nature of Australia's fauna (including its fish), it will be critical to assess the susceptibility to infection of various Australian species in a non-target species (NTS) testing program (McColl et al, 2017). OIE recommends a three-stage approach to assess susceptibility of a species to infection with a specific pathogen: 1) the route of transmission is consistent with natural pathways for the infection; 2) the pathogenic agent has been adequately identified; and 3) the presence of the pathogenic agent constitutes an infection (OIE, 2021a).

3.2. Efficacy of the BCA

The suitability of potential exotic BCAs will inevitably be based on published scientific evidence collected from overseas work, and further work may be required for assessments at a local level (Henzell et al., 2008). Most uncertainty relates to the likely virulence, transmissibility, and persistence of a BCA in wild fisheries. To be effective as a BCA, the agent needs to cause high mortality in tilapia of all ages. Ideally the BCA would be a self-disseminating agent that has the ability to transmit efficiently. For this reason, spread primarily by waterborne routes would be advantageous, as would persistence of the agent in the environment following the death of a high proportion of hosts.

Ideally, the BCA would have no unfavourable interaction with other pathogens. Endemic pathogen(s) should not provide cross-protection against the BCA such as occurred in Australia where previous exposure to non-pathogenic Australian rabbit calicivirus RCV-A1 increased survival of the rabbits during outbreaks of RHDV (Strive et al., 2013, Cooke et al., 2018). Clearly, research would be required to systematically assess the possibility of interfering endemic agents and viral mutants and reassortants arising if a virus was the chosen BCA (Chaput et al., 2020). This would involve, for example, *meta*-transcriptomic analyses (Turnbull et al., 2020) of Australian tilapia populations to identify the presence of other viruses.

3.3. Other factors affecting selection of a BCA

Having assessed the safety and efficacy of the selected BCA, consideration can then be given to other criteria listed in Tables 1. A naturally occurring agent in wild and farmed tilapia would likely be more socially acceptable than a genetically modified organism (GMO). The use of a GMO would also require additional time for approval and processing. In addition, a BCA that killed tilapia relatively humanely and not causing undue pain or suffering would be preferable (Sharp and Saunders, 2011).

Lethal pathogens have never been used or approved as controls for invasive fish, so a delay would be expected due to the need for public and government approval processes for the pathogenic biocontrol of tilapia. The requirements for, and consequences of, an aquatic BCA are quite different from those of a terrestrial BCA. For example, the impacts of fish kills on water quality and food webs need to be managed (Brookes and Hipsey, 2019, Beckett et al., 2019). Applying hydrological, ecological, and epidemiological modelling to test different scenarios and to predict the outcomes of the introduction of a BCA into a new environment will help inform the BCA release strategy for the biocontrol of tilapia in Australia (Joehnk et al., 2020, Durr et al., 2019).

3.4. The essential information required for the potential biocontrol of tilapia

Recently, selection criteria for a potential BCA were developed for another invasive pest fish in Australia, the common carp. McColl and Sunarto (2020) emphasized that, in developing a viral biocontrol program for carp, two basic criteria had to be met: an understanding of the biology of the targeted pest species and the potential BCA. For tilapia in Australia, there is a lack of understanding of the biology of the species in Australian conditions, and much also remains to be learned about potential BCAs, be they viral or some other infectious agent. Table 2 summarizes the essential information required for a potential biocontrol program on tilapia. The table identifies information already acquired about the targeted pest ('Knowns'), but, more importantly, summarizes the essential additional information that will be necessary not only to understand tilapia biology in Australia, but also to select an appropriate BCA ('Unknowns').

4. Biocontrol agent candidate assessment findings

Tilapia pathogens fall into the general categories of viruses, bacteria, fungi, and parasites. Overall, this bioprospecting review found that a large number of bacteria, fungi, and parasites have been associated with natural disease outbreaks in tilapia worldwide. However, none were specific for tilapia and therefore were rejected as BCA candidates. More promisingly, a number of viruses have been reported in tilapia.

4.1. Viruses

At least nine viruses have been detected in tilapia (Machimbirike et al., 2019), the first DNA virus being Lymphocystis disease virus (LCDV) (Paperna, 1973, Weissenberg, 1965), while infectious pancreatic necrosis virus (IPNV) was the first RNA virus (Hedrick et al., 1983). Neither has been associated with high natural mortality in tilapia, excluding them from consideration as BCAs. Seven viruses have been associated with disease outbreaks in tilapia. These are TiLV (Eyngor et al., 2014), TiPV (Liu et al., 2020), Tilapia larvae encephalitis virus (TLEV) (Shlapobersky et al., 2010), Bohle iridovirus (BIV) (Ariel and Owens, 1997), nervous necrosis virus (NNV) (Bigarré et al., 2009), infectious spleen and kidney necrosis virus (ISKNV) (Subramaniam et al., 2016, Suebsing et al., 2016), and iridovirus-like agents (McGrogan et al., 1998, Smith et al., 1997). Subramaniam et al. (2016) suggested that the irido-like viruses reported by Smith et al. (1997) and McGrogan et al. (1998) could actually be ISKNV isolates which would reduce the list to

Table 2
The essential information required for the potential biocontrol of tilapia.

Information required	Knowns	Unknowns
Tilapia biology in Australia	• Tilapia biomass across Australia	• Future estimates of tilapia biomass • Genomic and transcriptomic study of tilapia in Australia
Epidemiology of the BCA	• Global epidemiology	• Laboratory epidemiology • BCA epidemiology under Australian conditions • Genome of the BCA
Safety of the BCA (species specificity)	• Overseas field outbreaks	• Susceptibility of Australian native fish to the BCA • Human safety
Efficacy of the BCA	• Overseas field outbreaks	• BCA-host interactions: o Virus transmission (R_0) o Virulence of different BCA isolates in different strains of tilapia including tilapia hybrids • Survey of Australian tilapia for endemic pathogen(s)
Epidemiological modelling of the release and spread of the BCA	• TiLV as a model for the tilapia BCA	• Extensive studies required
Evolution of the BCA	• Rabbit and carp biocontrol viruses	• Increasing virulence or innocuity?
Broad-scale control measure(s) to complement the BCA	• Regional measures available	• Could other BCAs complement the selected BCA? • Genetic biocontrol
Social risks		• Views of local affected populations versus the Australian-wide population
Ecological concerns	• Ecological risk assessment	• Environmental clean-up procedures after fish kill events • Prey switching
Economic drivers	• Cost-benefit analysis of proposed investment in tilapia biocontrol • Business case for tilapia biocontrol	• Current and future impact and control costs associated with tilapia in Australia • Potential effectiveness and feasibility of the release of a BCA
Restoration benefits from tilapia control		• Expert elicitation study on the ecological consequences of reduced tilapia

Adapted from McColl and Sunarto (2020)

six candidates. None of the Iridoviruses (LCDV, BIV, ISKNV and Iridolike viruses) nor NNV are specific to tilapia, and therefore, were not considered as suitable candidates for BCAs. On the other hand, TiLV, TiPV, and TLEV are believed to be species-specific to tilapia (Table 3).

4.1.1. *Tilapia lake virus (TiLV)*

TiLV, taxonomically assigned as *Tilapinevirus tilapiae* under the genus *Tilapinevirus* and the family *Amnoonviridae* (Adams et al., 2017, Bacharach et al., 2016a, Kuhn et al., 2019; ICTV, 2021), is an enveloped and negative-sense ssRNA virus (Bacharach et al., 2016b, Eyngor et al., 2014). No other viruses within the family *Amnoonviridae* have been reported in tilapia (ICTV, 2021). The 10-segmented 10 kb genome contains 14 functional genes encoding 14 proteins (Acharya et al., 2019). Alignment analyses of segment 1 (Taengphu et al., 2020) and segment 3 (Skornik et al., 2020) as well as whole-genome sequences (Jansen et al., 2018) from geographically different isolates revealed high nucleotide identity, suggesting that a new recently-evolved virus has emerged. A relatively recent reassortment event, particularly those of segments 5 and 6, complicates phylogenetic analysis by individual segments and illustrates the need to exercise caution when using the analysis to infer geographical origin and the movement of the virus (Chaput et al., 2020). TiLV was first reported to cause mass die-offs in farmed and wild tilapia in Israel as early as summer 2009 (Eyngor et al., 2014). Around the same time, similar disease outbreaks called syncytial hepatitis of tilapia (SHT) were reported from farmed tilapia (*O. niloticus*) in Ecuador (Ferguson et al., 2014). The samples which were collected in 2011–2012 tested positive for TiLV (Del-Pozo et al., 2016). Since then TiLV has been reported from 16 countries across four continents (Surachetpong et al., 2020), suggesting that the virus is able to survive in different ecological niches and climates.

Natural morbidity and mortality due to TiLV are restricted to tilapia and tilapia hybrids (Surachetpong et al., 2017, Eyngor et al., 2014). Affected farmed species includes Nile tilapia (*O. niloticus*) in Ecuador (Ferguson et al., 2014), Egypt (Fathi et al., 2017), India (Behera et al., 2018), Indonesia (Koesharyani et al., 2018), Thailand (Dong et al., 2017b, Surachetpong et al., 2017) and Uganda (Mugimba et al., 2018); grey tilapia hybrid (*O. niloticus* × *O. aureus*) in Israel (Eyngor et al., 2014); red tilapia (*Oreochromis spp*.) in Thailand (Dong et al., 2017b, Surachetpong et al., 2017) and red tilapia hybrid (*O. niloticus* × *O. mossambicus*) in Malaysia (Amal et al., 2018). A wide range of wild tilapiines including *Tilapia zilli, O. aureus, Sarotherodon (Tilapia) galilaeus* and *Tristamella simonis intermedia* from the Kinneret Lake in Israel (Eyngor et al., 2014), wild black tilapia (*Oreochromis* spp.) in Malaysia (Abdullah et al., 2018), wild Nile tilapia in Lake Victoria (Tanzania and Uganda) (Mugimba et al., 2018) and in Peru (OIE, 2018) have been affected by TiLV.

Other fish species co-cultured with tilapia have not been affected by TiLV. These include grey mullet (*Mugil cephalus*) and common carp (*C. carpio*) in Israel (Eyngor et al., 2014); grey mullet and thin-lipped mullet (*Liza ramada*) in Egypt (Fathi et al., 2017); rohu (*Labeo rohita*), catla (*Catla catla*), mrigal (*Cirrhinus mrigala*), milk fish (*Chanos chanos*) and pearl spot (*Etroplus suratensis*) in India (Behera et al., 2018). However, wild river barb (*Barbonymus schwanenfeldii*) was found to be TiLV-positive by RT-PCR in Malaysia (Abdullah et al., 2018). Clearly, there is a need to differentiate TiLV genomic RNA (gRNA) from mRNA, the latter indicating viral replication in the host, particularly in non-target species such as river barb that was gRNA-positive by RT-PCR.

Experimental infection of 10 warm-water fish species including giant gourami (*Osphronemus goramy*), snakeskin gourami (*Trichogaster pectoralis*), iridescent shark (*Pangasianodon hypophtthalmus*), walking catfish (*Clarias macrocephalus*), striped snakehead fish (*Channa striata*), climbing perch (*Anabas testudineus*), common carp (*C. carpio*), silver barb (*Barbodes gonionotus*), Asian sea bass (*Lates calcarifer*), and red hybrid tilapia (*Oreochromis* spp.) revealed that only red hybrid tilapia and giant gourami were affected by TiLV (Jaemwimol et al., 2018). The mortality of red hybrid tilapia infected with TiLV by intraperitoneal (IP) injection was 63–85 % and that of giant gourami was 100 %. Despite the cumulative mortality of giant gourami being significantly higher than that of tilapia, only 53.55 % (8/15) of giant gourami samples were TiLV-positive by RT-qPCR compared to 100 % (15/15) of those of tilapia, suggesting that not all dead giant gourami may have been infected with the virus. African cichlid (*Aulonocara* spp.) is susceptible to TiLV infection (Yamkasem et al., 2021a). However, cichlids endemic to India, *viz* pearlspot (*Etroplus suratensis*), orange chromide (*Pseudetroplus maculatus*), and canara pearlspot (*E. canarensis*), are not susceptible to TiLV infection (Thangaraj et al., 2022). Zebrafish are susceptible to TiLV infection via intraperitoneal injection but not cohabitation (Widziolek et al., 2020, Rakus et al., 2020).

Wide variations in mortality associated with TiLV have been reported in wild and farmed tilapia. For example, 0.71 % mortality in wild black tilapia (*O. niloticus*) and 15–25 % in farmed red hybrid tilapia (*O. niloticus* × *O. mossambicus*) have been reported in Malaysia (OIE, 2017b, Abdullah et al., 2018, Amal et al., 2018). Similarly, low mortality

Table 3
Summary of information for the candidate biocontrol agents worthy for further investigation.

Candidate biocontrol agents	Appropriateness			Effectiveness				Efficiency				
	Species specificity	Socially acceptable	Humane	Virulence in tilapia	Impacts on all ages of tilapia	Effectiveness in wild fisheries	Interactions with other pathogens	Transmission	Persists in the environment	Cost for research & development	Cost for manufacture & distribution	Public and government approval requirements
Tilapia lake virus (TiLV)	TiLV causes disease outbreaks and mortalities in farmed and wild tilapia, but not in other fish species co-cultured or sharing waterways with tilapia. Wild river barb was found to be TiLV-positive by RT-PCR and giant gourami was affected by TiLV via IP injection and co-habitation challenges.	TiLV is a naturally occurring virus in wild and farmed tilapia (not a GMO). Prototype vaccines are available to protect farmed and ornamental tilapia.	Acute mortality occurs within a few days post infection. Chronic up to 24 days and sub-clinical infection have also been observed.	Experimental infection of tilapia with TiLV conducted in geographically different regions resulted in consistently high levels of mortality. Wide variations in mortality associated with TiLV have been reported in wild and farmed tilapia, ranging from0.71% to 90%.	TiLV has been reported to cause mortality in all age groups of tilapia.	TiLV causes mortality of wild tilapia in Israel, Malaysia, Tanzania, Uganda, and Peru. The virus causes significant decline in tilapia populations in Kinneret Lake, Israel.	No antagonistic interactions have been observed during co-infections with other pathogens in tilapia. No other viruses within the family *Amnoonviridae* have been reported in tilapia.	Epidemiological findings and cohabitation mode of horizontal transmission demonstrates the ability of TiLV to spread by waterborne routes. Vertical transmission has also been observed.	Most likely but need to determine how long TiLV survives in the water and in dead fish. TiLV RNA has been detected in mucus, feces and water tanks containing TiLV-infected fish.	Medium-sized project to test the efficacy (virulence and transmission). Large project to test the safety (susceptibility of non-target species).	TiLV grows in cell cultures and could be transported in freeze dried formor cold at 4°C.	Expected delay due to public and government approval processes. Australia has very strong legislative mechanisms for approval of biocontrol agent (Biological Control Act 1984), which may facilitate the processes.
Tilapia parvovirus (TiPV)	It has only been reported in tilapia in China and Thailand.	Vaccine is not available.	TiPV causes acute mortality in tilapia within 11 days.	TiPV causes 60-70% mortality in cage-farmed tilapia.	TiPV has been reported in all size of adult tilapia.	Unknown	No other parvovirus has been reported in tilapia or any other fish species.	TiPV is contagious, spreading to six cities in three provinces in China.	Unknown	Little is known about the characteristics of the virus.	TiPV grows in cell culture.	Ditto above.
Tilapia larvae encephalitis virus (TLEV)	It has only been reported in tilapia in Israel.	Vaccine is not available.	The virus affects brain with clinical signs of a whirling syndrome.	High mortality rates of up to 96% and 80% in blue and red tilapia larvae, respectively.	TLEV has only been reported in larvae of tilapia.	Unknown	No other herpesvirus has been reported in tilapia.	TLEV is capable of both vertical and horizontal transmission.	Unknown	Little is known about the characteristics of the virus.	TLEV has not been isolated or cultured in cell lines.	Ditto above.

Key: Positive | Minor concerns | Major concerns

of 6.4 % and 9.2 % in farmed tilapia have been reported in Chinese Taipei (OIE, 2017a) and Egypt (Fathi et al., 2017), respectively, the latter experiencing "summer mortality" in which TiLV was detected but the causal link was inconclusive (Nicholson et al., 2017). Subclinical infections have been reported in farmed tilapia in Thailand (Senapin et al., 2018) as well as in wild and farmed tilapia in Lake Victoria (Tanzania and Uganda) (Mugimba et al., 2018). In contrast, TiLV has caused disease outbreaks in wild tilapia populations in Israel and decreased the annual yield of *Tilapia galilaeus* from the Kinneret Lake from 316 t in 2005 to 52, 8, and 45 tons in 2007, 2009, and 2010, respectively (Eyngor et al., 2014). Interestingly, although the lake hosts 27 species of fish encompassing members of the families *Cichlidae*, *Cyprinidae*, *Mugillidae*, and *Claridae*, only tilapia (*Cichlidae*) were affected. In farmed tilapia, the disease resulted in massive mortality in Israel (Eyngor et al., 2014), 10–80 % mortality in Ecuador depending on the tilapia strain (Ferguson et al., 2014), 20–90 % mortality in Thailand (Dong et al., 2017b, Surachetpong et al., 2017) and 80–90 % in India (Behera et al., 2018).

Experimental infection of tilapia with TiLV by intragastric, intra-coelemic, and IP routes, and by cohabitation conducted in geographically different regions resulted in consistently high levels of mortality. The mortality of Nile tilapia infected with TiLV via intragastric and intra-coelemic routes was 40–45 % and 70 %, respectively, which occurred from 6 to 15 days post infection (dpi) (Pierezan et al., 2020, Pierezan et al., 2019). The mortality of cohabitating tilapia was 55.71 % from 3 to 15 dpi (Liamnimitr et al., 2018) and 80 % from 4 to 9 dpi (Eyngor et al., 2014). Virus challenge by IP injection resulted in high mortality, ranging from 75 to 85 % which occurred from 2 to 10 dpi (Eyngor et al 2014), 66–88 % from 1 to 12 dpi (Tattiyapong et al., 2017), 63–85 % from 4 to 24 dpi (Jaemwimol et al., 2018) and 100 % from 3 to 7 dpi (Behera et al 2018).

The causes of the variation in mortality are not known, but they may be attributed to different species, strain or family of tilapia, culture systems or other environmental factors. For example, 80 % mortality in the Chitralada strain compared to 10–20 % mortality in all male Genetically Improved Farmed Tilapia (GIFT) have been reported in Ecuadorian farms, despite both being *O. niloticus* (Ferguson et al., 2014). Furthermore, host resistance to TiLV is highly heritable in families of the GIFT strain, suggesting that selective breeding to increase the resistance of farmed tilapia to TiLV is feasible (Barría et al., 2020). Clinical outbreaks of TiLV have been reported in summer at water temperature of 22 to 32 °C in Israel (Eyngor et al., 2014), ≥25 °C in Egypt (Fathi et al., 2017) and 25 to 27 °C in Ecuador (Ferguson et al., 2014), suggesting that temperature plays an important role in TiLV outbreaks. Co-infection of TiLV with other pathogens including *Aeromonas spp*., particularly *A. veronii*, may also affect the severity and outcome of the disease (Amal et al., 2018, Nicholson et al., 2017, Rao et al., 2021). Although stocking density, dissolved oxygen levels and pond production cycles have been considered as risk factors of TiLV disease in aquaculture settings, no single factor has been attributed to TiLV outbreaks (Ali et al., 2020, Kabuusu et al., 2017). In controlled laboratory conditions, mortality is also dose-dependent, in which mortalities of 48.89 % and 77.78 % were observed in *O. mossambicus* IP-injected with low (10^3 $TCID_{50}$/mL) and high (10^5 $TCID_{50}$/mL) doses of TiLV, respectively (Waiyamitra et al., 2021). It is estimated that the LD_{50} of TiLV by IP injection was 5.7×10^4 $TCID_{50}$ (Yang et al., 2018).

Although small fish are more susceptible to TiLV infection than larger fish (Roy et al., 2021), all age groups of tilapias appear to be susceptible to TiLV. Fertilized eggs, larvae, fry, fingerlings, juveniles, adults and broodstocks of tilapia have tested positive for, or been affected by, TiLV (OIE, 2017c, OIE, 2018, Dong et al., 2017a, Behera et al., 2018, Eyngor et al., 2014, Ferguson et al., 2014, Pulido et al., 2019, Surachetpong et al., 2017, Yamkasem et al., 2019). Cumulative mortality of broodstock was 5–10 % while that of fry was 90–100 % (Yamkasem et al., 2019), suggesting that the maturity of the host's immune system may play a role in the outcome of the disease. TILV has also been detected in reproductive organs including ovary and testis, suggesting that TiLV can be vertically transmitted. The detection of TiLV RNA in mucus (Liamnimitr et al., 2018), feces and water tanks containing TiLV-infected fish (Pierezan et al., 2019) and cohabitation mode of horizontal transmission (Eyngor et al., 2014, Liamnimitr et al., 2018) demonstrates the ability of TiLV to spread by waterborne routes, an important pathway for a successful biocontrol agent of aquatic invasive fish.

Natural co-infections of TiLV and other pathogens including parasites, bacteria (*Aeromonas hydrophila, A. veronii, A. isthiosmia, A.*

enteropelogenes, Streptococcus agalactiae) and virus (*Tilapia parvovirus*, TiPV) have been reported in farmed tilapia (Yamkasem et al., 2021b, Amal et al., 2018, Basri et al., 2020, Nicholson et al., 2017, Nicholson et al., 2020, Rao et al., 2021, Surachetpong et al., 2017). Mortality rates due to TiLV outbreaks among tilapia farms in Thailand were 20 %-90 %, in which higher rates were associated with secondary bacterial and parasitic infections (Surachetpong et al., 2017). Co-infections of TiLV and *A. veronii* in farmed red hybrid tilapia in Malaysia resulted in 25 % mortality (Amal et al., 2018) while that of *TiLV, A. hydrophila* and *S. agalactiae* was 70 % (Basri et al., 2020). An experimental challenge in tilapia, in which co-infection of TiLV and *A. hydrophila* resulted in 93 % mortality while those of either TiLV or *A. hydrophila* alone was 34 % and 6.7 %, respectively (Nicholson et al., 2020) supported the reported high rate of mortality during co-infections in farmed tilapia. These results are also consistent with those of other bacterial and viral co-infections in tilapia, in which multiple infections have a synergistic effect that resulted in increased severity of the disease and higher rate of mortality in tilapia (Dong et al., 2015, Abdel-Latif et al., 2020).

Mathematical modelling estimated the reproductive number (R_0) for Nile tilapia infected with TiLV at 2.6×10^5 $TCID_{50}$/fish via cohabitation was 2.59, indicating that the virus was spreading within a tilapia population and the incidence of the disease was increasing under the test conditions (Yang et al., 2018). Furthermore, the authors estimated that the population of Nile tilapia decreased to 12 % of the initial population size after 16 dpi. These epidemiological findings suggest that TiLV is contagious and once established has the ability to persist in the environment and causes repeated outbreaks in tilapia populations.

TiLV causes disease outbreaks and mortalities in farmed and wild tilapia populations, but not in other fish species co-cultured or sharing waterways with tilapia (Eyngor et al., 2014, Surachetpong et al., 2017, Behera et al., 2018, Fathi et al., 2017), suggesting that TiLV is specific to tilapia. Though tilapia and its hybrids are the only species known naturally to be affected by TiLV, viral genomic RNA has also been detected by RT-PCR in healthy wild river barb (Abdullah et al., 2018) and mortality in giant gourami experimentally infected with TiLV has been reported (Jaemwimol et al., 2018). However, only 53.55 % (8/15) of giant gourami samples were TiLV-positive by RT-qPCR compared to 100 % (15/15) of those of tilapia, suggesting that not all dead giant gourami may have been infected with the virus. The huge difference of mortality rate of giant gourami infected with TiLV by IP injection (100 %) and co-habitation (5 %) further raises questions if the giant gourami is a true alternative host for TiLV. Although TiLV appears to be specific for tilapia, and although there are no native Australian fish belonging to the families *Cichlidae* (tilapia), *Osphronemidae* (gourami) or *Cyprinidae* (carp and barb), rigorous non-target species testing would be required before the use of any viral biocontrol could be considered. This has been the case with the proposed viral biocontrol agents for carp (McColl et al., 2017) and would be equally applicable for tilapia biocontrol to overcome concerns about the specificity of TiLV.

4.1.2. Tilapia parvovirus virus (TiPV)

Recently, a novel virus tentatively named TiPV has emerged in cage-cultured tilapia in China (Liu et al., 2020). TiPV is a spherical, 30 nm in diameter, non-enveloped virus with a linear, non-segmented, ssDNA genome (4269 bp) which consists of two major ORFs encoding NS1 and VP1 proteins. The virus is tentatively classified into a newly proposed genus of Chapparvovirus within the family *Parvoviridae* (ICTV, 2021). The first outbreaks of the disease were reported in farmed Nile tilapia from August to September 2015 in Hubei, China. Since then, it has been reported from six cities across three provinces in China. The disease affected adult tilapia resulting in 60–70 % mortality. Clinical signs of diseased fish include anorexia, lethargy, darting or corkscrew movements, haemorrhages on the body surface, lower jaw, anterior abdomen and fin bases, exophthalmia and pronounced ocular lesions. Most outbreaks occurred at water temperatures of 28–30 °C, but samples collected at water temperature from 22 to 32 °C have also been reported positive for TiPV, suggesting that temperature may play a role in disease outbreaks. The virus has been isolated on tilapia brain cells allowing further studies including experimental infection, in which the virus caused 90 % mortality within 11 days at 28 °C, similar to those naturally observed in cage culture systems. In November 2020, TiPV was detected in juvenile red tilapia during a disease outbreak associated with TiLV in Thailand (Yamkasem et al., 2021b). Owing to the nature of the outbreak (co-infection with TiLV), the role of TiPV in this outbreak is unknown.

4.1.3. Tilapia larvae encephalitis virus (TLEV)

Based on morphological, biophysical and very limited phylogenetic analyses, TLEV resembles a herpes-like virus (Shlapobersky et al., 2010). The virus has been associated with a high mortality rate in tilapia larvae including laboratory-reared blue tilapia (*O. aureus*), *O. niloticus* and *S. galilaeus*, in Israel. The disease is characterised by a whirling syndrome (a spiral swimming behaviour), darkened skin in blue tilapia and pale skin in red tilapia followed by high mortality rates of up to 96 % and 80 % in blue and red tilapia larvae, respectively. The virus was capable of both vertical transmission and horizontal transmission through water from infected fish (Sinyakov et al., 2011). After the first outbreaks of TLEV in tilapia larvae in Israel a decade ago (Shlapobersky et al., 2010, Sinyakov et al., 2011), the virus was never reported again either in Israel or in other countries, raising a question of whether the virus still persists in the environment. The virus has only been associated with mortalities in tilapia larvae in hatcheries, suggesting that the impact of TLEV in adult tilapia and its effectiveness in wild fisheries may be insignificant. TLEV has not been isolated in cell cultures, hindering further characterisation of the virus, and therefore, the cost for research and development as well as manufacture and distribution are major concerns.

4.1.4. Nervous necrosis virus (NNV)

NNV is the causative agent of viral nervous necrosis (VNN) otherwise known as viral encephalopathy and retinopathy (VER), a lethal disease of many marine and freshwater fish species associated with vacuolation of the central nervous system and the retina (Yoshikoshi and Inoue, 1990, OIE, 2019b). NNV is a small non-enveloped virus with positive sense ssRNA molecules. It belongs to the genus *Betanodavirus* within the family *Nodaviridae* (Mori et al., 1992). Following the first report of VNN outbreaks in Nile tilapia larvae in France (Bigarré et al., 2009), the disease has also been associated with mortality of tilapia larvae in Thailand (Keawcharoen et al., 2015), Indonesia (Yanuhar et al., 2018), and Egypt (Taha et al., 2020). The disease has been reported in more than 50 species belonging to 32 families from 12 different orders (OIE, 2019b). Furthermore, 177 marine species are susceptible to the virus and natural disease outbreaks of VNN have been reported in 62 marine and 12 freshwater fish species (Bandin and Souto, 2020).

4.1.5. Iridoviruses

Family *Iridoviridae* consists of two sub-families: *Alphairidovirinae* (*Lymphocystivirus, Ranavirus* and *Megalocytivirus*) which infect ectothermic vertebrates (bony fish, amphibian, and reptiles) and *Betairidovirinae* (*Iridovirus* and *Chloriridovirus*) which infect insects and crustaceans (Chinchar et al., 2017). Four iridoviruses including LCDV (*Lymphocystivirus)*, Bohle iridovirus (*Ranavirus*), ISKNV (*Megalocytivirus*) and Irido-like viruses, which are possibly ISKNV isolates, have been reported in tilapia. LCDV infection was first reported in South American cichlid in Guatemala (Weissenberg, 1965) and in African tilapia in East Africa (Paperna, 1973). The virus has been associated with the formation of wart-like growths, but mortalities have not been recorded in tilapia. Lymphocystiviruses infect more than 100 species of marine and freshwater fish (Chinchar et al., 2017).

Bohle iridovirus was first isolated from metamorphs of the ornate burrowing frog (*Limnodynastes ornatus* Gray,1842) in Bohle, North Queensland, Australia (Speare and Smith, 1992). Since then, the virus has been shown to infect amphibians, reptiles, and fish including tilapia (*O. mossambicus*) (Ariel and Owens, 1997), suggesting that BIV are

capable of infecting hosts from different classes (Chinchar et al., 2009, Chinchar et al., 2017). Natural disease outbreaks in tilapia associated with ISKNV infections have been reported in Canada, the USA and Thailand (McGrogan et al., 1998, Subramaniam et al., 2016, Dong et al., 2015, Suebsing et al., 2016, Smith et al., 1997). Mortalities of 50–75 % among Nile tilapia fry (Subramaniam et al., 2016) and up to 50 % in adults (Dong et al., 2015) were much lower than those in one of the main host, mandarin fish (*Siniperca chuatsi*), where mortality was up to 100 % (He et al., 2000). ISKNV is not only highly pathogenic in mandarin fish, but also able to infect 13 cultured and 39 wild marine fish species in the South China Sea (Wang et al., 2007) as well as freshwater fish (Chinchar et al., 2017).

4.2. Bacteria

Bacteria are potentially deadly pathogens for both wild and cultured fish and are responsible for mass mortality events in aquaculture facilities across the globe (Ibrahim, 2020). However there are no bacteria specific for tilapia (Plumb and Hanson, 2011). Six major bacterial pathogens associated with mortality events in tilapia have been documented and include the following genera: *Streptococcus, Aeromonas, Flavobacterium, Francisella*, *Edwardsiella* and *Pseudomonas* (Bromage et al., 1999, Anshary et al., 2014, Raj et al., 2019, Tartor et al., 2021, Plumb and Hanson, 2011, Ibrahim, 2020). These bacteria have not only caused natural outbreaks in other freshwater fish (Pękala-Safińska, 2018), but also in marine fish species (Toranzo et al., 2005). Therefore, all are inappropriate as BCA candidates.

4.3. Fungi

The most common fungal infection in freshwater fish is Saprolegniosis (El-Deen et al., 2018, Torto-Alalibo et al., 2005), while the fungal disease considered the most detrimental to freshwater, brackish water, wild and farmed fish throughout the world appears to be *Aphanomyces invadans* (Afzali et al., 2015). Interestingly, *O. niloticus* (Afzali et al., 2015) and *O. mossambicus* (Lilley et al., 1998) appear to be resistant to this deadly fungus while other tilapia species including *O. andersoni, O. machrochir, T. rendalli* and *T. sparrmanii* (OIE, 2019a) and at least 94 other fish species have been identified as susceptible to *A. invadans*. Likewise, none of the Saprolegnia and Branchiomyces detected in mass mortalities of tilapia are species-specific to tilapia.

4.4. Parasites

Numerous fish parasites exist which cause mass mortality in cultured tilapia, particularly in young ages. In addition to the damage caused by *O. mossambicus* in Australia, it appears that some of their exotic parasites have likely been co-introduced from African rivers and tributaries as four species of parasites - three monogeneans (*Cichlidogyrus tilapiae, C. sclerosus, C. halli)* and one trichodinid (*Trichodinia sp*) - have been reported on both African native and introduced Australian tilapia (Wilson et al., 2019). The most serious monogenean parasites in tilapia, *Gyrodactylus* sp., and the most numerous protozoans, *Trichodina* sp., are not species-specific to tilapia. A novel Myxosporean parasite, *Myxobolus bejeranoi,* has only been reported in tilapia hybrid (*O. aureus* male × *O. niloticus* female), which is an important aquaculture species in Israel (Lövy et al., 2018). However, the effectiveness of *Myxobolus* spp. in wild fisheries is unknown. In fact, every parasite found in aquaculture facilities are present in wild fish populations but most of them are not associated with disease outbreaks (Valladao et al., 2018) and therefore the species specificity of *Myxobolus* spp. is a major concern.

5. Discussion

A wide range of pathogens associated with disease outbreaks and mortalities in tilapia were assessed for their potential as BCAs for tilapia in Australia. No bacteria, fungi or parasites are considered as being host specific to tilapia. Although many bacteria (e.g. *Salmonella* spp. for rodents), fungi (chytrid fungus for frogs) and parasites (protozoa for rats) have been proposed and tested as potential BCAs for vertebrates pests, only viruses have demonstrated efficacy and been successfully released (Saunders et al., 2010). To date, there have only been three successful viral biocontrols of vertebrate pests: FPLV (parvovirus) contributing to the elimination of cats on Marion Island, and MYXV (myxoma virus) and RHDV (calicivirus) to control the feral rabbit population in Australia and New Zealand (Saunders et al., 2010, McColl et al., 2014). The remarkable success of MYXV and RHDV in the biological control of rabbits in Australia has led to ongoing research into similar solutions for other vertebrate aquatic pests including carp and recently tilapia.

Out of nine viruses detected in tilapia, six viruses (LCDV, IPNV, BIV, VNN, ISKNV and Irido-like viruses which are possibly ISKNV isolates) were first reported in species other than tilapia and therefore are not suitable as BCA candidates. However, three viruses originally reported in tilapia (TLEV, TiPV and TiLV) are apparently specific to tilapia, and therefore are categorised in this review as being tentatively worthwhile biocontrol candidates for further investigation. TLEV was categorised under a 'watching brief'. This means that TLEV was not currently selected for further investigation but will be watched as a possible future BCA through the international literature and scientific networks. TiPV is the first and only parvovirus known to infect fish and information on the virus is limited. However, it has been reported in two countries. Therefore, TiPV was categorised as 'tentatively worthwhile' for further investigation. TiLV was considered the most promising potential BCA candidate and was categorised as 'worthwhile for active further investigation'. These findings have been used to inform the cost-benefit analysis and business case for tilapia biocontrol in Australia. The successful identification of TiLV as the most promising BCA candidate and the positive cost-benefit ratio of 1 to 2.81 from this project suggest that the investment in tilapia biocontrol research is likely to be worthwhile (Hardaker and Chudleigh, 2021).

CSIRO has already imported TiLV isolates into Australia's high-containment laboratory (CSIRO ACDP) and developed the capability to work with this exotic virus in a laboratory setting. To demonstrate the efficacy of TiLV as a potential BCA for tilapia in Australia, the susceptibility of two tilapia species present in Australian waterways (*O. mossambicus* and *T. mariae*) to TiLV is being tested. If the two tilapia species are susceptible to TiLV, work would progress following a process similar to approved rabbit biocontrol (IA-CRC, 2014) and currently underway for carp biocontrol (NCCP, 2019). This process broadly consists of the following components: safety and efficacy testing, initially, followed by hydrological, ecological, epidemiological and economic modelling, and development of optimised release strategies. Social and ecological risk assessments, bioethical issues and public acceptance will be needed to support an application to release a new BCA against tilapia in Australia. If a new tilapia BCA is approved for release in Australia, a structured collaborative program of release strategies, clean-up, and post-release monitoring and evaluation will be developed similar to the program for carp control (McColl and Sunarto, 2020).

Further work including the identification of other broad-scale control measure(s) such as genetic control to complement the virus would need to be considered (Wedekind, 2019). Australia is currently investing in research to investigate these broadly applicable technologies for managing invasive fish species. A prerequisite for genetic biocontrol approaches is a thorough assessment of the genetic diversity of Australian tilapia (population genomics analyses). This is important as there is already significant evidence of hybridisation occurring among wild tilapia populations (Ovenden et al., 2014). The simultaneous use of two or more classical control methods could also provide a more effective means of controlling invasive tilapia. Such methods could include electrofishing, trapping, the use of chemical attractants, and habitat restoration.

6. Conclusions

Nine viruses have been reported in tilapia, but only three (TLEV, TiPV and TiLV) are considered to be specific for tilapia. TiLV is considered as the most promising potential BCA candidate and its susceptibility testing in two tilapia species present in Australian waterways is underway.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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CHAPTER 2: SUSCEPTIBILITY OF AUSTRALIAN-ORIGIN MOZAMBIQUE TILAPIA (*OREOCHROMIS MOSSAMBICUS*) AND SPOTTED TILAPIA (*PELMATOLAPIA MARIAE*) TO TILAPIA LAKE VIRUS[3]

2.1 INTRODUCTION

The term tilapia encompasses a large number of fish species within the family *Cichlidae*. The group includes one of the most important freshwater aquaculture species globally (FAO 2019) and one listed in the top 100 of the world's worst invasive species (IUCN 2014). Tilapia are invasive pest fish in some Australian waterways where they damage freshwater ecosystems through habitat alteration, including eutrophication. They negatively impact native fish indirectly by competition for habitat, and directly through disease transmission and predation of eggs and larvae (Canonico et al. 2005).

At present, there are feral populations of *O. mossambicus* (commonly known as Mozambique tilapia) and *Pelmatolapia mariae*, commonly referred to as spotted tilapia or black mangrove cichlid) with different distributions in Australia (reviewed by Sunarto et al. 2022). The Nile tilapia (*O. niloticus*) which is the most important aquaculture species in other countries, is not present in Australia. Following release from an aquarium in 1977, *O. mossambicus* has established in eastern catchments of Queensland from Brisbane to Cairns, in Western Australia, and, since 2014, in Cudgen Lake on the NSW far north coast (NSW DPI 2023). This is a highly fecund mouth-brooding species that can grow to 40 centimetres (cm) in length, adapt to a wide range of environmental conditions and hybridise with related species (CISS 2023). *P. mariae* is present in northern Queensland and a self-sustaining population inhabits a heated power station pondage at Hazelwood, Victoria. This species is of lower concern compared to *O. mossambicus* as a less fecund substrate spawner with a lower growth rate to a maximum size of 30 cm. The economic impact of tilapia in Queensland was estimated to be $1.2–13.6 million, with the potential to be significantly higher if tilapia spread to additional waterways, in particular the Murray–Darling Basin (MDB) and the Gulf of Carpentaria (Hardaker and Chudleigh 2021).

Tilapia lake virus (TiLV) has recently emerged as the cause of high mortality disease of *Oreochromis spp*. and their hybrids, as well as other tilapia species with a range extending to 16 countries and four continents (Jansen et al. 2018). The virus was first identified in Israel in 2014 following a decreased wild catch of tilapia since 2009 and disease outbreaks on farms. Early cases of TiLV were retrospectively identified in the same time frame in Ecuador (reviewed by Surachetpong et al. 2020). TiLV has a 10 kilobase single-stranded negative sense RNA genome with 10 segments and enveloped virions that are spherical and 75–80 nanometres (nm) in diameter (Bacharach et al. 2016). The virus is classified as species *Tilapia tilapinevirus*, genus *Tilapinevirus* within the family *Amnoonviridae*, which is most closely related to the *Orthomyxoviridae* (ICTV 2021). Disease caused by TiLV generally causes high mortality in farmed and wild fish, with the impact of the local conditions leading to variability from 10% to 100% (Aich et al. 2022). Affected fish have widespread pathology reflecting systemic infection, including severe pathology with syncytial cells and necrosis in the liver (Pierezan et al. 2020).

The host range of TiLV beyond tilapia is limited with natural infection and evidence of disease limited to giant gourami (*Osphronemus goramy*) and detection of the virus in the absence of disease in a few other species (reviewed by Surachetpong et al. 2020). At present, there is no information about the

[3] Work conducted at EMAI for CSIRO; report prepared by Paul Hick and Peter Kirkland

susceptibility of *P. mariae* to TiLV and given the genetic variability of *O. mossambicus* and its propensity to hybridise, the susceptibility status of tilapia of Australian origin needs to be established. There is the possibility of using TilV as a biocontrol agent to minimise the impact of feral tilapia in Australia (Sunarto et al. 2022). The objective of this study was to evaluate the susceptibility of Australian-origin *O. mossambicus* and *P. mariae* to infection and disease caused by TiLV as an initial step in evaluating its suitability for biocontrol.

2.2 MATERIALS AND METHODS

The study was conducted according to the following approvals: 1) Queensland Department of Agriculture and Fisheries' Notice of approval for scientific research (restricted imported matter) permit no. PRID000536, 20; 2) James Cook University's Animal Ethics Committee, project ID A2631; 3) Department of Agriculture, Fisheries and Forestry's Notice of approval to use restricted imported biological material for in-vivo use 2022/093, including approval to transfer material imported on Import Permit 001005013; 4) NSW Department of Primary Industries Biosecurity permits RDOC22/140889, RDOC22/140676 and RDOC22/139388; 5) Elizabeth Macarthur Agricultural Institute Animal Ethics Committee, project ID 339; and 6) Elizabeth Macarthur Agricultural Institute Institutional Biosafety Committee, M22/04.

2.2.1 SOURCE OF TILAPIA

Twelve wild *O. mossambicus* (>10 cm) were collected via cast net in Louisa Creek in Townsville and Plantation Creek in the Burdekin region, assisted by Dr Geoff Collins of Ozfish. The same number and length of wild *T. mariae* were collected via electrofishing in Lake Tinnaroo on the Atherton Tablelands, assisted by Terry Valence of Tropical River Consulting. Fish broodstock were brought back to James Cook University (JCU) and divided into two groups, with six fish per group. Each group of *O. mossambicus* was held in a 700 litre (L) recirculating system. Environmental enrichment devices, including pebbles, PVC pipes and clay pots were introduced into the tanks to simulate their natural environment. Each group of *T. mariae* was held in a 1000 L recirculating system. Unlike *O. mossambicus*, *T. mariae* are not mouth brooders and require substrate to lay their eggs, therefore sand and logs were introduced into the tanks to simulate their natural environment, in addition to the environmental enrichment devices including pebbles, PVC pipes and clay pots. *T. mariae* can be very territorial and aggressive during breeding, so a breeding pair was separated from the other broodstock and placed in their own 1000 L recirculating system.

Water temperature was set at between 27–28 °C and lighting was set to a 12-hour light cycle to provide optimal conditions for breeding. Broodstock was fed a combination of fish pellets and algae wafters and water exchanges were regularly undertaken to maintain good water quality. When the *O. mossambicus* frylings hatched, they were quickly separated into a nursery tank to avoid predation by the other broodstock. *T. mariae* 'parent' their frylings, so these frylings were left in the breeding tank to be protected by the parents. Frylings were initially fed micron fry food and transitioned to algae flakes and wafers as they grew to fingerling size. Once both species reached fingerling size, they were packaged and shipped via air freight to the Elizabeth Macarthur Agricultural Institute (EMAI) for infection challenge. The fish were transferred to a purge tank for 24 hours before being packed in plastic bags containing aquarium water with Aqui-S (0.75 ml/100 L) and filled with oxygen gas before sealing. The transport from Townsville to EMAI by road and air was managed by a commercial animal transport company (Dogtainers) and completed in less than 12 hours.

2.2.2 EXPERIMENT DESIGN

The fish were randomly assigned to 24 aquariums according to the experiment design described in Table 4. Two random sequences of integers between 1 and 110 were generated to assign the fish of each species to tanks[4] based on the order they were removed from the transport bags. Intraperitoneal injection was used to establish TiLV infection and assess disease pathology. Additionally, some of the injected fish were cohabited with naïve conspecifics to test a natural route of infection for each species (Table 4). The ratio of injected and naïve cohabiting conspecifics in tanks 13 to 24 was varied

[4] Using a random number generator found at https://www.random.org/sequences

to provide different infection pressures. A small second experiment was used to assess immersion exposure to cell culture-derived TiLV (Table 5). The fish were observed twice a day to identify clinical signs of disease and determine the cumulative mortality over 14 days for injection and 21 days for cohabitation. Fish were euthanised if there was evidence of moderate to severe clinical signs. Additional tanks of injected fish provided samples independent of those used for survival analysis, that is tanks 1, 2, 7 and 10 for daily skin mucus swabs and tanks 9 and 12 for non-survival samples on days 2, 4, 6 and 8.

Table 1. Experiment design for assessment of the susceptibility of Oreochromis mossambicus and Pelmatolapia mariae to infection with TiLV. All fish that were challenged by injection received a dose of 5.1 x 10^4 $TCID_{50}$/fish. Weight and length of each group are mean and standard deviation.

Tank ID	Species	n	Challenge method	Size (at challenge)	
				Weight (grams)	Total length (mm)
1	*O. mossambicus*	10	Negative control, intraperitoneal injection	7.0 +/- 1.6	76.0 +/- 7.5
2	*P. mariae*	10	Negative control, intraperitoneal injection	4.4 +/- 2.7	58.3 +/- 10.2
3	*O. mossambicus*	10	Negative control, 5 x intraperitoneal injection with 5 x cohabitors	7.7 +/- 4.4	72.8 +/- 18.4
4	*O. mossambicus*	10	Negative control, 5 x intraperitoneal injection with 5 x cohabitors	11.7 +/- 4.7	88.8 +/- 15.8
5	*P. mariae*	10	Negative control, 5 x intraperitoneal injection with 5 x cohabitors	5.3 +/- 1.7	59.3 +/- 8.0
6	*P. mariae*	9*	Negative control, 5 x intraperitoneal injection with 5 x cohabitors	6.1 +/- 2.3	63.6 +/- 8.7
7	*O. mossambicus*	10	Intraperitoneal injection + daily skin mucus swabbing	6.8 +/- 2.2	71.3 +/- 8.2
8	*O. mossambicus*	10	Intraperitoneal injection	8.5 +/- 4.4	75.9 +/- 16.1
9	*O. mossambicus*	12	Intraperitoneal injection + longitudinal sampling	7.1 +/- 2.3	71.8 +/- 10.3
10	*P. mariae*	10	Intraperitoneal injection + daily skin mucus swabbing	5.9 +/- 3.6	61.0 +/- 9.8
11	*P. mariae*	10	Intraperitoneal injection	5.6 +/- 2.9	61.3 +/- 11.1
12	*P. mariae*	12	Intraperitoneal injection + longitudinal sampling	5.2 +/- 1.4	59.4 +/- 7.6
13	*O. mossambicus*	10	Cohabitation. 5 TiLV injected donors with 5 naïve cohabitors	6.3 +/- 3.3	61.9 +/- 7.9
14	*O. mossambicus*	7*	Cohabitation. 4 TiLV injected donors with 3 naïve cohabitors	6.3 +/- 2.7	70.9 +/- 10.0
15	*O. mossambicus*	8	Cohabitation. 4 TiLV injected donors with 4 naïve cohabitors	5.3 +/- 1.2	66.3 +/- 2.8
16	*O. mossambicus*	8	Cohabitation. 4 TiLV injected donors with 4 naïve cohabitors	6.4 +/- 1.0	66.0 +/- 9.8
17	*O. mossambicus*	6	Cohabitation. 2 TiLV injected donors with 4 naïve cohabitors	5.5 +/- 1.3	65.7 +/- 4.0
18	*O. mossambicus*	6	Cohabitation. 2 TiLV injected donors with 4 naïve cohabitors	5.5 +/- 1.9	64.3 +/- 7.2
19	*P. mariae*	10	Cohabitation. 5 TiLV injected donors with 5 naïve cohabitors	6.3 +/- 3.3	63.5 +/- 11.0
20	*P. mariae*	10	Cohabitation. 5 TiLV injected donors with 5 naïve cohabitors	5.1 +/- 1.8	59.3 +/- 7.2
21	*P. mariae*	8	Cohabitation. 4 TiLV injected donors with 4 naïve cohabitors	4.8 +/- 1.4	57.9 +/- 57.9
22	*P. mariae*	8	Cohabitation. 4 TiLV injected donors with 4 naïve cohabitors	6.3 +/- 3.5	65.9 +/- 9.8
23	*P. mariae*	6	Cohabitation. 2 TiLV injected donors with 4 naïve cohabitors	4.7 +/- 1.2	58.3 +/- 6.3
24	*P. mariae*	6	Cohabitation. 2 TiLV injected donors with 4 naïve cohabitors	4.8 +/- 1.2	56.5 +/- 4.2

**pre-trial mortality reduced the intended number in these tanks*

Table 2. Experiment design for assessment of the susceptibility of Oreochromis mossambicus and Pelmatolapia mariae to infection with TiLV by immersion exposure. The fish were challenged by immersion were one month older than those challenged by IP injection and cohabitation challenge and had been held in the facility during this time. The indicated dose of TiLV was used for immersion in a 2 L volume for 1 hour before addition of the residual inoculum to the aquaria. Weight and length of each group are mean and standard deviation.

Tank ID	Species	n	Challenge method	Size (at challenge)		End trial		
				Weight (grams)	Total length (mm)	Weight (grams)	Total length (mm)	time (days)
1	*O. mossambicus*	2	Negative control, aquarium water and cell culture medium	11.0 +/- 4.0	87.5 +/- 9.5	12.0 +/- 3.0	89.5 +/- 5.5	8
2	*P. mariae*	2	Negative control, aquarium water and cell culture medium	10.5 +/- 3.5	72.0 +/- 8.0	13.5 +/- 4.5	86.0 +/- 14.0	14
3	*O. mossambicus*	8	TiLV high dose, 6.3 x 10^4 $TCID_{50}$/mL final concentration	12.0 +/- 6.0	85.3 +/- 15.1	15.5 +/- 7.6	93.8 +/- 16.9	7,8
4	*O. mossambicus*	7	TiLV lower dose, 6.3 x 10^3 $TCID_{50}$/mL final concentration	15.4 +/- 3.6	94.3 +/- 9.9	21.0 +/- 6.4	103.6 +/- 10.8	7,8
5	*P. mariae*	8	TiLV high dose, 6.3 x 10^4 $TCID_{50}$/mL final concentration	9.1 +/- 3.6	71.9 +/- 9.1	10.7 +/- 4.1	78.1 +/- 11.0	14
6	*P. mariae*	2	TiLV lower dose, 6.3 x 10^3 $TCID_{50}$/mL final concentration	6.5 +/- 1.4	63.8 +/- 4.6	10.3 +/- 1.2	76.3 +/- 3.9	14

2.2.3 AQUARIUM MANAGEMENT

Aquariums were set-up in the high-security animal housing at EMAI (Block 14.2) with modifications to liquid waste management to meet biosecurity requirements (e.g. blocked floor drains for periodic discharge of chemically disinfected water to an Actini high- temperature steriliser). Central air handling was used to maintain the air temperature at 28 °C +/- 1 °C. A 12-hour light–dark cycle was provided using fluorescent lighting. Two rooms each contained wire racking to hold 12 aquariums, a reservoir aquarium water tank (400 L), and wastewater collection barrels (3 x 200 L). Aquarium water was prepared from the municipal supply (salinity 0) by holding it for more than 24 hours in the reservoir tank, dosing with Prime (SeaChem), allowing continuous circulation through a cannister filter with activated charcoal (Eheim), and the temperature was maintained at 28 °C with an aquarium heater (Eheim).

The aquariums were polyethylene plastic containers with 50–55 L aquarium water covered with clear plastic to minimise losses to evaporation, the risk of aerosol and droplet cross-contamination, and the escape of fish. The target range for water temperature (28–30 °C) was maintained by managing air temperature. The temperature of each tank was recorded every 15 minutes using an iButton (Thermochron) and by daily measurement from an aquarium thermometer (Marina). Each of the 24 tanks contained an air-driven sponge filter (Aquatopia) and a submerged internal filter (Hailea BT1000). The internal filters were seeded with an active biofilter 24 hours before the addition of fish. This seeding was established by cycling a mix of ceramic biofilter media and filter wool (AquaOne) in cannister filters (Fluval) on a 400 L aquarium with the daily addition of chemical ammonia and water quality adjustments until a stable nitrogen cycle was established.

Fish were observed and fed twice daily with a maintenance ration of approximately 1% body weight/day, consisting predominantly of algae wafers (Hikari) and some cichlid staple pellets (Hikari). Water quality was tested using the freshwater master test kit (API) with daily measures of some parameters (total ammonia nitrogen TAN, pH) and others as required to monitor the nitrogen cycle (nitrite, nitrate). Water quality was maintained by adding sodium bicarbonate to increase the pH when it was less than 6.8 and by exchange of 25–50% of the water volume as required, or every 10 days. Cross-contamination between tanks was minimised by having separate equipment designated to each tank (bucket, net, bags of food) and by changing gloves before handling different tanks.

2.2.4 TILV ISOLATE AND IN VITRO CULTURE

The E11 cell line was sourced from CellBank Australia (Cat. no. 01110916, Lot number 16B043). Cells were grown using with Liebovitz-15 (L15) medium containing 2 millimolar (mM) glutamine (Gibco), 10% fetal bovine serum (FBS) and antibiotics (penicillin 100 units/mL, Streptomycin 100 µg/mL and amphotericin B 0.25 µg/mL) in 75 cm^2 tissue culture flasks (Nunc). A refrigerated incubator without carbon dioxide maintained the temperature at 28 °C. Cells were harvested using 0.25% trypsin and split 1/4 to achieve a seeding rate of approximately 3 x 10^4 cells/cm^2.

TiLV was supplied by the CSIRO Australian Centre for Disease Preparedness (CSIRO ACDP), CSIRO (MTA 2022092079) from an isolate originally obtained from diseased hybrid tilapia (*O. niloticus* crossed with *O. aureus*) in Israel (TiLV isolate 8440/8464, KoVax Ltd, Import Permit IP-0001005013). The material supplied was subjected to three passages in the E-11 cell line and was confirmed to be free from adventitious agents at the CSIRO ACDP. The TiLV identity was confirmed by conventional reverse transcriptase polymerase chain reaction (RT-PCR) and Sanger sequencing of an amplicon generated using the assay described by Dong et al. (2017).

AMPLIFICATION OF TILV

Three x 75 cm^2 flasks with E11 cells at approximately 90% confluence were rinsed with serum-free L15 and inoculated with one of three 10-fold dilutions of the supplied material (1/20 to 1/2000). Dilutions were prepared in serum-free L15 and a total volume of 2 millilitres (mL) was adsorbed on the monolayers for 2 hours at 28 °C before making the total medium volume up to 30 mL with L15 maintenance medium (L15 with 2% FBS and antimicrobials). The cultures were harvested after five days when cytopathic effect (CPE) was evident in approximately 100% of cells for the least dilute inoculum and 70% and 50% for the higher dilutions. Growth of TiLV was confirmed by RT-qPCR and

later by titration. The cell culture supernatant was clarified by centrifugation at 3,000 revolutions per minute (rpm) for 20 minutes at 4 °C and the supernatant was stored in aliquots at −80 °C (virus preparations P567 and P568 derived from the 1/200 and 1/2000 dilutions of inoculum).

2.2.5 RT-QPCR FOR TILV

The primers and probe, with an amplification target in segment 3, were used to detect TiLV RNA (Waiyamitra et al. 2018). Briefly, nucleic acids were extracted from 50 microlitres (µl) of liver tissue homogenate, swab fluid or cell culture supernatants, or from 100 µl of aquarium water using the MagMax-96 viral RNA isolation kit (Ambion) and a magnetic particle- handling system (Kingfisher-96, Thermo) according to the manufacturer's directions. RT-qPCR reactions were prepared using 5 µl of nucleic acid in 25 µl reactions, prepared with the Ag Path-ID one-step RT-PCR kit (AM1005, Ambion) and run according to the cycling conditions specified for the mastermix using a 7500 Fast Real-Time PCR System in standard mode for a total of 45 cycles (Applied Biosystems). An exogenous internal control was included in the extraction buffer for subsequent extraction, amplification and detection, concurrent with TiLV RNA in each reaction (Gu et al. 2014). The fluorescence threshold was set manually at 0.05 ∆Rn and the background was automatically adjusted (ABI 7500 software v.3). RT-qPCR results were expressed as cycle threshold (Ct) values and classified as negative if no amplification was observed after 45 cycles.

2.2.6 VIRUS ISOLATION AND TITRATION OF TILV

Cryopreserved E11 cells were thawed and seeded into 96-well plates at 1 x 10^4 cells/well with 150 µl of L15 maintenance medium. Serial 10-fold dilutions of the TiLV stock were prepared and 50 µl of each dilution were added to eight wells of a plate. The plates were sealed and incubated at 28 °C. After seven days, the cells were examined for evidence of CPE and the titre was calculated according to the standard TCID50 method (Reed and Muench 1938).

To test for infectious TiLV in select experimental samples derived from the in vivo trials, tissue homogenate, swab fluid or aquarium water was clarified by centrifugation and the supernatant was filtered with a 22 µm syringe filter. A 0.5 mL aliquot of the samples was adsorbed for 1 hour onto an 80% confluent E11 cell monolayer in duplicate tissue culture tubes (approximately 5 cm^2) before the addition of 2.5 mL of L15 maintenance medium. Tubes were examined for CPE at five and seven days, in the absence of cytopathology were passaged twice before assigning a negative result. When CPE was observed, the cell culture supernatant was tested by RT-qPCR and a positive result was assigned to samples if the quantity of TiLV RNA exceeded that in the original sample (as indicated by a Ct value >3 less than the original sample). Negative and positive samples were concurrently assessed in control tubes.

2.2.7 IN VIVO INFECTION CHALLENGE WITH TILV

All fish were anaesthetised using benzocaine in aerated aquarium water at 40 mg/L for *P. mariae* and 60 mg/L for *O. mossambicus*. The length and weight of each fish was measured at the time of challenge and at the completion of the trial for the fish that survived.

INJECTION CHALLENGE

Fish were injected using a 27-gauge needle to administer 100 µl to the caudo-ventral peritoneal cavity. Fish that were injected and intended for cohabitation challenge experiments were identified by excising the caudal point of the dorsal fin while anaesthetised. The inoculum was prepared as a dilution of TiLV stock P567 in serum-free L15 with an initial target of 1 x 10^5 $TCID_{50}$/fish based the titre of the virus stock. The negative control injection was clarified cell culture supernatant from uninfected E11 cells diluted 1/10 in L15. Fish to be challenged by cohabitation were anaesthetised for weight and length measurement at the same time, but were not exposed to TiLV or a negative control injection prior to addition to the aquariums containing injected donor fish.

IMMERSION CHALLENGE

A 0.5 mL aliquot of P567 was thawed, diluted 1/5 and inoculated by adsorption onto E11 cells that were approximately 60% confluent in a 75 cm^2 flask. After four days when there was cytopathology in

most cells, the supernatant was collected, clarified by centrifugation, and pooled with 4 x 5 mL vials of P567 that had been stored at −80 °C and thawed at room temperature. This provided 45 mL of TiLV that was used for immersion challenge. Two buckets were prepared with an approximate 1/100 dilution (22 mL mixed with 2 L clean aquarium water) and two buckets with a 1/1000 dilution prepared by mixing 200 mL of the first dilution with 1.8 L clean aquarium water. This provided a single replicate at each dose for each species (*O. mossambicus* and *P. mariae*). The fish were kept at relatively high stocking density in the 2 L TiLV inoculum buckets for 1 hour at 28 °C before returning to the aquariums with clean water. The water used as the inoculum was also added to the aquarium with the fish. The remainder of the undiluted inoculum and samples of the immersion challenge water were collected at the end of 1 hour and were tested by RT-qPCR and titration. Fish in the negative control group were immersed in a 2 L volume prepared by adding 22 mL L15 maintenance medium to 2 L of clean aquarium water.

SAMPLE COLLECTION

Water samples were collected once a day for 14 days from every aquarium using a transfer pipette to take 3 mL from the middle of the water column and placing it in a 5 mL sterile screw-topped container. Samples of skin mucus were collected daily from all fish in some aquariums for 10 days after challenge using flocked swabs (Deltalab) which were placed into 3 mL of Deltalab virus transport medium. To obtain these samples, all the fish were removed from the aquarium using a soft nylon net (AquaOne) and placed in a small container of clean aquarium water from the reservoir. Individual fish were then held briefly in the net for swabbing before returning to their original aquarium. The swab was moved with gentle pressure and rotation across one lateral body surface from the pectoral fin to the tail. Nets were rinsed in clean aquarium water between fish, but there was no further attempt to reduce cross-contamination between fish from the same aquarium.

Tissue samples were obtained at the completion of the trial or when fish were euthanised or died due to disease. Fish with advanced disease were euthanised using an initial anaesthetic dose of benzocaine in aquarium water and a subsequent overdose of benzocaine (>120 mg/L). At necropsy, 0.15–0.30 g of fresh tissue was collected from the caudal liver. The spleen and anterior kidney were preserved in >5 times volume RNALater (Invitrogen) for a different project and the remainder of the tissues were preserved for histopathology with >10 times volume of 10% neutral buffered formalin. The only sample taken from fish that were found to have died suddenly was fresh liver that was stored in a 1.5 mL cryovial.

All samples that were collected in the animal houses were transferred from chilled storage on ice to minus 80 °C storage within 2 hours of collection. Fresh liver tissues were prepared as homogenates immediately prior to extraction of nucleic acids by adding 0.5 mL of L15 medium without supplements, grinding manually with a micropestle in conical bottom laboratory tubes and clarifying by centrifugation.

2.2.8 HISTOPATHOLOGY

Tissues were fixed in 10% neutral buffered formalin and decalcified with 0.5 M (ethylenediaminetetraacetic acid (EDTA)) pH 7.8. Selected fish, including fish used for pre-trial health checks and negative control samples were prepared according to standard histologic methods for hematoxylin and eosin staining and examination by a veterinary pathologist. Cassettes contained liver and other key tissues (brain, gill, heart, spleen, muscle, kidney, gastrointestinal tract) dissected from selected fixed tissues.

2.2.9 STATISTICS

Data were collated and simple descriptive statistics and charts were prepared using Excel (Microsoft). Weight and length data were compared using t-tests. Kaplan–Meier survival curves for mortality data were prepared in Rstudio (2023.03.0 Build 386) using the ‘survival’ and ‘survminer’ packages (Therneau 2023) with log rank tests used to test for significance.

2.3 RESULTS

2.3.1 PREPARATION AND QUANTIFICATION OF TILV INOCULUM

The virus stock P567 used in this study was produced after one additional passage in E11 cells, i.e. fourth passage of TiLV isolate 8440/8464. The titre was 8.7 x 10^6 $TCID_{50}$/mL (Ct value 14.5). After dilution, the titre used for the injection challenge was 5.1 x 10^5 $TCID_{50}$/mL for an aliquot at the time of dilution and another held chilled in the aquatic facility until after the final injection. This confirmed the expected dose was administered in each injection (5.1 x 10^4 $TCID_{50}$/fish). The TiLV negative status of the control inoculum was confirmed by PCR and virus titration. The TiLV preparation used to prepare immersion challenges diluted 1/100 and 1/1000 in aquarium water had a titre of 6.3 x 10^6 $TCID_{50}$/mL. After 1 h of holding fish for immersion challenge in 2 L volumes these dilutions had the following Ct values for TiLV RNA: *O. mossambicus* high dose 24.3, 1/10 dose 27.8; *P. mariae* high dose 23.5, 1/10 dose 27.4.

2.3.2 MANAGEMENT AND HEALTH OF FISH

There was no mortality during transport. Fish of both species accepted food and were apparently healthy based on appearance and behaviour the day after transfer to the aquariums. During one week of acclimation, there was mortality of 3 x *O. mossambicus* (2.6%) and 1 x *P. mariae* 1 (0.8%) which was within expectations for transfer into biosecure aquarium housing. Pre-trial health screens, including histopathology for a random selection of *O. mossambicus* and *P. mariae*, indicated good health (n=3 and n=6, respectively). No evidence of TiLV was detected in these fish by RT-qPCR.

At the time of challenge, the *O. mossambicus* were 7.2 +/- 3.4 grams (mean +/- standard deviation) and 71.6 +/- 13.1 mm in total length (TL) (n=107). The *P. mariae* were 5.3 +/- 2.5 g and 60.1 +/- 9.3 mm TL (n=110). The size of groups of fish in each aquarium after random distribution is shown in Table 4. At immersion challenge, the fish were four weeks older and had grown compared to the fish used in the injection and cohabitation experiment (Table 5).

Water quality was maintained by the biofilters with pH adjustment and scheduled water exchange providing TAN $\leq$ 0.25 mg/L, pH 6.8–7.0, nitrite 0 and nitrate $\leq$ 40 mg/L and water temperature was maintained in the target range of 28–30 °C (+/-1 °C) for all daily observations throughout the trial period.

OBSERVATION OF CLINICAL DISEASE

There was no mortality in the 48-hour period after anaesthesia for measurement, injection and fin clipping. Fish in all tanks returned to eating the following day. Fish injected with the negative control inoculum remained healthy for the duration of the experiment, including those that were subjected to daily swabbing where good health was confirmed by growth over a 14-day period. There was significant growth of the negative control fish for both species over 21 days, including a mix of fish that received a negative control injection with fin clip and also the cohabitors (Table 6).

Evidence of disease was first observed 48 hours after injection in both species. Affected fish stopped eating and had reduced activity compared to the negative controls. Colour variation was frequently observed in affected fish, evident predominantly as pale colouration with some darkly coloured fish compared to controls and their colour prior to disease. Progression of clinical signs included fish that ceased active attempts to retreat to a hide when the aquarium was disturbed, with weak and/or uncoordinated swimming. Some fish developed ulceration and reddening of the skin that, in a few cases, included erosion of the opercula. These signs were considered advanced disease and triggered euthanasia. In many instances, fish progressed from mild disease to death within the 12–14-hour observation interval or had progressed from mild disease to being moribund (lost ability to maintain position in the water column, unable to swim) at the time of euthanasia. Of the fish that died during the trial, 53.4% were found dead, compared with 46.6% that were euthanised due to clinical signs of disease. Immersion challenge caused clinical signs in *O. mossambicus,* but not *P. mariae*. The incubation period was longer than injection with the fish apparently healthy for five days. Severe clinical signs were first evident on day 7, after which disease progressed rapidly.

Cumulative mortality was 100% for *O. mossambicus* challenged with TiLV by injection compared with 0% for negative controls (Figure 2). Most injected fish died within five days and the last by day 9 (n=50). The first mortality of a cohabiting *O. mossambicus* was on day 6, with the peak of mortality between days 9 and 12. All cohabiting *O. mossambicus* (n=24) died regardless of the number of injected donor fish in the tank. Immersion challenge also led to 100% mortality of this species with the onset of mortality on day 7, two days after appetite first decreased (Figure 3). All fish progressed to severe disease regardless of the dose and were euthanised by day 8 (n=15 in total).

Table 3. Weight and length of fish over the 14-day TiLV challenge period. Data are mean and standard deviation for the negative control fish in Tanks 1 and 2 (which also had daily swabbing). P. mariae was the only species with survivors at Day 14 where there was a significant decline in body condition (Tanks 10 and 11). All O. mossambicus died rapidly after challenge so TiLV growth data were not relevant.

Treatment group		**Species**	**Measure**	**Challenge (Day 0)**		**End of trial**			**p**
				n		**n**	**Day 14**	**Day 21**	
Injection, daily swabs, sample at 14 days	Negative control	*O. mossambicus*	weight (g)	10	6.5 +/- 2.2	10	8.0 +/- 2.1	-	0.08
			length (mm)		74.0 +/- 9.3		79.7 +/- 7.7	-	0.09
		P. mariae	weight (g)	10	4.5 +/- 2.7	10	6.5 +/- 3.2	-	0.08
			length (mm)		58.1 +/- 10.2		65.6 +/-10.1	-	0.14
	TiLV challenge	*P. mariae*	weight (g)	20	5.2 +/- 2.3	12 (survivors)	3.3 +/- 0.9	-	<0.05
			length (mm)		59.8 +/- 8.9		58.0 +/- 4.4	-	0.46
Injection and cohabitation, sample at 21 days	Negative control	*O. mossambicus*	weight (g)	20	9.8 +/- 4.7	16	-	13.5 +/- 5.3	<0.05
			length (mm)		81.3 +/- 18.0		-	121.1 +/- 57.5	<0.01
		P. mariae	weight (g)	20	5.5 +/- 2.1	16	-	8.4 +/- 3.2	<0.01
			length (mm)		60.7 +/- 8.4		-	69.4 +/- 8.6	<0.01
	TiLV challenge	*P. mariae*	weight (g)	26 (cohabitors)	5.3 +/- 2.5	13 (survivors)	-	7.5 +/- 2.7	<0.01
			length (mm)		60.0 +/- 8.6		-	68.9 +/- 8.9	<0.01

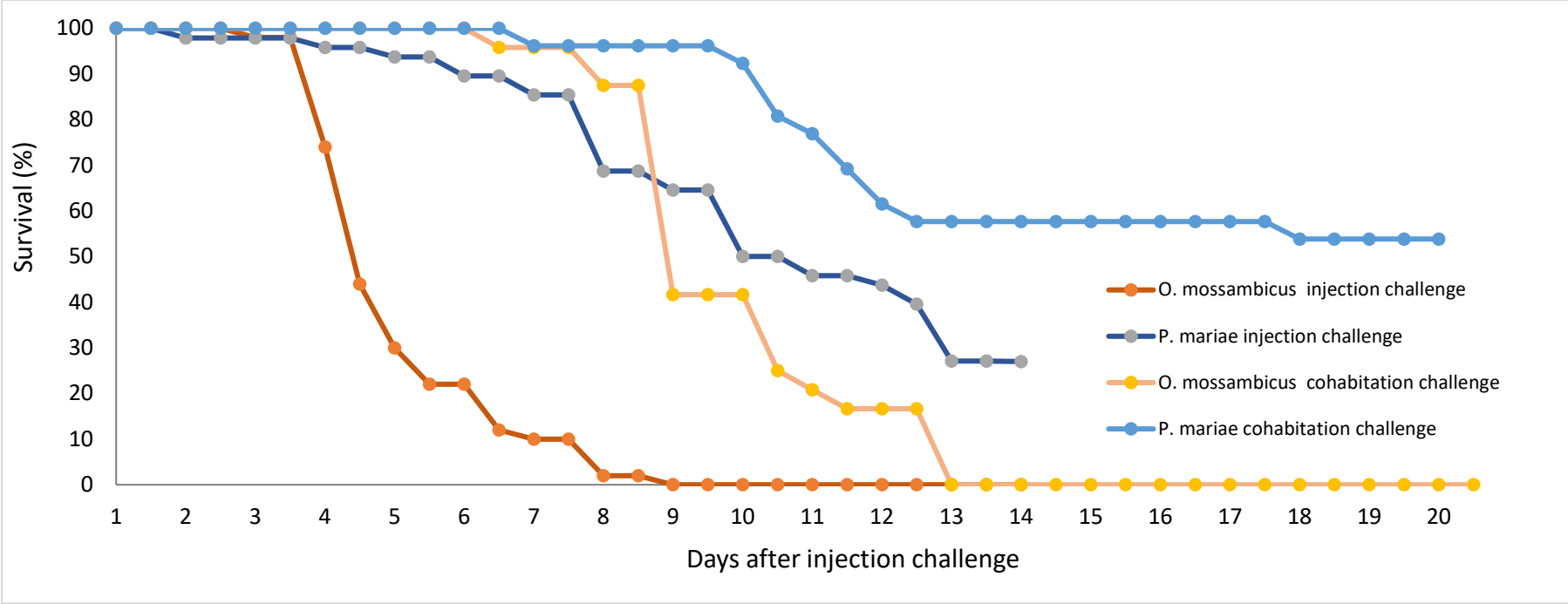

Figure 1. Mortality of Oreochromis mossambicus and Pelmatolapia mariae after challenge with TiLV by injection of cohabitation. Data are cumulative survival (pooled across the aquaria described in Table 4) based on 12h observation intervals. O. mossambicus by injection (n=50) or by cohabitation (n=24) and P. mariae by injection (n=48) or by cohabitation (n=26). There was no mortality in any negative control tanks, data not plotted.

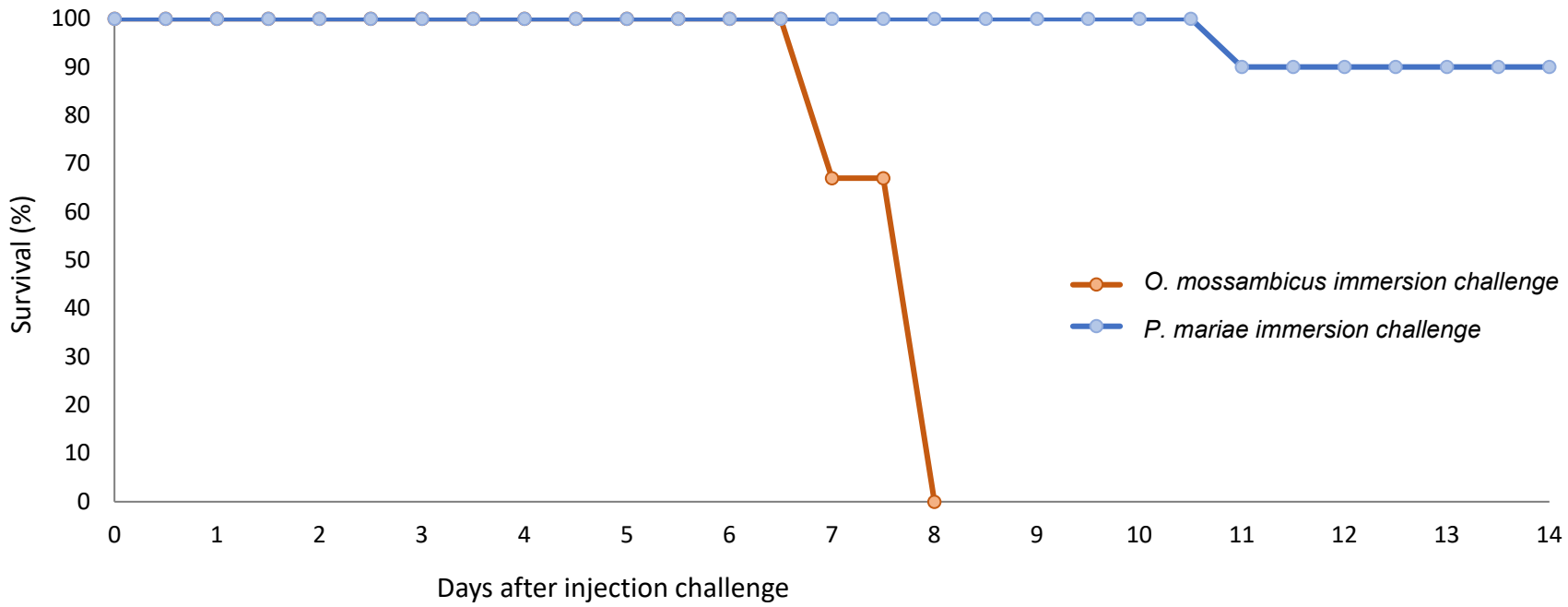

Figure 2. Survival after immersion challenge, data are pooled for the two doses i.e. Oreochromis mossambicus (n=15) and Pelmatolapia mariae (n=10). The single P. mariae that dies was negative for TiLV while the TiLV RNA load in all dead O. mossambicus was very high.

The cumulative mortality of *P. mariae* injected with TiLV was 63% at day 14 (n=48). Each of the surviving fish had evidence of disease through reduced activity, prolonged reduction in appetite and reduced body condition (Table 5). If the trial continued beyond 14 days for these injected fish, it is likely that they would have been euthanised based on persistent inappetence and loss of condition meeting a criterion for severe disease. The cumulative mortality of cohabiting *P. mariae* at the completion of this phase of the trial on day 21 was 46% (n=26, Figure 2). One of the 14 survivors at day 21 appeared underweight and dark in colour and tested positive for TiLV for a final outcome of 50% of cohabiting fish being infected and becoming diseased. The remaining cohabitors were apparently healthy by clinical assessment and on necropsy and there was no evidence of TiLV. There was a single mortality after immersion challenge of *P. mariae* without evidence of TiLV by RT-qPCR (Figure 3). The median survival time for *P. mariae* was longer than *O. mossambicus*, regardless of the challenge method (Figure 4). For *O. mossambicus*, the median survival time was longer after immersion challenge compared to injection (Table 7).

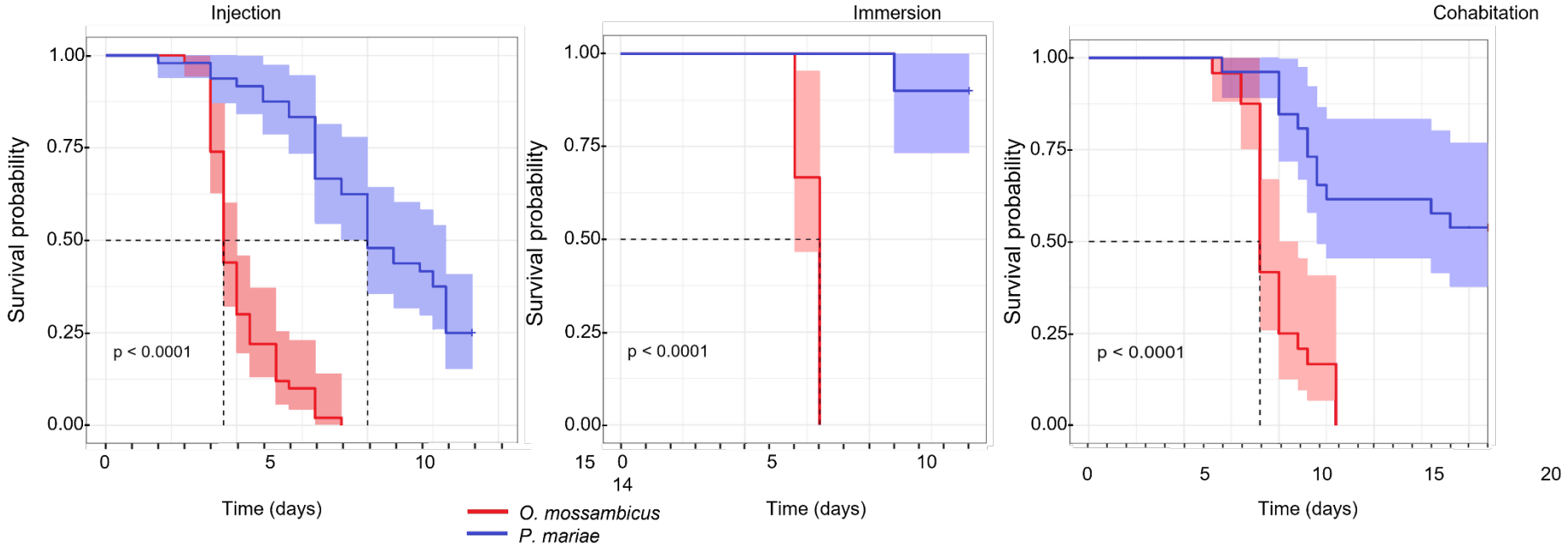

Figure 3. Kaplan-Meier survival curves for TiLV challenge by injection, immersion and cohabitation based on observation for mortality twice a day. The data show the median survival time (black dashed lines) for O. mossambicus and P. mariae with p-values indicating significance by the log-rank test.

Table 4. Kaplan-Meier survival analysis for O. mossambicus and P. mariae after different methods of challenging with TiLV. Median survival time in days was estimated from twice daily observation, see also Figure 4.

Challenge method	Species	n	No. died	Median survival time, days (95% confidence limits)		Log rank test
Injection	*O. mossambicus*	50	50	4.5	(4.5–5.0)	P<0.001
	P. mariae	48	35 (1)*	10.0	(10.0–13.0)	
Cohabitation	*O. mossambicus*	24	24	9.0	(9.0–10.0)	P<0.001
	P. mariae	26	12	n/a	(12.0–n/a)	
Immersion	*O. mossambicus*	15	15	8.0	(7.0–n/a)	P<0.001
	P. mariae	10	0 (1)*	n/a	–	

Species	Challenge method	n	No. died	Median survival time, days (95% confidence limits)		Log rank test
O. mossambicus	Injection	50	50	4.5	(4.5–5.0)	
	Cohabitation	24	24	9.0	(9.0–10.0)	P<0.001
	Immersion	15	15	8.0	(7.0–n/a)	

** There were two groups in which there was mortality of one P. mariae without TiLV infection (left censored data) at 48 h in the injection challenge and at day 12/13 for the immersion challenge. All other deaths were 'events' whereby TiLV infection was confirmed.*

Pathology was most marked in the liver, which had some degree of discolouration and reduced size in all affected fish for both species. An empty gastrointestinal tract with marked distention of the gall bladder and loss of body condition was seen consistently in clinically affected fish of both species (Figure 5). Splenomegaly and enlarged anterior kidney occurred infrequently. More frequently, the size of both organs was reduced in most clinically affected fish from both species compared to negative controls. Pronounced, generalised scale protrusion ('dropsy') was an additional clinical sign observed only after immersion challenge (Figure 6). Rapid decomposition of fish that died suddenly at ≥28 °C prevented detailed examination of the viscera for approximately half of the fish.

A1. A2.

B1. B2.

Figure 4. Necropsy observations at the time of euthanasia of TiLV challenged fish with clinical signs (A) and matched control fish that were euthanised at the time of death (B) for (1) O. mossambicus (2) P. mariae. Consistent findings in every clinically affected fish was gross pathology of the liver (arrow), empty gastrointestinal tract, distended gall bladder and reduced body condition.

A.

B.

Figure 5. Necropsy observations for O. mossambicus challenged with TiLV by immersion (A) and negative control fish (B). These images illustrate reduced size and discolouration of liver, empty gastrointestinal tract and enlarged gall bladder that was seen in all cases of disease and the dark skin colouration and scale protrusion seen in approximately half of the O. mossambicus after immersion challenge.

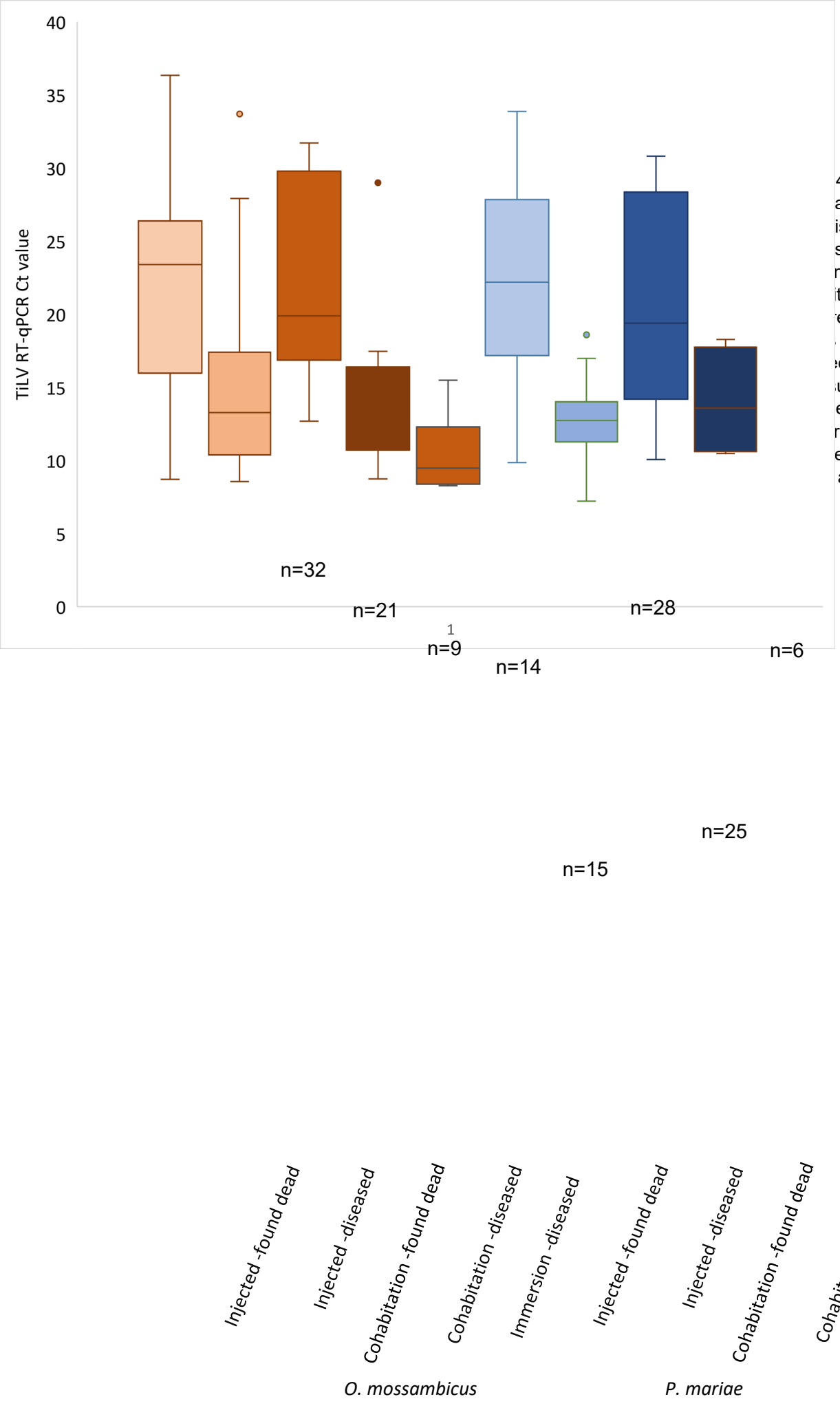

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Figure 6. Quantity of TiLV RNA in liver tissue at the time of death. Data are median and quartiles with the number of fish for which a PCR result was available in each category.

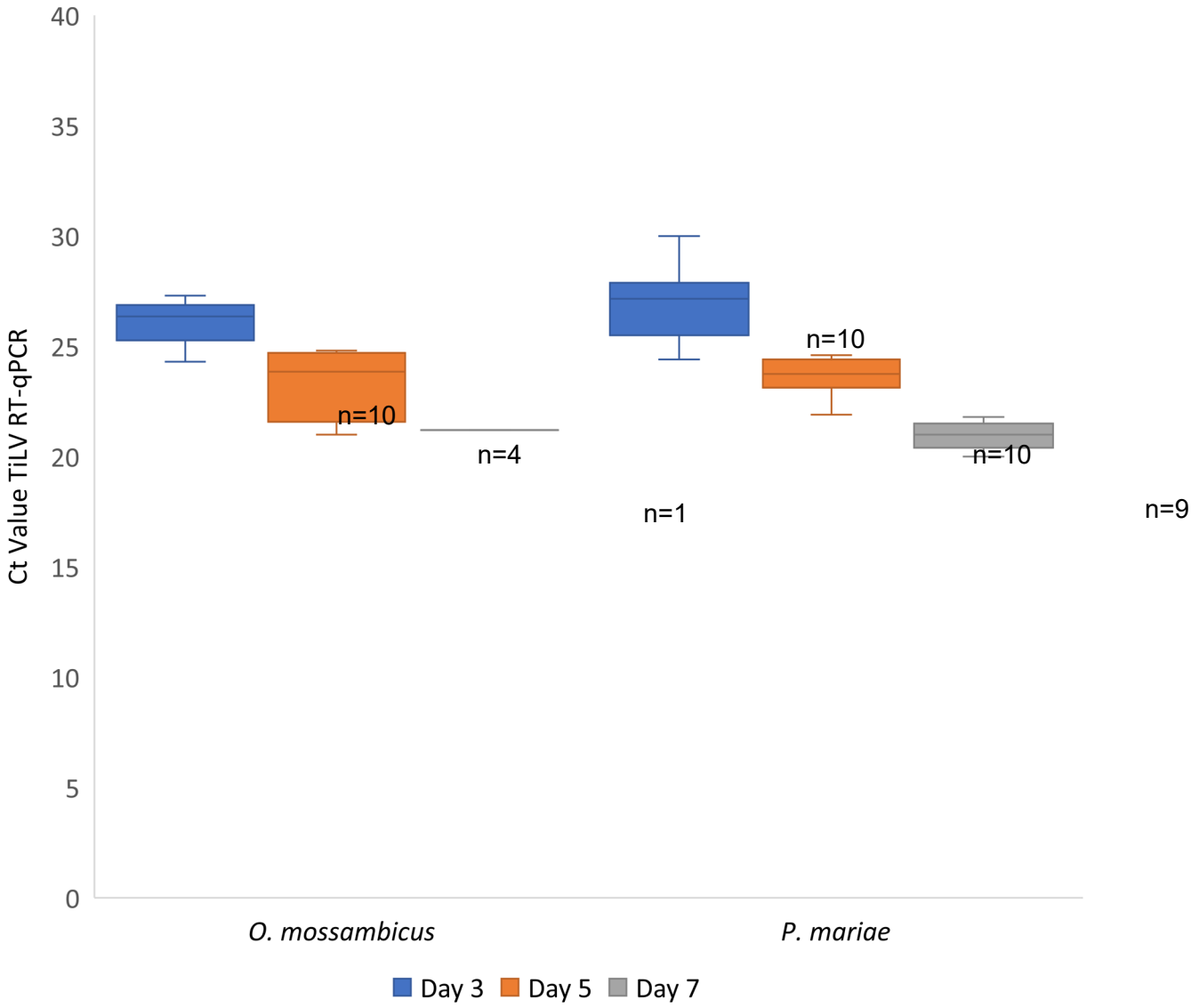

Figure 7. Detection of TiLV by RT-qPCR on skin swabs at different times post-infection by intraperitoneal injection. Data are Ct values with median and quartiles. Every challenged fish was positive for TiLV on all skin swabs tested. Reduced numbers of fish indicate mortality within the 10-day sampling period.

Table 5. Detection of TiLV RNA in aquarium water by RT-qPCR. Data are Ct values when TiLV was detected (samples are available for all tanks on Days 1–14).

Tank ID	Description			TiLV RT-qPCR result		
	Species	Challenge	Outcome	Day 5	Day 7	Day 9
1	*O. mossambicus*	Negative control, IP injection	All survive, all TiLV negative	Neg.	Neg.	Neg.
2	*P. mariae*	Negative control, IP injection	All survive, all TiLV negative	Neg.	Neg.	Neg.
3	*O. mossambicus*	Negative control, 5 x IP injection with 5 x cohabitors	All survive, all TiLV negative	Neg.	Neg.	Neg.
4	*O. mossambicus*	Negative control, 5 x IP injection with 5 x cohabitors	All survive, all TiLV negative	Neg.	Neg.	Neg.
5	*P. mariae*	Negative control, 5 x IP injection with 5 x cohabitors	All survive, all TiLV negative	Neg.	Neg.	Neg.
6	*P. mariae*	Negative control, 5 x IP injection with 5 x cohabitors	All survive, all TiLV negative	Neg.	Neg.	Neg.
7	*O. mossambicus*	IP injection + daily skin mucus swabbing	100% mortality day 5	31.08	Neg.	Neg.
8	*O. mossambicus*	IP injection	100% mortality day 8	28.7	30.99	Neg.
9	*O. mossambicus*	IP injection + longitudinal sampling	100% mortality day 5	31.14	Neg.	Neg.
10	*P. mariae*	IP injection + daily skin mucus swabbing	7/10 fish survive to day 14	31.3	30.08	27.96
11	*P. mariae*	IP injection	5/10 fish survive to day 14	32.93	30.25	28.4
12	*P. mariae*	IP injection + longitudinal sampling	Longitudinal sampling = empty tank at day 8	Neg.	30.51	Neg.
13	*O. mossambicus*	Cohabitation. 5 TiLV injected with 5 naïve cohabitors	100% mortality day 9	32.24	Neg.	37.32
14	*O. mossambicus*	Cohabitation. 4 TiLV injected with 3 naïve cohabitors	100% mortality day 12	37.93	30.09	28.2
15	*O. mossambicus*	Cohabitation. 4 TiLV injected with 4 naïve cohabitors	100% mortality day 13	31.2	Neg.	26.25
16	*O. mossambicus*	Cohabitation. 4 TiLV injected with 4 naïve cohabitors	100% mortality day 9	38.68	Neg.	30.87
17	*O. mossambicus*	Cohabitation. 2 TiLV injected with 4 naïve cohabitors	100% mortality day 14	30.71	40.84	Neg.
18	*O. mossambicus*	Cohabitation. 2 TiLV injected with 4 naïve cohabitors	100% mortality day 10	Neg.	Neg.	29.86
19	*P. mariae*	Cohabitation. 5 TiLV injected with 5 naïve cohabitors	All injected dead at day 10, 1 cohab survivor	35.05	28.61	Neg.
20	*P. mariae*	Cohabitation. 5 TiLV injected with 5 naïve cohabitors	All injected dead at day 10, 3 cohab survivors	34.25	30.53	30.57
21	*P. mariae*	Cohabitation. 4 TiLV injected with 4 naïve cohabitors	All injected dead at day 11, 4 cohab survivors	29.75	31.4	28.8
22	*P. mariae*	Cohabitation. 4 TiLV injected with 4 naïve cohabitors	All injected dead at day 12, 2 cohab survivors	Neg.	31.65	29.86
23	*P. mariae*	Cohabitation. 2 TiLV injected with 4 naïve cohabitors	Both injected dead at day 12, 2 cohab survivors	32.19	Neg.	Neg.
24	*P. mariae*	Cohabitation. 2 TiLV injected with 4 naïve cohabitors	Both injected dead at day 4, 2 cohab survivors	Neg.	40.04	Neg.

HISTOPATHOLOGY

No pathology was observed for fish of either species sampled pre-trial. Pathology for *O. mossambicus* was consistent with previous reports for TiLV in tilapia (Table 9). Additional findings included ulcerative glossitis, enteritis and gastritis, and necrotic dermatitis. *P. mariae* also displayed liver pathology consistent with disease caused by TiLV infection. The characteristic hepatocellular necrosis, syncytial cells and leukocyte infiltration evident in each species is illustrated in Figure 9. However, anterior kidney pathology included lymphocytic infiltrates without significant necrosis and brain haemorrhage was not observed in sections from *P. mariae*. Some fish of this species had multifocal chronic granulomas associated with the subcutis and posterior kidney, likely predating the TiLV infection.

Table 6. Pathological findings in each treatment group for O. mossambicus and P. mariae. Data are lesions present on histological examination (Y) or not observed (N).

Histopathological feature	*O. mossambicus*			*P. mariae*		
	Negative control	**Injection**	**Cohab.**	**Negative control**	**Injection**	**Cohab.**
Liver necrosis	N	Y	Y	N	Y	Y
Liver syncytia	N	Y	Y	N	Y	Y
Liver cytoplasmic inclusions	N	Y	Y	N	Y	Y
Liver cytoplasmic vacuolation	N	Y	Y	N	Y	Y
Liver prominent nucleoli	N	Y	Y	N	Y	Y
Liver perivascular leukocytes	N	Y	Y	N	Y	Y
Kidney cytoplasmic eosinophilia	N	Y	Y	N	N	N
Kidney necrosis	N	Y	Y	N	N	N
Kidney lymphocytic infiltration	N	Y	Y	N	Y	Y
Pancreatic necrosis	N	Y	Y	N	Y	Y
Brain haemorrhage	N	Y	Y	N	N	N

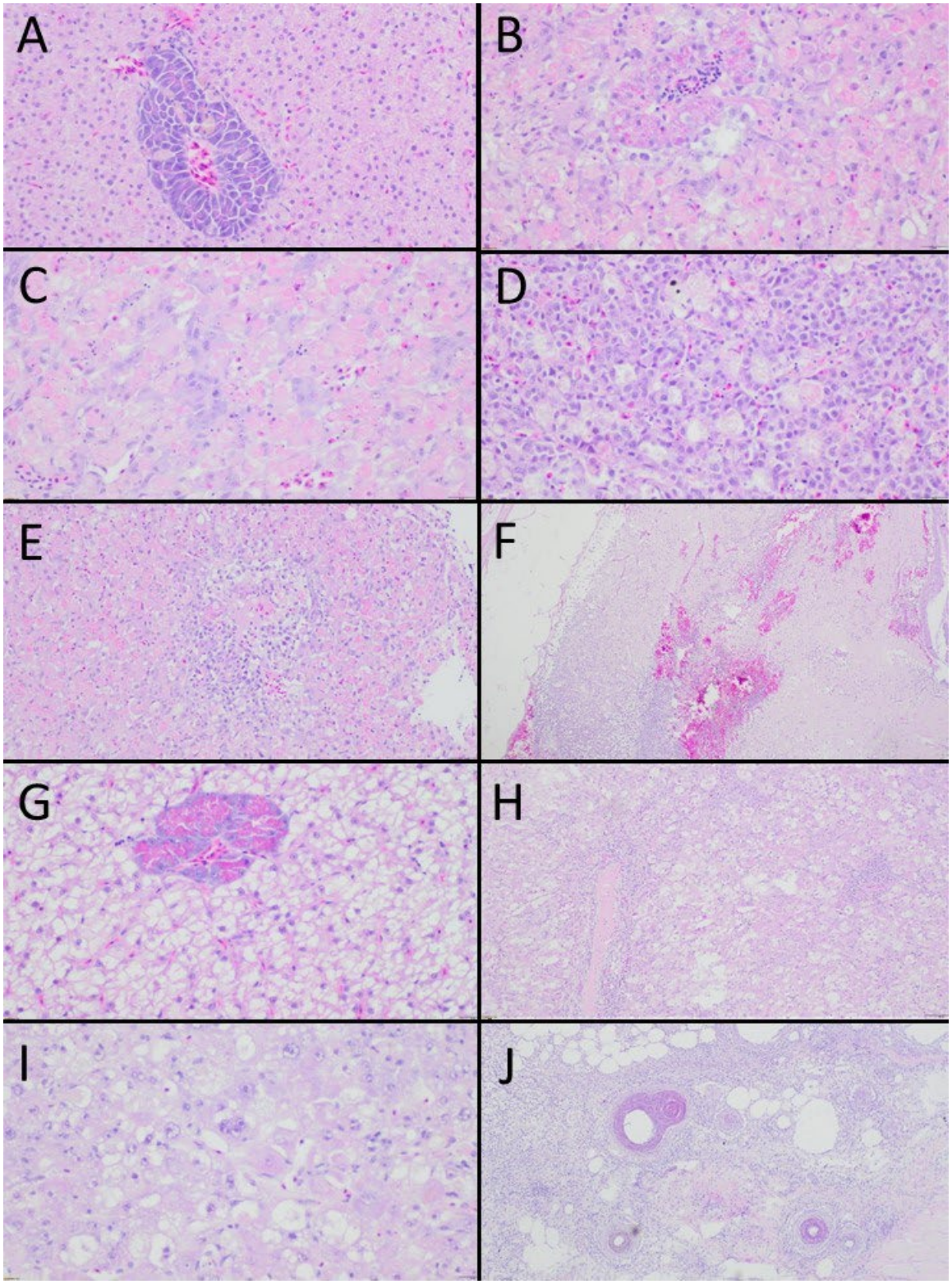

Figure 8. Photomicrograph of haematoxylin and eosin-stained sections liver and pancreas of O. mossambicus (A) and P. mariae (G) control fish. TiLV-infected fish with pancreatic and hepatocellular necrosis (O. mossambicus: B and P. mariae: H); syncytical cells (O. mossambicus: C and P. mariae: I); and perivascular leukocytic infiltrates in the liver (O. mossambicus: E). TiLV-infected O. mossambicus with cerebral haemorrhage (F) and kidney necrosis (D). TiLV-infected P. mariae with chronic granulomas, likely unrelated to TiLV infection (J).

2.4 DISCUSSION

This study provided evidence of the susceptibility of two species–*O. mossambicus* and *P. mariae*–to infection with TiLV; severe disease was directly attributable to this infection. The methods were consistent with the guidelines provided by World Organisation for Animal Health (WOAH), whereby evidence of susceptibility is derived from a non-invasive experiment procedure (cohabitation and immersion) and RT-qPCR provided evidence that TiLV was replicating in target tissues together with pathology consistent with the disease (WOAH 2019). A further important criterion in assessing the susceptibility of the species was provision of an adequate environment because it can affect the host's resistance or transmission of the pathogen. Here, the aquarium environment, temperature and nutrition supported growth and reproductive development (data not shown) in the absence of excessive background mortality or secondary disease. TiLV infection in experimental settings has predominantly used IP injection with the dose used in the present experiment being close to the lower end of the range used in other studies i.e. from $1x10^4$ (Pierezan et al. 2020), $1x\ 0^5$ (Eyngor et al. 2014; Jaemwimol et al. 2018) to 10^6 $TCID_{50}$/fish (Tattiyapong et al. 2017). This dose is justified by the large quantities of TiLV observed in infected fish and their environment. The cohabitation challenge in this study included fish being able to touch, as well as sharing water, which became contaminated when fish infected by injection were present. Demonstration of large quantities of TiLV in skin mucus suggests that close contact may be important for TiLV transmission. Additionally, when mortality occurred, there was evidence of cannibalism targeted towards the viscera (data not shown). With very high viral load in livers at the time of death, this route of infection is consistent with previous studies demonstrating TiLV infection by intragastric administration (Pierezan et al. 2019). Cohabitation provides a realistic transmission model to include the multiple potential exposure routes that would operate in field conditions and explains how *P. mariae* that were refractory to infection by immersion could become naturally infected. The immersion challenge (bath exposure) was unique to this study and with further investigation of the minimum dose and contact time, it will help understand pathways for spread of TiLV independent of live fish.

This study provides new information about the susceptibility of *P. mariae* to TiLV, with a current lack of evidence of the impacts of TiLV for this species. *Oreochromis* are the key tilapiine species susceptible to TiLV, and *O. mossambicus* hybrids have been impacted in aquaculture (Amal et al. 2018). Knowledge about the susceptibility of this species without hybridisation was recently established using an IP injection model (Waiyamitra et al. 2021). Australian genotypes of *O. mossambicus* had to be assessed for susceptibility due to the likely unique genetics arising over the long time period since their introduction to Australia from a small founder population. This species has a propensity to hybridise and has been subject to selective breeding for aquaculture or selection for fitness in adapting to different environments (Yáñez et al. 2020). Genetic variation within a host species is important to TiLV susceptibility as evidenced by selective breeding for strains of O*. niloticus* with reduced disease impacts (Barría et al. 2020).

The pathogenesis of disease was similar for both species in this study and was consistent with the descriptions in other susceptible species with replication of TiLV to high quantities in the liver leading to an acute high mortality disease with characteristic gross and histopathologic lesions (Lakshmi et al. 2023). The formation of syncytial cells and degeneration of hepatocytes is a consistent histopathological finding for disease caused by TiLV (Pierezan et al. 2020). When disease occurred in this study, it was severe and acute with histopathology demonstrating that the TiLV infection was the primary driver of the mortality. Higher mortality occurred in *O. mossambicus*, which were extremely susceptible to a systemic pathology associated with extensive virus replication. There was the suggestion of a different disease pathogenesis in some *P. mariae* that survived up to 14 days with persistent clinical signs leading to loss of body condition. There is the possibility that this severe loss of condition would lead to fatal disease progression in a different environment to these tank trials due to secondary disease or predation. The two species in this study also differed in the efficiency of TiLV transmission, with *O. mossambicus* completely susceptible in all conditions tested, while the efficiency in *P. mariae* was 50% for cohabitation and 0% for immersion. The identification of a spectrum of susceptibly to TiLV for different fish is apparent through susceptibility studies for a range of species (Thangaraj et al. 2022). For example, there was TiLV amplification and mortality of some angel fish

(Pterophyllum scalare) with pathology that consisted of necrosis rather than the typical syncytial cells (Paria et al. 2023). Meanwhile, amplification of TiLV without clinical signs and minimal pathology occurred in firemouth cichlid *(Thorichthys meeki),* while there was no TiLV replication or pathology in three-spot gourami *(Trichopodus trichopterus).* However, these studies only used IP injection challenge, leaving questions about the possibility of natural infection in any of the species tested.

The virulence of TiLV was high, producing an acute, high-mortality disease in infected fish of both species. The difference in mortality for *O. mossambicus* between the present study (100%) and the report of Waiyamitra et al. (2021) (49–78%) highlights the importance of factors other than dose and route of infection. To date, there is no evidence of differences in virulence for strains of TiLV (Pulido et al. 2019); however, this may become apparent as considerable viral genomic diversity is apparent (Tran et al. 2022). Important host factors include the age and size of the fish, with decreasing TiLV-related mortality demonstrated with increasing size (Roy et al. 2021). Of the important environmental conditions that impact TiLV disease pathogenesis, there is evidence that water temperature is important across a range from 22–32 °C (Adamek et al. 2023). There are limited data on the impact of other suboptimal water quality factors, including conditions involving oxygen concentration, pH and ammonia products and salinity; although stress generally is a risk factor for more severe disease (Dong et al. 2017). For this reason, experimental infection models cannot provide clear guidance on the expected mortality associated with a disease outbreak in the variable host-pathogen-environment scenarios that would be encountered in a biocontrol program.

There is a need for improved control of pest tilapia species that are degrading freshwater ecosystems in Australia and are refractory to available control measures, including physical removal in attempts at eradication (reviewed by Sunarto et al. 2022). New technologies, including genetic manipulation and sex determination, require further exploration. Identifying the susceptibility of the target species to a potentially fatal infection with TiLV is only the first consideration in assessing suitability as a biocontrol agent. A framework for integrating the social, economic and efficacy considerations of aquatic biocontrol has been established in the National Carp Control Plan (NCCP), which considered the suitability of Cyprinid herpesvirus 3 for biocontrol of feral carp in Australia (NCCP 2022).

2.5 CONCLUSION

Both tilapia species demonstrated susceptibility to infection with TiLV, although the subsequent disease expression was more acute and severe in *O. mossambicus* compared to *P. mariae* in the aquarium conditions used in this trial. A natural route of infection for TiLV used cohabitation to demonstrate a differential infection rate between *O. mossambicus*, which rapidly progressed to 100% mortality compared to *P. mariae*, which was limited to a 50% incidence of infection. Further, the different susceptibility to TiLV was highlighted by the absolute difference of 100% infection of *O. mossambicus* by immersion compared to no *P. mariae*. This study provides a proof-of-principal demonstration of the susceptibility of Australian genotypes of two tilapia species to TiLV as an initial step in assessing the suitably of this pathogen for biocontrol. Many factors will influence the rate of infection and severity of disease outside of the constraints in this study.

CHAPTER 3: BUSINESS CASE TO ADVANCE THE SELECTION AND TESTING OF NEW TILAPIA BIOCONTROL AGENTS IN AUSTRALIA

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BUSINESS CASE TO ADVANCE THE SELECTION AND TESTING OF NEW TILAPIA BIOCONTROL AGENTS IN AUSTRALIA

A REPORT BY TALIA HARDAKER & PETER CHUDLEIGH – AGTRANS RESEARCH

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Front cover image: Mozambique tilapia (*Oreochromis mossambicus*).

Executive Summary

Tilapia are listed in the top 100 of the world's worst introduced species. All tilapia species are considered pest species in Australia and pose a significant threat to native fish and Australian ecosystems. A study conducted in Queensland in 2008 suggested that the current economic impact costs of tilapia may lie between $1.2 million and $13.6 million per annum (2020/21 dollar terms). If targeted efforts to control tilapia are not undertaken the economic costs of tilapia in Queensland could increase to over $35.4 million per annum. Further, it is likely that, on a national scale, the impact costs could be significantly higher if tilapia are allowed to spread into other key Australian waterways, in particular the Murray-Darling Basin.

There is currently no single overall option for the control of tilapia. Ongoing research, development and extension (RD&E) is being funded and carried out by various research organisations in Australia to refine detection and control methods for tilapia. Biological control (biocontrol) was thought likely to be a potentially cost-effective and practical solution for the management for invasive fish species, including tilapia.

A current RD&E project, led by CSIRO for the Centre for Invasive Species Solutions (Project P01-B-003: *Tilapia biocontrol: prospecting and evaluation*), was funded to conduct a review of tilapia pathogens and assess their potential as biocontrol agents (BCAs, a process known as bioprospecting). This report, developed as part of Project P01-B-003, presents a business case and ex-ante cost-benefit analysis (CBA) to advance the selection of new tilapia BCAs for future management of feral tilapia in Australia.

The in depth, international review of potential tilapia BCAs covered a wide range of pathogens. Tilapia pathogens fall into the general categories of viruses, bacteria, parasites, and fungi. The review identified three tilapia viruses that were considered species-specific to tilapia and were categorised as tentatively worthwhile for further investigation. The three viruses were:

- Tilapia lake virus (TiLV),
- Tilapia parvovirus (TiPV), and
- Tilapia larvae encephalitis virus (TLEV).

TiLV was considered the most promising potential BCA candidates and was categorised as 'worthwhile for active further investigation'. CSIRO already have imported the virus and are currently developing the capability to work with TiLV in a laboratory setting. Further assessment of the safety and efficacy of BCAs for potential use in Australia requires rigorous testing and substantial, and often long-term, investment.

This business case proposes a six stage RD&E program (including the original investment in P01-B-003) to advance the selection and testing of new tilapia BCAs in Australia. The six stages would include:

- Stage 1: Bioprospecting and Evaluation (Project P01-B-003)
- Stage 2A: Efficacy Testing
- Stage 2B: RD&E on Complementary Tilapia Control Methods
- Stage 3: Safety Testing
- Stage 4: Planning and Modelling Optimal Release
- Stage 5: Other Assessments and Regulatory Approvals
- Stage 6: Nationally Coordinated Release and Clean-up

An ex-ante CBA was conducted to assess whether the proposed investment (the total costs of the research and development required to address the advancement of new BCAs to manage tilapia in Australia) would be paid for by the estimated potential benefits of the proposed BCA(s). Publicly available information on TiLV as the selected BCA was used within the analysis. The primary benefit of the proposed tilapia biocontrol investment is expected to be a net reduction in the total annual impact costs of tilapia to the Australian community and economy. This primary benefit would be driven by the release of a new tilapia BCA leading to a reduction in tilapia biomass and associated negative impacts.

Valuation of the primary impact involved making several uncertain assumptions as a number of key relationships along the pathways to impact were unknown. The total expected RD&E investment was estimated at $18.69 million (present value terms). The investment was estimated to produce total expected net benefits of $52.53 million (present value terms). This gave a net present value of $33.84 million, a benefit cost-ratio of 2.81 to 1, an internal rate of return of 9.3% and a modified internal rate of return of 7.1%. Investment criteria were estimated for the total investment, using a 5% discount rate, over a period of 50 years from the first year of investment in Project P01-B-003.

The positive investment criteria suggest that the initial investments (Stages 1 to 5) would be worthwhile given the estimates made of the current and future potential impact and control costs of tilapia in Australia, likely pathways to impact for proposed new BCAs, the RD&E investment and associated timelines required, and the risks involved. Further, the proposed investment can be staged conditionally (stop/go points) so that, as the investment proceeds along a particular pathway, the direction of the RD&E could be changed according to any past success and any new information available. This may avoid or minimise any potential losses and maximise the chances of significant impacts being delivered.

The successful identification of BCA candidates and the positive ex-ante CBA results from Project P01-B-003 indicate that the proposed investment in tilapia biocontrol RD&E is likely to be worthwhile and should be viewed favourably by the Centre for Invasive Species Solutions, potential funding partners, and other tilapia biocontrol and/or management stakeholders.

Contents

1. Introduction

Tilapia is the common name for a large number of species within the cichlid tribe Tilapiini (Russell, Thuesen, & Small, 2010). Tilapia were first introduced to Australia in the 1970s as an ornamental fish (Queensland Government, 2021). There have been three species of tilapia introduced to Australia, the Mozambique tilapia (*Oreochromis mossambicus*), the black mangrove cichlid (*Pelmatolapia mariae,* formerly *Tilapia mariae*), and the redbelly tilapia (*Coptodon zillii*) (Native Fish Australia, n.d.).

Tilapia are listed in the top 100 of the world's worst introduced species. All tilapia species are considered pest species in Australia and pose a significant threat to native fish and Australian ecosystems.

There are a range of control measures currently available for use on tilapia, but most are situation specific. Management tools include containment and/or exclusion, physical removal through netting, electrofishing, angling/ line fishing, draining of waterbodies, and chemical removal (poisons) (Centre for Invasive Species Solutions (CISS), 2021a). However, in the majority of situations and in the absence of effective ongoing management, unless the entire population and any possible source of reintroduction are removed, the highly flexible reproductive capacity of tilapia will see the population quickly return to original numbers (CISS, 2021a). There is currently no single overall option for the control of tilapia.

Ongoing research, development and extension (RD&E) is being funded and carried out by various research organisations in Australia to refine detection and control methods for tilapia (Department of Employment, Economic Development and Innovation, 2011). Biological control (biocontrol) was thought likely to be a potentially cost-effective and practical solution for the management for invasive fish species, including tilapia. Past and current RD&E has indicated that combinations of viral biocontrol and genetic technologies are emerging as the best technologies to cause a major decline in invasive fish numbers, and in some cases even lead to complete eradication.

Australia previously has had success using biocontrol to reduce populations of invasive European rabbits through the release of the myxoma virus (1950) and variants of rabbit haemorrhagic disease virus (RHDV) (1995 and 2017). Both the myxoma virus and RHDV variants have had significant positive impacts on Australian ecosystems and agricultural industries, reducing up to 90% of the wild rabbit population initially and providing estimated economic benefits of A$70 billion for the industries over 60 years (Cooke, Chudleigh, Simpson, & Saunders, 2013).

A current RD&E project, led by CSIRO for CISS (Project P01-B-003: *Tilapia biocontrol: prospecting and evaluation*), was funded to conduct a review of tilapia pathogens and assess their potential as biocontrol agents (BCAs, a process known as bioprospecting).

This report was developed under Project P01-B-003 and presents a business case and ex-ante cost-benefit analysis (CBA) to advance the selection of new tilapia BCAs for future management of feral tilapia in Australia.

2. Review of Potential Tilapia Biocontrol Agents

2.1 Overview

The following Sections provide a brief description of the tilapia bioprospecting work undertaken by CSIRO under CISS Project P01-B-003: *Tilapia biocontrol: prospecting and evaluation.* These sections include a summary of the key findings relevant to the current business case to advance the selection of new tilapia BCAs for future management of feral tilapia in Australia. The full review document, titled 'Tilapia pathogens with emphasis on potential biological control agents for invasive tilapia in Australia', is included in Appendix 1.

2.2 Biocontrol Agent Assessment Criteria

Biocontrol agent assessment criteria adapted from Henzell, Cooke, & Mutz (2008) and Peacock (2015) for rabbit biocontrol in Australia were used to assess the appropriateness, effectiveness, and efficiency of potential BCAs for tilapia. The full details of the BCA framework are described in Appendix 1. Table 1 below shows the final BCA assessment criteria used in the tilapia biocontrol review.

Table 1: Tilapia biocontrol agent assessment criteria

1.	**Appropriateness** 1.1. Species specificity – the BCA should not infect, let alone affect, any non-target species in Australia. 1.2. Socially acceptable – the nature and biological action of the BCA needs to be acceptable to the community. For example, is the agent naturally occurring in tilapia and is a vaccine available to protect other ornamental cichlids? 1.3. Humane – the BCA should cause rapid death.
2.	**Effectiveness** 2.1. Virulence – the BCA needs to cause high mortality in tilapia. Survivors are likely to seroconvert, become more resistant and may confer the resistance on their offspring through maternal immunity. This would likely lead to recovery of the tilapia populations. 2.2. Impacts on all ages – ideally the BCA needs to provide high impact on juvenile and adult tilapia. 2.3. Effectiveness in wild fisheries – the BCA needs to provide great impact in wild tilapia populations, e.g. regardless of the effect of temperature/season. 2.4. No antagonistic interaction with other pathogens – for example cross-protection by closely related pathogens that may be endemic.
3.	**Efficiency** 3.1. Transmission – the BCA would have the ability to transmit efficiently to other fish and have the capacity to spread through the local, regional, and national tilapia populations (self-disseminating). 3.2. Persists in the environment – the BCA should persist despite death of a high proportion of hosts and once established causes repeated outbreaks. 3.3. Cost for research and development – e.g. benefits should exceed the cost of testing the safety and efficacy of the candidates, risk assessment and cost-benefit analysis. 3.4. Cost for manufacture and distribution – preferably, the organism(s) could be cultured, prepared, and stored in large quantities to allow effective distribution. 3.5. Public and government approval requirements – i.e. are there any significant differences between biocontrol options, e.g. genetically modified organisms (GMOs) as a genetic biocontrol option also requires additional approval.

Source: Reproduced from CSIRO review: Tilapia pathogens with emphasis on potential biological control agents for invasive tilapia in Australia (CISS Project P01-B-003) (see Appendix 1).

2.3 Biocontrol Agent Assessment Findings

The in-depth, international review of potential tilapia BCAs covered a wide range of pathogens. Tilapia pathogens fall into the general categories of viruses, bacteria, parasites, and fungi. Specific details of the complete set of tilapia pathogens identified and assessed against the BCA assessment criteria can be found in the full review in Appendix 1.

Table 2 (below) shows the identified candidate tilapia pathogens reviewed against the BCA assessment criteria using a traffic light rating system. Overall, the bioprospecting review found that a large number of bacteria, fungi, and parasites have been associated with natural disease outbreaks in tilapia worldwide. However, none of them were species-specific to tilapia and therefore were rejected as BCA candidates. More promisingly, nine viruses have been reported in tilapia. Six of them were found to have first been reported in species other than tilapia and therefore were assessed as not suitable as BCA candidates. The other three viruses, originally reported in tilapia were:

- Tilapia lake virus (TiLV),
- Tilapia parvovirus (TiPV), and
- Tilapia larvae encephalitis virus (TLEV).

All three viruses were considered to be species-specific to tilapia and were categorised in the review as being tentatively worthwhile biocontrol candidates for further investigation. Table 3 (below) describes a set of summary information for the three tilapia pathogens that were assessed as being tentatively worthwhile biocontrol candidates for further investigation.

TiLV was considered the most promising potential BCA candidate and was categorised as ‘worthwhile for active further investigation’. CSIRO already have imported the virus and are currently developing the capability to work with TiLV in a laboratory setting. The project team currently plans to test TiLV’s susceptibility in tilapia sourced from QLD waters in January 2022.

TiPV was categorised as ‘tentatively worthwhile’ for further investigation. TiPV is the first and only parvovirus known to infect fish. The virus also has been isolated in cell cultures, allowing future testing of the virus including experimental challenge.

TLEV was categorised under a ‘watching brief’. This means that TLEV was not selected for further investigation right now but will be watched as a possible future BCA through the international literature and scientific networks.

Table 2: Candidate pathogens reviewed against biocontrol agent assessment criteria

Candidate pathogen	Appropriateness			Effectiveness				Efficiency				
	Species specificity	Socially acceptable	Humane	Virulence in tilapia	Impacts on all ages of tilapia	Effectiveness in wild fisheries	Interactions with other pathogens	Transmission	Persists in the environment	Cost for research & development	Cost for manufacture & distribution	Public and government approval requirements
Tilapia lake virus (TiLV)												
Tilapia parvovirus (TiPV)												
Tilapia larvae encephalitis virus (TLEV)												
Nervous Necrosis Virus (NNV)												
Bohle Iridovirus (BIV)												
Infectious spleen and kidney necrosis virus (ISKNV)												
Streptococcus agalactiae												
Streptococcus iniae												
Aeromonas hydrophila												
Aeromonas veronii												
Flavobacterium columnare												
Francisella sp.												
Edwardsiella tarda												
Pseudomanas sp.												
Aphanomyces invadans												
Saprolegenia sp.												
Branchiomyces												
Gyrodactylus cichlidarum												
Gyrodactylus olsoni												
Gyrodactylus imperialis												
Trichodinia sp.												
Myxobolus bejeranoi												

Key: Positive | Minor concerns | Major concerns

Source: Reproduced from CSIRO review: Tilapia pathogens with emphasis on potential biological control agents for invasive tilapia in Australia (CISS Project P01-B-003) (see Appendix 1).

Table 3: Summary of information for the candidate biocontrol agents worthwhile for further investigation[(a)]

Candidate virus	Appropriateness			Effectiveness				Efficiency				
	Species specificity	Socially acceptable	Humane	Virulence in tilapia	Impacts on all age of tilapia	Effectiveness in wild fisheries	Interactions with other pathogens	Transmission	Persists in the environment	Cost for research & development	Cost for manufacture & distribution	Public and government approval
Tilapia lake virus (TiLV)	TiLV causes disease outbreaks and mortalities in farmed and wild tilapia, but not in other fish species co-cultured or sharing waterways with tilapia (Eyngor et al., 2014, Surachetpong et al., 2017). However, wild river barb was found to be TiLV-positive by RT-PCR (Abdullah et al., 2018) and giant gourami was affected by TiLV via IP injection and co-habitation challenges (Jaemwimol et al., 2018).	TiLV is a naturally occurring virus in wild and farmed tilapia (not a GMO). Good management practices (Jansen et al., 2018) and biosecurity measures (OIE, 2018a) are in place and prototype vaccines are available to protect farmed and ornamental tilapia (Zeng et al., 2021).	Acute mortality occurs within a few days post infection (Eyngor et al., 2014). Chronic up to 24 days and sub-clinical infection have also been observed (Jaemwimol et al., 2019, Senapin et al., 2018).	Experimental infection of tilapia with TiLV conducted in geographically different regions resulted in consistently high levels of mortality. However, wide variations in mortality associated with TiLV have been reported in wild and farmed tilapia, ranging from very low mortalities (0.71% in Malaysia and 6.4% in Chinese Taipei) to relatively high mortality (80% in Israel, 20-90% in Thailand, and 80-90% in India).	TiLV has been reported to cause mortality in all age groups of tilapia (Yamkasem et al., 2019).	TiLV causes mortality of wild tilapia, for example, declines in tilapia populations in the Sea of Galilee, Israel (Eyngor et al., 2014). TiLV have also been reported in wild tilapia from Malaysia, Tanzania, Uganda, and Peru.	No antagonistic interactions have been observed during co-infections in tilapia (Abdel-Latif et al., 2020). Co-infections of TiLV and *A. hydrohila* caused 93% mortality of tilapia compared to either TiLV (34%) and *A. hydrophila* (6.7%) alone (Nicholson et al., 2020). No other viruses within the family *Amnoonviridae* have been reported in tilapia (ICTV, 2018).	Epidemiological findings and cohabitation mode of horizontal transmission (Eyngor et al., 2014, Liamnimitr et al., 2018) demonstrates the ability of TiLV to spread by waterborne routes. Vertical transmission has also been observed (Yamkasem et al., 2019).	Most likely but need to determine how long TiLV survives in the water and in dead fish. TiLV RNA has been detected in mucus (Liamnimitr et al., 2018), feces and water tanks containing TiLV-infected fish (Pierezan et al., 2019). Persistent or latent infection has not been reported.	Medium-sized project to test the efficacy (virulence and transmission). Large project to test the safety (susceptibility of non-target species).	TiLV grows in cell cultures and could be transported in freeze dried form or cold at 4°C.	Viral biocontrol agent has never been used or approved for use against invasive fish, and therefore, public and government approval for the viral biocontrol in tilapia is a major concern. However, Australia has very strong legislative mechanisms for approval of biocontrol agent (Biological Control Act 1984) which may facilitate the process.

Tilapia parvovirus (TiPV)	It has only been reported in tilapia in China (Liu et al., 2020) and Thailand (Yamkasem et al., 2021).	Vaccine is not available.	Experimental infection showed TiPV causes 90% mortality in tilapia within 11 days.	TiPV causes 60-70% mortality in cage-farmed tilapia.	TiPV has been reported in all size of adult tilapia.	Unknown	No other parvovirus has been reported in tilapia or any other fish species.	TiPV is contagious, spreading to six cities in three provinces in China.	Unknown	TiPV is a newly emerging virus with only two publications available and therefore little is known about the characteristics of the virus.	TiPV grows in cell culture.	Ditto above.
Tilapia larvae encephalitis virus (TLEV)	It has only been reported in tilapia in Israel (Shlapobersky et al., 2010, Sinyakov et al., 2011).	Vaccine is not available.	The virus affects brain and the disease is characterised by a whirling syndrome (a spiral swimming behaviour).	High mortality rates of up to 96% and 80% in blue and red tilapia larvae, respectively.	TLEV has only been reported in larvae of tilapia.	Unknown	No other herpesvirus has been reported in tilapia.	TLEV is capable of both vertical transmission from the mother to their offspring and horizontal transmission through water from infected fish.	Unknown	Although it was reported a decade ago, only two publications are available and therefore little is known about the characteristics of the virus.	TLEV has not been isolated or cultured in cell lines.	Ditto above.

Source: Reproduced from CSIRO review: Tilapia pathogens with emphasis on potential biological control agents for invasive tilapia in Australia (CISS Project P01-B-003) (see Appendix 1).
(a) The references within Table 3 can be found in the Reference List for the full review in Appendix 1.

3. Discussion of Candidate Pathogens

3.1 BCA Candidates Assessed as Tentatively Worthwhile for Further Investigation

3.1.1 Worthwhile for Active Further Investigation: Tilapia lake virus (TiLV)

TiLV was first reported to cause mass die-offs in farmed and wild tilapia in Israel as early as 2009. Since then, TiLV has been reported in 16 countries across four continents, suggesting that the virus is able to survive in different ecological niches and climates. Epidemiological findings also suggest that TiLV is contagious and spreads through a waterborne route, an important transmission pathway for a potential biocontrol virus of fish.

Being an aquatic species, tilapia may have infection with other pathogen(s) that provide cross-protection against TiLV and affect its effectiveness as a BCA. Although both synergistic and antagonistic interactions occurring during co-infections of multiple pathogens have been reported in fish, antagonistic effects have not been observed in tilapia to date. Naturally occurring and experimentally induced co-infections of TiLV and other pathogens including *A. hydrophila*, *A. veronii*, *A. isthiosmia*, *A. enteropelogenes*, and *S. agalactiae* showed higher rates of mortality in tilapia, suggesting that multiple infections in tilapia have a synergistic effect.

TiLV causes disease outbreaks and mortalities in both farmed and wild tilapia populations, but not in other fish species co-cultured or sharing waterways with tilapia, suggesting that TiLV is species-specific to tilapia. Although there are no native Australian fish belonging to the families *Cichlidae* (tilapia), *Osphronemidae* (gourami) or *Cyprinidae* (carp and barb), because the host range of a virus is difficult to predict, and aquatic ecosystems are complex, rigorous non-target species testing is needed before the use of any viral biocontrol.

Therefore, two major concerns for a successful biocontrol, safety (species-specificity) along with efficacy (virus virulence and transmission), will be rigorously tested in the proposed next stages of the assessment process.

3.1.2 Tentatively Worthwhile: Tilapia parvovirus (TiPV)

Tilapia parvovirus (TiPV) is a newly emerging virus identified in China and Thailand which has been reported to cause 60-70% mortality in tilapia but not in other fish species. First observed in Hubei province (China), TiPV now has been reported in six cities across three provinces, suggesting that the virus is rapidly spreading. The virus also has been isolated in tilapia brain cells, allowing further characterisation of the virus including through experimental challenge. In the challenge, TiPV caused 90% mortality in tilapia within 11 days.

TiPV is the first and only parvovirus known to infect fish and is considered to be species-specific to tilapia. Therefore, TiPV has been categorised as potentially worthwhile for further investigation as a BCA candidate for control of tilapia in Australia. A watching brief for new information/ data on TiPV in the international literature was recommended. Further information on the identification and assessment of TiPV as a BCA candidate can be found in Appendix 1.

3.1.3 Watching Brief: Tilapia larvae encephalitis virus (TLEV)

Outbreaks of TLEV were first reported in tilapia larvae in Israel approximately a decade ago. The virus has never been reported again either in Israel or in other countries, raising the question of whether the virus still persists in the environment. The virus has only been associated with mortalities in tilapia larvae in hatcheries, suggesting that the impact of TLEV in adult tilapia and its effectiveness in wild fisheries are unknown. TLEV has not been isolated in cell cultures, hindering further characterisation of the virus, and therefore, the cost for research and development. In additional, manufacture and distribution of TLEV are major concerns.

TLEV is considered to be species-specific to tilapia and has been categorised as potentially worthwhile for further investigation as a BCA candidate for control of tilapia in Australia. A watching brief for new information/ data on TLEV in the international literature was recommended. Further information on the identification and assessment of TLEV as a BCA candidate can be found in Appendix 1.

4. Proposed RD&E Investment for Biocontrol Candidates Assessed as Worthwhile

Rigorous testing of safety and efficacy is a well-accepted practice in Australia for BCAs and requires substantial, and often long-term, investment. The following Section describes the likely next Stages of RD&E investment that would be required to advance the selection, testing and potential release of a new tilapia BCA, specifically either TiLV or TiPV, for management of invasive tilapia in Australia. Further detail (including relevant references) is described in the full BCA review in Appendix 1.

The RD&E stages and investment costs are likely to be similar for both TiLV and TiPV should the Environment and Invasives Committee (EIC) and individual jurisdictions (States and Territory) support additional investment to progress tilapia biocontrol RD&E. With the exception of the bioprospecting work carried out under CISS Project P01-B-003, the additional RD&E investment to advance any given candidate BCA (e.g. TiLV or TiPV) would be independent and additive if more than one agent was to be investigated.

4.1 Advancing Tilapia Biocontrol RD&E

- Stage 1: Bioprospecting and Evaluation

CISS Project P01-B-003: *Tilapia biocontrol: prospecting and evaluation* represents Stage 1 of the RD&E investment required to identify and advance the selection of a new BCA for tilapia in Australia. To progress any tilapia BCA candidates identified as worthwhile for further investigation, it will be essential to formally and thoroughly evaluate the agent.

As of August 2021, TiLV had been selected for active further investigation and additional research already had commenced (see Stage 2A below).

- Stage 2A: Efficacy Testing

Where, based on the findings of Stage 1, the EIC and individual jurisdictions (States and Territory) support additional investment to progress tilapia biocontrol RD&E, the next stage of the project (Stage 2) would be testing the candidate BCA's efficacy (virus virulence and transmission). Further, alongside the efficacy testing, RD&E is required to systematically assess the possibility of interfering endemic viruses and also the possibility of reassortments (Chaput et al., 2020). This would involve, for example, meta-transcriptomic analyses (Turnbull et al., 2020), of other viruses in Australian tilapia populations.

CSIRO already have imported TiLV and are developing the capability to work with it in a laboratory setting. The project team will commence testing of TiLV's susceptibility in tilapia sourced from QLD waters in January 2022. Additional and independent investment in similar RD&E would be required should stakeholders choose also to progress TiPV as a potential tilapia BCA.

- Stage 2B: RD&E on Complementary Tilapia Control Methods

Further work including the identification of other broad-scale control measures, such as genetic control, to complement the virus would need to be considered. A number of genetic technology options for broad-scale control may be applicable for tilapia, and some are currently under investigation. These include genetic biocontrol such as 'gene drives' and/or self-stocking incompatible male systems (CISS Project P01-B-005). Australia is currently investing in RD&E to investigate these broadly applicable technologies for managing invasive fish species. A prerequisite for genetic biocontrol approaches is a thorough assessment of the genetic makeup and diversity of Australian tilapia (population genomics analyses). This is important as there already is significant evidence of hybridisation occurring among wild populations.

It would be beneficial if the findings of any successful RD&E into complementary control measures could be built into Stage 4 (if available) and Stage 6 to ensure the greatest control can be achieved (i.e. optimal and maximum reduction of tilapia biomass in Australian waterways).

- Stage 3: Safety Testing

Stage 3 would involve testing the safety (susceptibility of non-target species) of TiLV as a BCA. If successful, the data generated from the efficacy (Stage 2) and safety (Stage 3) trials on the virus then will provide input to development of an epidemiological model for TiLV.

The findings from the Stage 2 and 3 RD&E investment represent an important stop/go decision point for any future investment to further advance a new BCA toward release as a practical tool for tilapia control in Australia.

Note: if resources committed to tilapia biocontrol RD&E in the future permitted, it would be possible that Stage 3 could be undertaken concurrently with Stage 2 (A and B). This would reduce the overall timeframe for the proposed tilapia biocontrol RD&E.

- Stage 4: Planning and Modelling Optimal Release

If the findings from Stage 2 and 3 RD&E indicate that the proposed BCA (e.g. TiLV) could be used as a safe and effective tilapia BCA in Australia, the epidemiological model then will be used as a key part of further RD&E required to determine the optimal release strategy, or strategies, for the virus (Stage 4). Understanding and optimising potential release strategies will provide critical input for planning, coordinating, and costing any actual future release of a new BCA, pending necessary approvals. It is likely that work conducted in Stage 4 would be predominantly QLD-centric and would be modelled on work associated with European Carp undertaken as part of the recent National Carp Control Program (NCCP).

- Stage 5: Other Assessments and Regulatory Approvals

Social and ecological risk assessments will be needed to support an application to release a new BCA against tilapia in Australia. Application for release of any such tilapia BCA would be made through the Commonwealth Department of Agriculture, Water, and the Environment (DAWE) and Australian Pesticide and Veterinary Medicines Authority (APVMA).

Applications for regulatory approval to release a new tilapia BCA in Australia would rely heavily on data and information generated through investment in Stages 1 to 4. Further, the outcomes of the required applications (e.g. approval, approval with conditions, approval in principle with additional information/ data required, and/or non-approval) represent another important stop/go point for further investment in tilapia biocontrol.

- Stage 6: Nationally Coordinated Release and Clean-up

If a new tilapia BCA is approved for release in Australia, a structured collaborative program of release strategies and planning and coordination of any clean-up will be developed. This stage of investment (Stage 6) also will address bioethical issues and public acceptance of viral biocontrol of tilapia. This stage also will need to include investment for activities to support effective and efficient biocontrol release such as post-release monitoring and additional RD&E focused on the development of new virus variants in subsequent years as fish develop natural immunity/ resistance to the BCA.

To date, a viral BCA has never been used or approved for use against aquatic invasive species in Australia[1]. Therefore, public and government approval is considered a major concern. Australia has very strong legislative mechanisms for approval of BCA including the Commonwealth Biological Control Act 1984 along with Acts in the States and Territories as well as numerous international conventions. The legislation requires procedures to demonstrate that:

1) There is an urgent need to control the pest,
2) The BCA will likely reduce the impacts caused by the invasive species, and
3) The release of the BCA will not negatively affect the environment and the non-target species sharing the waterways.

4.2 Other Activities

To ensure that effective future control of tilapia does not depend solely on the successful advance and release of a single candidate pathogen BCA, it will be important for invasive species researchers to remain up to date and informed on other potential tilapia biocontrol candidates.

Specifically, as the RD&E on TiLV progresses, some investment should be made to maintain the watching brief on the other tilapia BCAs that were assessed as being tentatively worthwhile, such as TELV and TiPV. Further, information on other existing or emergent tilapia pathogens in the international literature should continue to be monitored and ongoing professional engagement and networking between invasive species experts/ researchers and other stakeholders should be facilitated wherever practical.

[1] It is worth noting that cyprinid herpes virus 3 (CyHV-3) as a BCA to control feral European carp in Australian waterways currently is under review by the Australian Government following the National Carp Control Program (NCCP) undertaken between 2016 and 2021. More information can be found at: https://carp.gov.au/

4.3 Proposed Tilapia Biocontrol RD&E Timeframes

The following GANTT chart outlines the proposed project schedule for further investment over the next ten years for Stages 1 to 4 of tilapia biocontrol RD&E. The chart was developed based on RD&E for TiLV but similar stages and RD&E time periods also would apply to TiPV should additional investment be made to progress safety and efficacy testing of an additional candidate BCA.

Activities	Financial Year											
	2020-21	2021-22	2022-23	2023-24	2024-25	2025-26	2026-27	2027-28	2028-29	2029-30	2030-31	2031-32
Stage 1 Tilapia biocontrol - Bioprospecting												
Stage 2A Testing the efficacy of TiLV												
Stage 2B Complementary genetic technologies												
Stage 3 Testing the safety of TiLV												
Stage 4 Modelling and release strategy												

Note: Investment in RD&E to further the epidemiological modelling of TiLV and optimal release strategies (Stage 4) is likely to be dependent on success of the RD&E under Stages 1 to 3 (stop/go point).

5. Estimated Economic Benefits of Recommended Tilapia Biocontrol Candidates

Based on a study conducted in Queensland (Greiner & Gregg, 2008), it was estimated that the current economic impact costs of tilapia may lie between $1.2 million and $13.6 million per annum (2020/21 dollar terms). If targeted efforts to control tilapia are not undertaken to prevent the future spread of tilapia, the economic costs could increase to over $35.4 million per annum. Further, it is likely that, on a national scale, the impact costs could be significantly higher were tilapia to spread into other key Australian waterways, in particular the Murray-Darling Basin. Without intervention this scenario is considered highly likely.

There is currently no single overall option for the control of tilapia in Australia. Ongoing RD&E is being funded and carried out by various research organisations to refine detection and control methods for tilapia. Biocontrol is thought to be a potentially cost-effective and practical solution for the management for invasive fish species, including tilapia.

An ex-ante cost-benefit analysis (CBA) was conducted to assess whether the proposed investment (the total costs of the research and development required to address the advancement of new BCAs to manage tilapia in Australia) would be paid for by the estimated potential benefits of the proposed BCA(s).

To date, Project P01-B-003 has successfully identified three potential tilapia biocontrol candidates categorised as tentatively worthwhile for further investigation. TiLV currently is considered the most promising potential biocontrol candidate and was categorised as ‘worthwhile for active further investigation’. CSIRO already have imported the virus and are currently developing the capability to work with TiLV in a laboratory setting. Thus, information on TiLV was used as the selected BCA within the CBA.

The CBA was set within a staged risk management framework of investment. The approach included identifying and describing the six stages of RD&E for the proposed tilapia biocontrol investment, RD&E objectives, planned activities, expected outputs and outcomes. Potential impacts associated with the expected outcomes then were identified and categorised as economic, environmental, and social impacts. The primary impact is expected to be a net reduction in the total annual impact costs of tilapia to the Australian community and economy through a reduction in tilapia biomass.

Valuation of the primary impact involved making several uncertain assumptions as a number of key relationships along the pathways to impact were unknown. The total expected RD&E investment was estimated at $18.69 million (present value terms). The investment was estimated to produce total expected net benefits of $52.53 million (present value terms). This gave a net present value of $33.84 million, a benefit cost-ratio of 2.81 to 1, an internal rate of return of 9.3% and a modified internal rate of return of 7.1%.

Care should be taken when interpreting the results of the ex-ante analysis. It is important to note that the expected release and subsequent impact of a new tilapia BCA, such as TiLV, would not occur until approximately 22 years after the first year of investment in Project P01-B-003. Given that the investment criteria became positive between 25 and 30 years after the first year of investment, this indicates that implementation of a new tilapia BCA would create benefits sufficient to cover the costs of the proposed tilapia biocontrol RD&E investment within five to ten years of release of the BCA.

Further, it is important to remember that the ex-ante analysis was conducted within a risk management framework and that the results are expected values. This means that it is theoretically possible for the total proposed investment in tilapia biocontrol to be made (approximately $45.6 million in nominal dollars) and for there to be no benefits realised. That is, the new agent is released and is unsuccessful in reducing tilapia impact costs. However, the risk of this is very minimal as the proposed tilapia biocontrol RD&E investment has been planned as a staged investment with a

number of key stop/ go points that would enable funding partners, researchers and other stakeholders to adjust and/ or redirect the RD&E to alternative and more promising directions. Also, the knowledge generated through Stages 1 to 3 are likely to contribute to increased scientific knowledge and research capacity associated with management of pest tilapia in Australia.

The positive investment criteria suggest that the initial investments (Stages 1 to 5) would be worthwhile given the estimates made of the current and future potential impact and control costs of tilapia in Australia, likely pathways to impact for proposed new BCAs, the RD&E investment and associated timelines required, and the risks involved. Further, the proposed investment can be staged conditionally (stop/go points) so that, as the investment proceeds along a particular pathway, the direction of the RD&E could be changed according to any past success and any new information available. This may avoid or minimise any potential losses and maximise the chances of significant impacts being delivered.

The successful identification of BCA candidates and the positive ex-ante CBA results from Project P01-B-003 indicate that the proposed investment in tilapia biocontrol RD&E is likely to be worthwhile and should be viewed favourably by the Centre for Invasive Species Solutions, potential funding partners, and other tilapia biocontrol and/or management stakeholders.

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Abbreviations and Acronyms

BC	Business Case
BCA	Biological Control Agent
Biocontrol	Biological Control
CBA	Cost-Benefit Analysis
CISS	Centre for Invasive Species Solutions
EIC	Environmental and Invasives Committee
MDB	Murray Darling Basin
NSW	New South Wales
QLD	Queensland
RD&E	Research, Development and Extension
RHDV	Rabbit Haemorrhagic Disease Virus
TiLV	Tilapia Lake Virus
TiPV	Tilapia Parvovirus
TLEV	Tilapia Larvae Encephalitis Virus
WA	Western Australia

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Appendices

Appendix 1: P01-B-003 Tilapia Biocontrol Review

Tilapia pathogens with emphasis on potential biological control agents for invasive tilapia in Australia

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Abstract

Originating in Africa, tilapia (Pisces, *Cichlidae*) now have a worldwide distribution and are both a prime model system for evolutionary biology as well as an important aquaculture species in over 135 countries. In contrast, Mozambique tilapia (*Oreochromis mossambicus*) is also listed in the top 100 of the world's worst invasive alien species and has been documented to have severe impacts on freshwater ecosystems primarily through displacement of native species and habitat alteration. In Australia, both *O. mossambicus* and the lesser-known spotted tilapia (*Tilapia mariae*) have established significant populations within Queensland waters, and recent incursions into northern New South Wales are of great concern. Eradication attempts using a combination of electrofishing and piscicide poisons are rarely successful in open waterways, and given their invasive nature, there is a lack of demonstrated broad-scale effective control mechanisms for tilapia. Biological control (biocontrol) where it is feasible can be a cost-effective, safe (species specific) and practical solution to managing invasive species because it does not require reapplication of chemicals or poisons, and once established should be self-sustaining. Based on the development of previous viral biocontrol strategies for rabbit and carp, we used a robust assessment framework for bioprospecting of biocontrol agents and found that tilapia lake virus (TiLV), and possibly tilapia parvovirus (TiPV), may offer the potential of biocontrol for invasive tilapia in Australia. TiLV causes high mortality in wild and cultured tilapia, but not in other species, and spreads through a waterborne route - an important transmission pathway for a successful viral biocontrol of fish. However, safety and efficacy, two major concerns for a successful biocontrol virus, need to be taken into consideration before the use of any exotic biocontrol virus is considered. Here, we describe a systematic approach to assess known pathogens for their suitability as potential agents for biological control of tilapia and outline the possible next steps to further investigate the top candidates.

Keywords: viral biocontrol, biological control agent (BCA), tilapia lake virus (TiLV), tilapia parvovirus (TiPV), tilapia, aquatic invasive species.

1. Introduction

Tilapia refers to a group of subtropical to tropical tilapiine fish of the family *Cichlidae*, one of the most species-rich families of vertebrate (Kocher, 2004). Tilapia are grouped into three genera according to parental care patterns: *Oreochromis* (maternal mouthbrooders), *Sarotherodon* (paternal or biparental mouthbrooders), and *Tilapia* (substrate-spawners) (Trewavas, 1982a, Trewavas, 1982b). The African cichlids represent a paraphyletic assemblage known as the haplotilapiine lineage (Dunz and Schliewen, 2013) which is comprised of more than 3,000 species and is naturally distributed across Africa to Madagascar, the Middle East, Southern India, Sri Lanka, Central and South America (Snoeks, 2000, Turner et al., 2001). The lineage gave rise to the spectacular East African cichlid radiation (EAR), a phenomenon where a single lineage diversified into many ecologically varied species in a short span of time. The rapid radiation of cichlid fish in the East Africa Great Lakes, namely Lakes Tanganyika, Malawi and Victoria, evolved almost 2,000 unique species in the last 10 million years, making the African cichlids an ideal model system for understanding the mechanism of vertebrate evolution and speciation (Kocher, 2004, Seehausen, 2006, Trewavas, 1947). The adaptive nature of cichlids also contributed to the successful spread of tilapia worldwide. Originating in Africa, tilapia have been introduced into all five continents (Asia, North and South America, Europe and Australia) since the 1930s for different reasons including biological control of aquatic weeds and insects, as baitfish for certain capture fisheries, as ornamental species, for restocking to augment capture fisheries, and as an aquaculture commodity (Canonico et al., 2005, De Silva et al., 2004). Tilapia not only enjoy an international reputation as a prime model system in evolutionary biology (Kocher, 2004, Kornfield and Smith, 2000) but also as the second most important aquaculture commodity after carp (FAO, 2019), despite being listed in the Global Invasive Species Database among the top 100 of the world's worst invasive alien species (GISD, 2006, Lowe et al., 2000).

Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) farming is considered the world's oldest aquaculture venture which can be traced to ancient Egypt dating back over 4000 years (Gupta and Acosta, 2004). The first scientific trials of tilapia culture in modern history were recorded in Kenya in 1924. Since then, tilapia culture has expanded worldwide, initially with Mozambique tilapia, *O. mossambicus* (Peters, 1852) during the 1940s and 1950s, and then the more productive Nile tilapia during the 1960s up to the 1980s. Mozambique tilapia were introduced from East Africa to Indonesia for aquaculture purposes in 1939. During World War II, tilapia was introduced to Singapore, Malaysia, and Taiwan by the Japanese, who had brought the fish from the island of Java, Indonesia. From Malaysia they spread to Thailand (1949) and subsequently from Thailand to the Philippines (1950), India (1952), Bangladesh (1954) and Japan (1954). Multiple introductions of *O. mossambicus* to Asia and the Pacific have significantly increased the animal protein production in the regions (Lin, 1977). However, *O. niloticus* became the preferred species due to its higher growth rate and greater consumer appeal (Smith and Pullin, 1984). Nile tilapia have been established as the main farmed tilapia not only in Asia including China (1978), but also in America (Brazil in 1971 and the United States in 1974). Currently, tilapia have been farmed in over 135 countries with global production estimated at 4.5 million metric tonnes and valued at US$7.5 billion (FAO, 2019). Interestingly, over 90 percent of the global tilapia production comes from the top ten of tilapia-producing countries including China, Indonesia, Egypt, the Philippines, Thailand, Bangladesh, and Vietnam, of which only Egypt is in the native range of these fish. Various species of tilapia exhibit high value aquaculture traits including high fecundity, rapid growth rate, tolerance to adverse water quality, and relative resistance to disease and other stressors (De Silva et al., 2004). Tilapias are also known as the 'aquatic chicken' because of their affordable and high-yield source of protein that can be raised in a wide range of production systems – from subsistence backyard ponds to high intensity farms. Tilapia have made a significant contribution to food production, poverty alleviation and livelihood support in Asia and the Pacific (De Silva et al., 2004), and in terms of volume they are the second most important aquaculture commodity after carp (FAO, 2019).

Mozambique tilapia are a maternal mouthbrooder native to eastward flowing rivers of central and southern Africa which include Eswatini, Lesotho, Malawi, Mozambique, South Africa, Zambia and Zimbabwe (Trewavas, 1982a, Trewavas, 1982b). *O. mossambicus*, which can grow up to 40 cm long and 1.1 kg, has been considered as a "model invader" because it is aggressive, has an extraordinary environmental adaptability (it tolerates wide ranges of salinity, temperature and dissolved oxygen), phenotypic plasticity (ability to modify cranial and dental structures to fit the food type available), high hybridization capacity and rapid reproduction due to its maternal mouthbreeder status (Pérez et al., 2006). Although it has been considered as an invasive species in Australia, the Bahamas, Dominican Republic, Mexico and the United States of America (USA), its invasiveness in other countries where Mozambique tilapia has been introduced is unknown (GISD, 2006).

In Australia, tilapia have caused severe impacts on the natural environment primarily through displacement of native species, habitat alteration, predation, and as a vector of diseases and non-native parasite transmission (Hutchison et al., 2011, IA-CRC, 2012a, Russell et al., 2012b, Russell et al., 2010, Wilson et al., 2019). The establishment of tilapia is currently predominantly in Queensland, but recent incursions into northern New South Wales freshwater ecosystems have caused concern for waterway managers. The nearby Murray-Darling Basin (MDB), Australia's largest river basin that spans four of the most populous states, has also been assessed as suitable habitat (>50%) to support Mozambique tilapia (Hutchison et al. 2012). Despite the importation of live tilapia into Australia being prohibited since 1963, the ornamental *O. mossambicus* from either Singapore or Indonesia were released by a Brisbane aquarist in 1977 (Bluhdorn and Arthington, 1989, McKay, 1977, McKay, 1978). Since then, the species has been reported to establish in many eastern catchments in Queensland, from Brisbane in the south to Cairns in the north (Figure 1). The population in the Burnett catchment is of particular concern since this is only two kilometres from the headwaters of MDB. Another area at high risk of invasion is the Gulf of Carpentaria (GoC) (IA-CRC, 2012b), of which both the lesser known tilapia *T. mariae* and *O. mossambicus* recently established in the Walsh River catchment of the GoC in 2017 and 2019, respectively (B. Holmes, *unpubl. data*). In addition, the species has also been established in Western Australia. They were first found in an ornamental pond in Geraldton in 1978 and have since colonised the Gascoyne, Chapman, Minilya and Lyndon Rivers, all of which constitute part of the Pilbara Drainage (Morgan et al., 2004).

Spotted tilapia (*T. mariae* Boulenger, 1899) is a freshwater and estuarine cichlid native to West Africa and has been introduced to and become established in Australia, the United States and Russia (Courtenay and Robins, 1973, Ivoylov, 1986, Cadwallader et al., 1980). In contrast to *O. mossambicus*, which is a maternal mouthbrooder*, T. mariae* is substrate-spawners – the female lay their eggs on hard substrate which will be fertilised by the male (Russell et al., 2012a). Due to its relatively low growth rate and fecundity, high natural mortality and small maximum size (32 cm long and 550 g) compared to other tilapia species, however, it is not extensively cultured locally and globally (Bradford et al., 2011). Introduction of spotted tilapia for aquaculture purposes has been reported in Russia (Ivoylov, 1986), but information on its production and geographical distribution is not available. Nevertheless, the attractively coloured *T. mariae* is a desirable ornamental fish and is most likely present in aquaria in many countries outside its natural range. Spotted tilapia was established in the USA as a result of escapes or intentional releases from ornamental fish farms in Dade County, Florida between 1972 and 1974 (Hogg, 1974, Hogg, 1976, Courtenay and Robins, 1973). Currently, the species is naturalised in Florida, reported in Arizona and Nevada, and its presence in Texas is uncertain (Nico and Neilson, 2020).

The history of the introductions of *T. mariae* to Australia remain unclear (Bradford et al., 2011). The species was first found in the cooling pond of Hazelwood power station in temperate Victoria in 1978 (Cadwallader et al., 1980), where they persist in the heated water being charged by the station. During the 1980s, the species was also detected near Cairns, North Queensland and has since become established in surrounding river catchments and estuaries between Innisfail and Cairns

(Webb, 2007). Recent spread of the species to the western-flowing Walsh River in North Queensland in 2017 has increased the risk of invasion across the Gulf of Carpentaria catchments and across to the Northern Territory in northern Australia (Figure 1).

Nile tilapia is a highly invasive fish that plagues a variety of ecosystems in more than 100 countries and coincides with its use as an aquaculture species (De Silva et al., 2004, GISD, 2021, Valdez-Moreno et al., 2019, Welch, 2020). The species is not yet established in Australia. Due to its effective mouthbrooding reproductive strategy and extraordinary environmental adaptability, *O. niloticus* presents the same significant risk as *O. mossambicus* if it is established in Australia. Nile tilapia have been reported to cause severe impacts on native biodiversity and ecosystems in which they are introduced (Canonico et al., 2005). These include alteration of water quality, the dynamic of nutrient and eutrophication, predation of eggs and young of other fish species, and extinction of native fish species including *O. esculentus* and *O. variabilis* from Lake Victoria (Goudswaard et al., 2002, Starling et al., 2002).

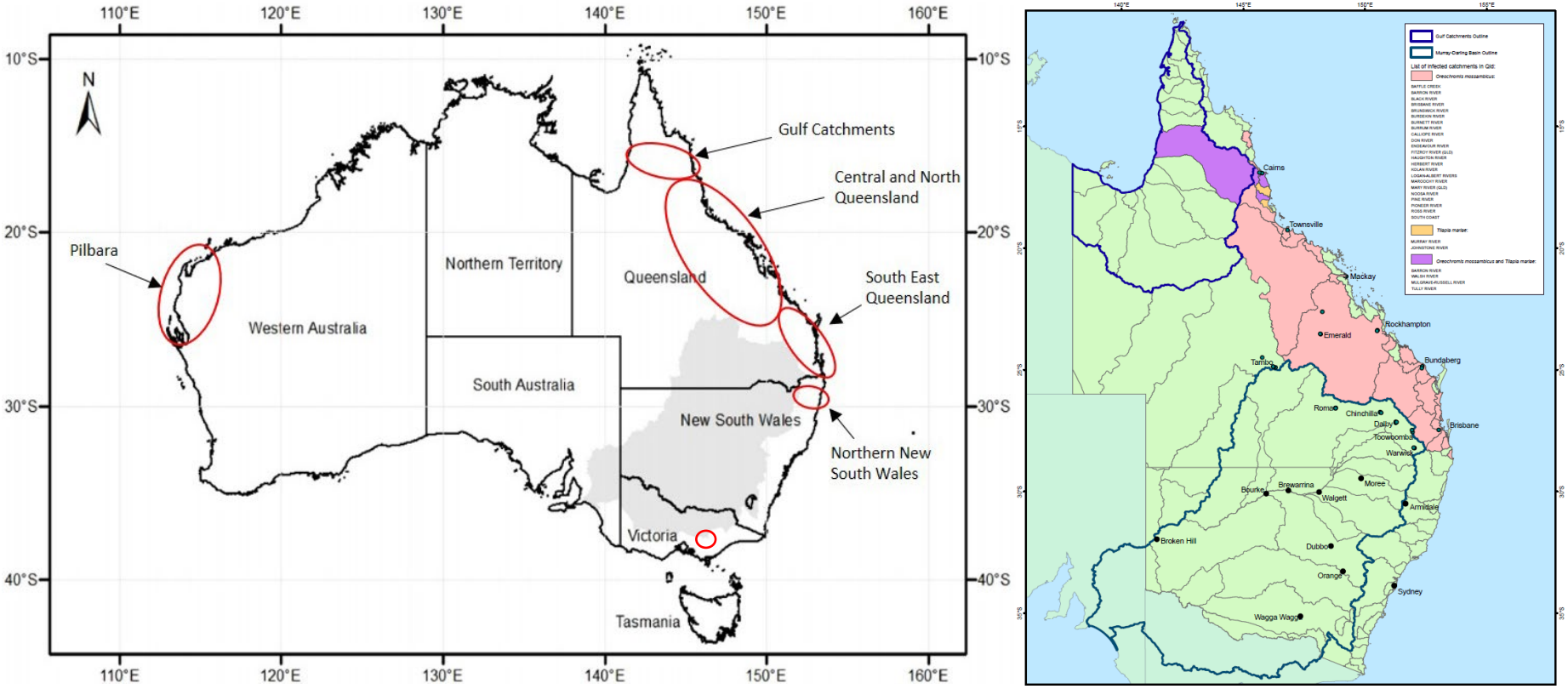

Figure 1. Geographical distribution of tilapia in Australia. Left panel: red circles indicate approximate spread of tilapia across Australia and grey shaded area indicates the Murray-Darling Basin (adapted from Jha et al., 2013). Right panel: *Oreochromis mossambicus* and *Tilapia mariae* distribution in Queensland (Source: Queensland Department of Agriculture and Fisheries).

1. Management of invasive fish in Australia

While we now have an effective environmental DNA (eDNA) surveillance tool (Noble et al., 2015) for early detection and mapping of the distribution of tilapia (developed under the stewardship of the Invasive Animals Cooperative Research Centre, IA-CRC), current management mechanisms are inadequate to control tilapia once an incursion has occurred. Indeed, it is now clear that current education programs are failing to stop the spread and options for management post-incursion are extremely limited. Eradication attempts are routinely attempted through the use of a combination of electrofishing and piscicide poisons, are rarely successful in open waterways, and are often unsuccessful for tilapia given their invasive nature. Eradication was only thought to be achieved for one infestation of *T. mariae* (Eureka Creek, Mitchell River Catchment) using a combination of electrofishing and poison (rotenone) (Pearce et al., 2009) in a restricted area of the creek. However, the detection of *T. mariae* in the same section of Eureka Creek again in 2019 now casts doubt over the original attempts. Eradication of infestations in other systems (e.g., Fitzroy River Catchment) has not been possible. Indeed, there is a lack of demonstrated effective control mechanisms for tilapia and for invasive fishes in general. Thus, there is a critical need to research, develop and evaluate other potential tilapia control agents where establishment has already occurred.

Where feasible, biocontrol can be a cost-effective, safe and practical solution to manage invasive species at the landscape scale because it does not require reapplication of chemicals or poisons, and once established should be self-sustaining. An excellent example of this is the use of myxoma virus (MYXV) and rabbit haemorrhagic disease virus (RHDV), which were released in 1950 and 1995, respectively, as BCAs for rabbit in Australia. Both MYXV and RHDV have been of massive benefit to Australian ecosystems and agricultural industries, leading to initial reductions of ~ 90% of rabbit population in some areas. It has to be noted that viral biocontrol is never a silver bullet that can be expected to eradicate its host entirely, and that may eventually lose effectiveness due to the ongoing evolution between the host and the pathogen (Di Giallonardo and Holmes, 2015b, Strive and Cox, 2019). This notwithstanding, the sustained reductions of rabbit populations and impacts by the two rabbit BCAs resulted in an estimated benefit of A$70 billion to Australia's agricultural industries in the 60 years between 1950 and 2010 (Cooke et al., 2013). Based on the success with the use of MYXV and RHDV to reduce the impacts of invasive rabbit in Australia, spring viremia of carp virus (SVCV; Rhabdovirus) was proposed as a potential BCA for common carp (*Cyprinus carpio*) (Stevenson, 1978), which are regarded as the most devastating invasive fish in Australia. However, subsequent research found that SVCV was not species-specific to carp (Family *Cyprinidae*). The virus not only infected other fish species within the Family *Cyprinidae* (goldfish, tench), but also those of other families including sheatfish (*Siluridae*), guppy (*Poecilliidae*) and Northern pike (*Esocidae*) (Crane, 1995). Therefore, SVCV was inappropriate as a BCA and its investigation as a potential BCA for carp was terminated.

In 2000, an Australia's *National Management Strategy for Carp Control* was adopted by Carp Control Coordinating Group (CCCG) (CCCG, 2000b). Its companion document, a "Strategic Research Plan" (*Future Directions for Research into Carp*), was developed to support the Strategy (CCCG, 2000a). The documents recognised that the existing techniques to control carp such as poisoning and physical removal are often effective in small scale but need to develop cost-effective control strategies on broad scale. The "Strategic Research Plan" identified possible techniques for controlling carp including habitat manipulation, genetic control, and carp specific pathogens. In the mid-2000s, an investigation of koi herpesvirus (KHV) (Hedrick et al., 2000), taxonomically known as cyprinid herpesvirus 3 (CyHV-3) (Waltzek et al., 2005), as a potential BCA was proposed as part of an integrated carp control program (Fulton, 2006, McColl et al., 2007).

CyHV-3 was first reported in Israel and Germany in 1998 (Hedrick et al., 2000) and subsequently spread to at least 28 countries across Europe, America, Africa, and Asia (OIE, 2021) including Indonesia, from which an isolate was transferred to Australia's high-containment laboratory, the

CSIRO Australian Centre for Disease Preparedness (formerly known as Australian Animal Health Laboratory). Subsequent research funded by the Australian Government through IA-CRC showed that the Indonesian KHV C07 isolate was highly virulent in carp sourced from Australian waters (Sunarto et al., 2011) and the virus was specific to carp (McColl et al., 2017). The results encouraged further investigation of CyHV-3 as a potential BCA as part of the National Carp Control Plan (NCCP, 2019), which is currently underway. The $15 million plan, which is funded by the Australian Department of Agriculture, Water and the Environment (DAWE) through the Fisheries Research and Development Corporation (FRDC) will determine the feasibility of using CyHV-3 as a BCA for carp in Australia with focus on options for maximising the reduction of carp populations while minimising impacts to industries, communities, and the environment. The FRDC's NCCP is one of several important inputs that will inform a decision by the Australian, state and territory governments on the virus. In addition to the plan, a final decision on carp biocontrol will require further public consultation and regulatory approval including the *Agriculture and Veterinary Chemicals Code Act 1994, Environment Protection and Biodiversity Conservation Act 1999, Biological Control Act 1984,* and *Biosecurity Act 2015*.

Recently, a combination of viral biocontrol and genetic technologies are emerging as the best technologies to cause a major decline in fish numbers, and in some cases even lead to complete eradication (Thresher et al., 2014a, Thresher et al., 2014b). Based on our experience with viral biocontrol in rabbit and carp (McColl et al., 2014, McColl and Sunarto, 2020, Kerr et al., 2021), here we describe a systematic approach to assess known pathogens for their suitability as potential agents for biological control of tilapia and outline the possible next steps to further investigate the top candidates.

1. Biocontrol agent assessment criteria

Biological control agent (BCA) assessment criteria adapted from Henzell et al. (2008) and Peacock (2015) for rabbit biocontrol in Australia were used to assess the appropriateness, effectiveness, and efficiency of potential BCAs for tilapia (Table 1). Safety and efficacy are the two major concerns in assessing the potential of BCA. Species-specificity is an important determinant for the safety of a potential biocontrol agent, whereas virulence and transmission are important for the efficacy (Di Giallonardo and Holmes, 2015a). Therefore, to be considered as a potential BCA candidate, the agent should at least meet these three key determinants – species-specificity (criteria 1.1), high levels of virulence (criteria 2.1), and effective transmission (criteria 3.1).

Table 1. Biocontrol agent assessment criteria

1. Appropriateness

1.4. Species specificity – the BCA should not infect, let alone affect, any non-target species in Australia.

1.5. Socially acceptable – the nature and biological action of the BCA needs to be acceptable to the community. For example, is the agent naturally occurring in tilapia and is a vaccine available to protect other ornamental cichlids?

1.6. Humane – the BCA should cause rapid death.

2. Effectiveness

2.5. Virulence – the BCA needs to cause high mortality in tilapia. Survivors are likely to seroconvert, become more resistant and may confer the resistance on their offspring through maternal immunity. This would likely lead to recovery of the tilapia populations.

2.6. Impacts on all ages – ideally the BCA needs to provide high impact on juvenile and adults tilapia.

2.7. Effectiveness in wild fisheries – the BCA needs to provide great impact in wild tilapia populations, e.g. regardless of the effect of temperature/season.

2.8. No antagonistic interaction with other pathogens – for example cross-protection by closely related pathogens that may be endemic.

3. Efficiency

3.6. Transmission – the BCA would have the ability to transmit efficiently to other fish and have the capacity to spread through the local, regional, and national tilapia populations (self-disseminating).

3.7. Persists in the environment – the BCA should persist despite death of a high proportion of hosts and once established causes repeated outbreaks.

3.8. Cost for research and development – e.g. benefits should exceed the cost of testing the safety and efficacy of the candidates, risk assessment and cost-benefit analysis.

3.9. Cost for manufacture and distribution – preferably, the organism(s) could be cultured, prepared, and stored in large quantities to allow effective distribution.

3.10. Public and government approval requirements – i.e. are there any significant differences between biocontrol options, e.g. GMO as a genetic biocontrol option also requires additional approval.

There are a number of factors to consider in this assessment and these are described here with comments on how they influence the selection of a BCA for tilapia in Australia. The BCA assessment using the criteria below and summarised in Table 1 is a complex process and the selection criteria used in this review may not cover all aspects of the assessment. Another limitation of the assessment is they involve subjective scoring, which affects the consistency of the results. For example, how many studies should have been done to justify the application of the criteria and what the criteria were for assignment into the category of “positive”, “minor concerns”, and “major concerns”? Safety and efficacy are obviously the two major concerns in assessing the appropriateness and effectiveness of BCAs, respectively. However, the expected delay due to public and government approval processes for a viral biocontrol is also a major concern.

In term of appropriateness (safety), the BCA should have a narrow host range and affect only tilapia but no other fish species, aquatic animals, terrestrial animals or humans sharing/using the waterways (Peacock, 2015). The BCA should also be socially acceptable (criteria 1.2). Social acceptability can be difficult to define, depend on different perspectives and may change overtime, but risk perception and animal welfare considerations are common topics in this context (Schirmer and Clayton, 2018, Mankad et al., 2019, Wilkinson and Fitzgerald, 1997). Having biosecurity measures in place and vaccine(s) available to protect non-target populations such as ornamental cichlids is therefore considered highly desirable. A naturally occurring agent in wild and farmed tilapia would also be expected to be relatively more socially acceptable than a genetically modified organism. The agent should have the ability to kill tilapia relatively humanely (criteria 1.3), e.g. by causing rapid death and shortening the period of experiencing pain and suffering as much as possible (Sharp and Saunders, 2011).

To be effective as a BCA, the agent would ideally cause high mortality in tilapia of all ages (criteria 2.1 and 2.2). The effectiveness of the agent in wild fisheries (criteria 2.3) is difficult to assess accurately because of the paucity of data on the impacts of tilapia pathogens as most studies were focused on aquaculture. Therefore, an agent that has been reported to cause serious disease outbreaks in wild tilapia fisheries and associated with the decline of populations is considered positive in this context, whereas those that were only observed in farmed tilapia, but not in wild populations, is considered less preferable. Ideally, the BCA would have no antagonistic interaction with other pathogens (criteria 2.4. Although both synergistic and antagonistic interactions occurring during co-infections of multiple pathogens in fish have been reported (Kotob et al., 2016), antagonistic interactions have not been observed during co-infections in tilapia (Abdel-Latif et al., 2020). It has been proposed that multiple infections might have a synergistic effect that resulted in increased severity of the disease and higher rate of mortality in tilapia (Dong et al., 2015, Basri et al., 2020), which is positive in the context of biocontrol. In addition, cross-protection are likely to occur among closely related pathogens, and therefore the absence of other pathogens within the family is also considered positive. However, research is required to systematically assess the possibility of interfering endemic viruses and also the possibility of reassortments (Chaput et al., 2020). This would involve, for example, meta-transcriptomic analyses (Turnbull et al., 2020), of other viruses in Australian tilapia populations.

Ideally the BCA would have the ability to transmit efficiently to other fish and have the capacity to spread through the local, regional, and national tilapia populations (self-disseminating) (criteria 3.1). The BCA that has the ability to spread by waterborne routes, an important pathway for a successful BCA of invasive fish, is considered positive. The BCA should persist in the environment despite death of a high proportion of hosts (criteria 3.2). The agent that could survive in the water and in dead fish or become latent in surviving hosts with the capacity to reactivate under certain conditions and transmit the disease to naïve fish is considered positive. Cost for research and development include cost of testing the safety and efficacy of the candidates, risk assessment and cost-benefit analysis (criteria 3.2). In the context of cost for manufacture and distribution, it is preferable if the agent could be cultured, prepared, and stored in large quantities to allow for large scale effective distribution (criteria 3.4). Lethal pathogens have never been used or approved as controls for invasive fish, and therefore, the expected delay due to public and government approval processes for the pathogenic biocontrol in tilapia as being major concern (criteria 3.5). The use of GMO as a genetic biocontrol option would also require additional approval and time for processing such.

Understanding the complexity of the processes and the limitations of the framework are important in terms of finding the most promising BCAs despite data gaps. The suitability for introduction of BCAs into Australia based on published scientific evidence collected overseas could be tenuous, and further work will be required for relevant assessments at a local level (Henzell et al., 2008). Most uncertainty relates to the likely virulence, transmissibility, and persistence of the BCAs in wild fisheries, which may differ from those in aquaculture settings. For example, lower host density in wild fisheries compared to high density in farmed tilapia may affect the transmission and survival of the agent in wild environment and influence the mortality rate of the host. Applying hydrological, ecological, and

epidemiological modelling to test different scenarios and predict the outcomes of introduction of BCAs into new environments found in Australia would also strengthen the decision platform (Joehnk et al., 2020, Durr et al., 2019).

2. Biocontrol agent candidate assessment findings

Tilapia pathogens fall into the general categories of viruses, bacteria, fungi, and parasites. Specific details of the tilapia pathogens identified and assessed against the BCA assessment criteria are described below. Table 2 shows the identified tilapia pathogens reviewed against the BCA assessment criteria using a traffic light rating system. Overall, the bioprospecting review found that a large number of bacteria, fungi, and parasites have been associated with natural disease outbreaks in tilapia worldwide. However, none of them were species-specific to tilapia and therefore were rejected as BCA candidates. More promisingly, nine viruses have been reported in tilapia. Six of them were found to have first been reported in species other than tilapia and therefore were assessed as not suitable as BCA candidates. The other three viruses, originally reported in tilapia namely tilapia lake virus (TiLV) (Eyngor et al., 2014), tilapia parvovirus (TiPV) (Liu et al., 2020) and tilapia larvae encephalitis virus (TLEV) (Shlapobersky et al., 2010), were considered to be species-specific to tilapia and categorised as being 'tentatively worthwhile BCA candidates for further investigation'. TiLV was considered the most promising potential BCA candidates and categorised as 'worthwhile for active further investigation'. TiPV was categorised as 'tentatively worthwhile' for further investigation. TLEV was categoried under a 'watching brief'. This means that TLEV was not selected for further investigation right now but will be watched as possible future BCA through the international literature and scientific networks. Table 3 describes a set of summary information for the three tilapia pathogens that were assessed as being 'tentatively worthwhile BCA candidates for further investigation'.

4.1. Viruses

At least nine viruses have been detected in tilapia (Machimbirike et al., 2019), the first virus being Lymphocystis disease virus (LCDV) (Paperna, 1973, Weissenberg, 1965). Infectious pancreatic necrosis virus (IPNV) was the first RNA virus reported in tilapia (Hedrick et al., 1983). Both viruses are not specific to tilapia and neither have been associated with natural high mortality in tilapia. For these reasons, they were excluded from further assessment as potential BCAs. Seven viruses have been associated with disease outbreaks in tilapia. These are TiLV (Eyngor et al., 2014), TiPV (Liu et al., 2020), TLEV (Shlapobersky et al., 2010), Bohle iridovirus (BIV) (Ariel and Owens, 1997), nervous necrosis virus (NNV) (Bigarré et al., 2009), infectious spleen and kidney necrosis virus (ISKNV) (Subramaniam et al., 2016, Suebsing et al., 2016), and iridovirus-like agents (McGrogan et al., 1998, Smith et al., 1997). Subramaniam et al. (2016) suggested that the Irido-like viruses reported by Smith et al. (1997) and McGrogan et al. (1998) could actually be ISKNV isolates which would reduce the list to six candidates. None of the Iridoviruses (LCDV, BIV, ISKNV and Irido-like viruses) and NNV are species-specific to tilapia, and therefore, were not considered as suitable candidates for BCAs. For example, natural disease outbreaks of VNN have been reported in 62 marine and 12 freshwater fish species (Bandin and Souto, 2020). On the other hand, TiLV, TiPV, and TLEV are believed to be species-specific to tilapia, and therefore worthy of further assessment as BCA candidates.

Tilapia lake virus (TiLV)

TiLV, taxonomically assigned as *Tilapia tilapinevirus* under the genus *Tilapinevirus* and the family *Amnoonviridae* (Adams et al., 2017, Bacharach et al., 2016a, Kuhn et al., 2019), is an enveloped and negative-sense ssRNA virus (Bacharach et al., 2016b, Eyngor et al., 2014). No other viruses within the family *Amnoonviridae* have been reported in tilapia (ICTV, 2018). The deca-segmented 10kb genome contains 14 functional genes encoding 14 proteins (Acharya et al., 2019). Alignment analyses of segment 1 (Taengphu et al., 2020) and segment 3 (Skornik et al., 2020) as well as whole-genome sequences (Jansen et al., 2018) from geographically different isolates revealed high nucleotide identity, suggesting that a new recently-evolved virus has emerged. A relative recent reassortment event, particularly those of segments 5 and 6, complicates phylogenetic analysis by individual segments and illustrates the need to exercise caution when using the analysis to infer geographical origin and the movement of the virus (Chaput et al., 2020). TiLV was first reported to cause mass die-offs in farmed and wild tilapia in Israel as early as summer 2009 (Eyngor et al., 2014). Around the same time, similar disease outbreaks called syncytial hepatitis of tilapia (SHT) were reported from farmed tilapia (*O. niloticus*) in Ecuador (Ferguson et al., 2014). The samples which were collected in 2011-2012 tested positive for TiLV (Del-Pozo et al., 2016). Since then TiLV has been reported from 16 countries across four continents comprising Egypt, Uganda, Tanzania, Israel, India, Bangladesh, Thailand, Malaysia, Indonesia, Philippines, Taiwan, USA, Mexico, Colombia, Ecuador and Peru (Surachetpong et al., 2020).

Natural morbidity and mortality due to TiLV are restricted to tilapia and tilapia hybrids (Surachetpong et al., 2017, Eyngor et al., 2014). Affected farmed species includes Nile tilapia (*O. niloticus*) in Ecuador (Ferguson et al., 2014), Egypt (Fathi et al., 2017), India (Behera et al., 2018), Indonesia (Koesharyani et al., 2018), Thailand (Dong et al., 2017b, Surachetpong et al., 2017) and Uganda (Mugimba et al., 2018); grey tilapia hybrid (*O. niloticus x O. aureus*) in Israel (Eyngor et al., 2014); red tilapia (*Oreochromis spp.*) in Thailand (Dong et al., 2017b, Surachetpong et al., 2017) and red tilapia hybrid (*O. niloticus x O. mossambicus*) in Malaysia (Amal et al., 2018). A wide range of wild tilapiines including *Tilapia zilli, O. aureus, Sarotherodon (Tilapia) galilaeus* and *Tristamella simonis intermedia* from the Kinneret Lake in Israel (Eyngor et al., 2014), wild black tilapia (*Oreochromis spp.*) in Malaysia (Abdullah et al., 2018), wild Nile tilapia in Lake Victoria (Tanzania and Uganda) (Mugimba et al., 2018) and in Peru (OIE, 2018b) have been affected by TiLV. Other fish species co-cultured with tilapia have not been affected by TiLV. These include grey mullet (*Mugil cephalus*) and common carp (*Cyprinus carpio*) in Israel (Eyngor et al., 2014); grey mullet and thin-lipped mullet (*Liza ramada*) in Egypt (Fathi et al., 2017); rohu (*Labeo rohita*), catla (*Catla catla*), mrigal (*Cirrhinus mrigala*), milk fish (*Chanos chanos*) and pearl spot (*Etroplus suratensis*) in India (Behera et al., 2018). However, wild river barb (*Barbonymus schwanenfeldii*) was found to be TiLV-positive by RT-PCR in Malaysia (Abdullah et al., 2018). Clearly, there is a need for differentiating TiLV genomic RNA (gRNA) from mRNA, which indicates viral replication in the host, particularly in non-target species such as river barb that was gRNA-positive by RT-PCR.

Experimental infection of 10 warm-water fish species including giant gourami (*Osphronemus goramy*), snakeskin gourami (*Trichogaster pectoralis*), iridescent shark (*Pangasianodon hypophtthalmus*), walking catfish (*Clarias macrocephalus*), striped snake-head fish (*Channa striata*), climbing perch (*Anabas testudineus*), common carp (*Cyprinus carpio*), silver barb (*Barbodes gonionotus*), Asian sea bass (*Lates calcarifer*), and red hybrid tilapia (*Oreochromis spp.*) revealed that only red hybrid tilapia and giant gourami were affected by TiLV (Jaemwimol et al., 2018). The mortality of red hybrid tilapia infected with TiLV by intraperitoneal (IP) injection was 63-85% and that of giant gourami was 100%. Despite the cumulative mortality of giant gourami being significantly higher than that of tilapia, only 53.55% (8/15) of giant gourami samples were TiLV-positive by RT-qPCR compared to 100% (15/15) of those of tilapia, suggesting that not all dead giant gourami may have been infected with the virus.

Wide variations in mortality associated with TiLV have been reported in wild and farmed tilapia. For example, 0.71% mortality in wild black tilapia (*O. niloticus*) and 15-25% in farmed red hybrid tilapia (*O. niloticus* x *O. mossambicus*) have been reported in Malaysia (OIE, 2017b, Abdullah et al., 2018, Amal et al., 2018). Similarly, low mortality of 6.4% and 9.2% in farmed tilapia have been reported in Chinese Taipei (OIE, 2017a) and Egypt (Fathi et al., 2017), respectively, the latter experiencing "summer mortality" in which TiLV was detected but the causal link was inconclusive (Nicholson et al., 2017). Subclinical infections have been reported in farmed tilapia in Thailand (Senapin et al., 2018) as well as in wild and farmed tilapia in Lake Victoria (Tanzania and Uganda) (Mugimba et al., 2018). In contrast, TiLV has caused disease outbreaks in wild tilapia populations in Israel and decreased the annual yield of *Tilapia galilaeus* from the Kinneret Lake from 316 tons in 2005 to 52, 8 and 45 tons in 2007, 2009, and 2010, respectively (Eyngor et al., 2014). Interestingly, although the lake hosts 27 species of fish encompassing members of the families *Cichlidae*, *Cyprinidae*, *Mugillidae*, and *Claridae*, only tilapia (*Cichlidae*) was affected. In farmed tilapia, the disease resulted in massive mortality in Israel (Eyngor et al., 2014), 10-80% mortality in Ecuador depending on the tilapia strain (Ferguson et al., 2014), 20-90% in Thailand (Dong et al., 2017b, Surachetpong et al., 2017) and 80-90% in India (Behera et al., 2018).

Experimental infection of tilapia with TiLV by intragastric, intra-coelemic, cohabitation and IP injection conducted in geographically different regions resulted in consistently high levels of mortality. The mortality of Nile tilapia infected with TiLV via intragastric and intra-coelemic route was 40-45% and 70%, respectively, which occurred from 6 to 15 days post infection (dpi) (Pierezan et al., 2020, Pierezan et al., 2019). The mortality of cohabitating tilapia was 55.71% from 3 to 15 dpi (Liamnimitr et al., 2018) and 80% from 4 to 9 dpi (Eyngor et al., 2014). Virus challenge by IP injection resulted in high mortality, ranging from 75-85% which occurred from 2 to 10 dpi (Eyngor et al 2014), 66-88% from 1 to 12 dpi (Tattiyapong et al., 2017), 63-85% from 4 to 24 dpi (Jaemwimol et al., 2018) and 100% from 3 to 7 dpi (Behera et al 2018). Mass mortality in wild populations have not been observed but high mortality above 80% have been consistently reported in farmed hybrid tilapia (*O. niloticus* x *O. aureus*) in Israel (Eyngor et al., 2014).

The causes of the variation in mortality are not known, but they may be attributed to different species, strain or family of tilapia, culture systems or other environmental factors. For examples, 80% mortality in the Chitralada strain compared to 10-20% mortality in all male Genetically Improved Farmed Tilapia (GIFT) have been reported in Ecuadorian farms, despite both being *O. niloticus* (Ferguson et al., 2014). Furthermore, host resistance to TiLV is highly heritable in families of the GIFT strain, suggesting that selective breeding to increase the resistance of farmed tilapia to TiLV is feasible (Barría et al., 2020). Clinical outbreaks of TiLV have been reported in summer at water temperature of 22 to 32 ^{o}C in Israel (Eyngor et al., 2014), ≥25 ^{o}C in Egypt (Fathi et al., 2017) and 25 to 27 ^{o}C in Ecuador (Ferguson et al., 2014), suggesting that temperature plays an important role in TiLV outbreaks. Co-infection of TiLV with other pathogens including *Aeromonas spp*., particularly *A. veronii*, may also affect the severity and outcome of the disease (Amal et al., 2018, Nicholson et al., 2017, Rao et al., 2021). Although stocking density, dissolved oxygen levels and pond production cycles have been considered as risk factors of TiLV disease in aquaculture settings, no single factor has been attributed to TiLV outbreaks (Ali et al., 2020, Kabuusu et al., 2017). In controlled laboratory conditions, mortality is also dose-dependent, in which mortalities of 48.89% and 77.78% were observed in *O. mossambicus* IP-injected with low (10^3 $TCID_{50}$/mL) and high (10^5 $TCID_{50}$/mL) doses of TiLV, respectively (Waiyamitra et al., 2021). It is estimated that the LD_{50} of TiLV by IP injection was 5.7 x 10^4 $TCID_{50}$ (Yang et al., 2018).

Although small fish are more susceptible to TiLV infection than larger fish (Roy et al., 2021), all age groups of tilapias appear to be susceptible to TiLV. Fertilized eggs, larvae, fry, fingerlings, juveniles, adults and broodstocks of tilapia have tested positive for, or been affected by, TiLV (OIE, 2017c, OIE,

2018b, Dong et al., 2017a, Behera et al., 2018, Eyngor et al., 2014, Ferguson et al., 2014, Pulido et al., 2019, Surachetpong et al., 2017, Yamkasem et al., 2019). Cumulative mortality of broodstock was 5-10% while that of fry was 90-100% (Yamkasem et al., 2019), suggesting that the maturity of the host's immune system may play a role in the outcome of the disease. Early developmental stages of tilapia including fertilized eggs, larvae and fry have tested positive for TiLV (Dong et al., 2017a, Yamkasem et al., 2019). Furthermore, the authors reported that TILV was also detected in reproductive organs including ovary and testis, suggesting that TiLV can be vertically transmitted. The detection of TiLV RNA in mucus (Liamnimitr et al., 2018), feces and water tanks containing TiLV-infected fish (Pierezan et al., 2019) and cohabitation mode of horizontal transmission (Eyngor et al., 2014, Liamnimitr et al., 2018) demonstrates the ability of TiLV to spread by waterborne routes, an important pathway for a successful biocontrol agent of aquatic invasive fish.

Natural co-infections of TiLV and other pathogens including parasites, bacteria (*Aeromonas hydrophila, A. veronii, A. isthiosmia, A. enteropelogenes, Streptococcus agalactiae*) and virus (*Tilapia parvovirus*, TiPV) have been reported in farmed tilapia (Yamkasem et al., 2021, Amal et al., 2018, Basri et al., 2020, Nicholson et al., 2017, Nicholson et al., 2020, Rao et al., 2021, Surachetpong et al., 2017). Mortality rates due to TiLV outbreaks among tilapia farms in Thailand were 20%-90%, in which higher rates were associated with secondary bacterial and parasitic infections (Surachetpong et al., 2017). Co-infections of TiLV and *A. veronii* in farmed red hybrid tilapia in Malaysia resulted in 25% mortality (Amal et al., 2018) while that of *TiLV, A. hydrophila* and *S. agalactiae* was 70% (Basri et al., 2020). An experimental challenge in tilapia, in which co-infection of TiLV and *A. hydrophila* resulted in 93% mortality while those of either TiLV or *A. hydrophila* alone was 34% and 6.7%, respectively (Nicholson et al., 2020) supported the high rate of mortality during co-infections in farmed tilapia. These results are also consistent with those of other bacterial and viral co-infections in tilapia, in which multiple infections have a synergistic effect that resulted in increased severity of the disease and higher rate of mortality in tilapia (Dong et al., 2015, Abdel-Latif et al., 2020).

Tilapia parvovirus virus (TiPV)

Recently, a novel virus tentatively named TiPV has emerged in cage-cultured tilapia in China (Liu et al., 2020). Furthermore, the authors reported that TiPV is a spherical 30 nm in diameter, non-enveloped virus with linear, non-segmented, ssDNA genome (4269 bp) which consists of two major ORFs encoding NS1 and VP1 proteins. The virus is tentatively classified into a newly proposed genus of Chapparvovirus within the family *Parvoviridae*. The first outbreaks of the disease were reported in farmed Nile tilapia from August to September 2015 in Hubei, China. Since then, it has been reported from six cities across three provinces in China. The disease affected adult tilapia resulting in 60-70% mortality. Clinical signs of diseased fish include anorexia, lethargy, darting or corkscrew movements, haemorrhages on the body surface, lower jaw, anterior abdomen and fin bases, exophthalmia and pronounced ocular lesions. Most outbreaks occurred at water temperatures of 28-30^{o}C, but samples collected at water temperature from 22 to 32^{o}C have also been reported positive for TiPV, suggesting that temperature may play a role in disease outbreaks. The virus has been isolated on tilapia brain cells (TiB) allowing further studies including experimental infection, in which the virus caused 90% mortality within 11 days at 28^{o}C, similar to those naturally observed in cage culture systems. In November 2020, TiPV was detected in juvenile red tilapia during a disease outbreak associated with TiLV in Thailand (Yamkasem et al., 2021). Due to the nature of the outbreak (co-infection with TiLV), the role of TiPV in this outbreak is unknown.

Tilapia larvae encephalitis virus (TLEV)

Based on morphological, biophysical and very limited phylogenetic analyses, TLEV resembles a herpes-like virus (Shlapobersky et al., 2010). The virus has been associated with a high mortality rate in tilapia larvae including laboratory-reared blue tilapia (*O. aureus*), *O. niloticus* and *S. galilaeus*, in Israel. The disease is characterised by a whirling syndrome (a spiral swimming behaviour), darkened skin in blue tilapia and pale skin in red tilapia followed by high mortality rates of up to 96% and 80% in blue and red tilapia larvae, respectively. The virus was capable of both vertical transmission and horizontal transmission through water from infected fish (Sinyakov et al., 2011).

Nervous Necrosis Virus (NNV)

NNV is the causative agent of viral nervous necrosis (VNN) otherwise known as viral encephalopathy and retinopathy (VER), a lethal disease of many marine and freshwater fish species associated with vacuolation of the central nervous system and the retina (Yoshikoshi and Inoue, 1990, OIE, 2019c). NNV is a small non-enveloped virus with a diameter of 25-30 nm and the genome is composed of positive sense ssRNA molecules known as RNA1 (3.1 kb) and RNA2 (1.4 kb). It belongs to the genus *Betanodavirus* within the family *Nodaviridae* (Mori et al., 1992). The greatest impact of the disease is in marine fish including Japanese parrotfish (*Oplegnathus fasciatus*), groupers (*Epinephalus akaara, E. fuscogutatus, E. malabaricus, E. moara, E. septemfasciatus, E. tauvina, E. coioides and Cromileptes altivelis),* barramundi (*Lates calcarifer*), European sea bass (*Dicentrarchus labrax),* turbot (*Scopthalmus maximus)*, and striped jack (*Pseudocaranx dentex)*. However, mortality associated with VNN has also been reported in several freshwater fish species including tilapia. Following the first report of VNN outbreaks in Nile tilapia larvae in France (Bigarré et al., 2009), the disease has also been associated with mortality of tilapia larvae in Thailand (Keawcharoen et al., 2015), Indonesia (Yanuhar et al., 2018), and Egypt (Taha et al., 2020). The disease has been reported in more than 50 species belonging to 32 families from 12 different orders (OIE, 2019c), but a recent review suggests that the host range is continuously increasing (Bandin and Souto, 2020). Furthermore, Bandin and Souto (2020) reported that 177 marine species are susceptible to the virus and natural epizootic outbreaks have been reported in 62 of them. In addition, natural outbreaks of VNN have also been reported in 12 freshwater species belonging to 12 families from six different orders.

Iridoviruses

Iridoviridae is a family of large 150-200 nm in diameter, non-enveloped, icosahedral viruses with a dsDNA genome of 103 to 220 kbp (Chinchar et al., 2017). The family consists of two sub-families: *Alphairidovirinae* (*Lymphocystivirus, Ranavirus* and *Megalocytivirus*) which infect ectothermic vertebrates (bony fish, amphibian, and reptiles) and *Betairidovirinae* (*Iridovirus* and *Chloriridovirus*) which infect insects and crustaceans. Four iridoviruses including LCDV (*Lymphocystivirus)*, Bohle iridovirus (*Ranavirus*), ISKNV (*Megalocytivirus*) and Irido-like viruses, which are possibly ISKNV isolates, have been reported in tilapia.

LCDV infection was first reported in South American cichlid *Cichlasoma synspilum* Hubbs, 1953 in Guatemala (Weissenberg, 1965) and in African tilapia (T*. amphimelas, T. esculenta, T. variabilis* and *Haplochromis sp.*) in East Africa (Paperna, 1973). The virus has been associated with the formation of wart-like growths composed of clusters of cells up to 5 mm in diameter primarily on the skin, but sometimes in internal organs. Although morbidity may be high, mortalities have not been recorded in tilapia. Lymphocystiviruses infect more than 100 species of marine and freshwater fish (Chinchar et al., 2017).

Bohle iridovirus was first isolated from metamorphs of the ornate burrowing frog (*Limnodynas*tes *ornatus* Gray,1842) in Bohle, in North Queensland, Australia (Speare and Smith, 1992). Since then, the virus has been shown to infect introduced and Australian native species across three classes of ectothermic vertebrates (amphibians, reptiles, and fish). BIV-associated mortalities approaching 100% over 60 days in tilapia fry (*O. mossambicus*) were first reported in an aquatic animal health laboratory in North Queensland, Australia (Ariel and Owens, 1997). The diseased fish exhibited corkscrew-like swimming patterns (‘spinning’), which led to the disease being named the ‘spinning tilapia’ (ST) syndrome. Experimental infection of barramundi fingerlings (*Lates calcarifer* Bloch, 1790) with BIV by bath-exposure or inoculation in both freshwater and seawater resulted in 100% mortalities (Moody and Owens, 1994). Two Australian anurans, *Limnodynastes terraereginae* and *Litoria Latopalmata,* tadpoles were highly susceptible to BIV (Cullen et al., 1995). The virus was also found to be

extremely virulent in hatchling tortoises *Elseya latisternum* and *E. krefftii* via intracoelomic challenge (Ariel et al., 2015), suggesting that BIV are capable of infecting hosts from different classes (Chinchar et al., 2009, Chinchar et al., 2017).

ISKNV is not only highly pathogenic in mandarin fish (*Siniperca chuatsi* Basilewsky, 1855) (He et al., 2002, He et al., 2001, He et al., 2000), but also able to infect 13 cultured and 39 wild marine fish species in the South China Sea (Wang et al., 2007) as well as freshwater fish (Chinchar et al., 2017). Natural disease outbreaks in tilapia associated with ISKNV infections have been reported in Canada, the USA and Thailand (McGrogan et al., 1998, Subramaniam et al., 2016, Dong et al., 2015, Suebsing et al., 2016, Smith et al., 1997). The authors reported that the diseased fish showed lethargy, gill palor, and coelomic distension due to ascites. Histopathological findings such as hypertrophic cells (megalocytes) with cytoplasmic inclusions in the spleen and kidney, and also in other tissues, suggest that ISKNV causes systemic disease which affects multiple internal organs. Mortalities of 50-75% among Nile tilapia fry (Subramaniam et al., 2016) and up to 50% in adults (Dong et al., 2015) were much lower than those in one of the main host, mandarin fish, where mortality was up to 100% (He et al., 2000).

4.2 Bacteria

Bacteria are potentially deadly pathogens for both wild and cultured fish and are responsible for mass mortality events in aquaculture facilities across the globe (Ibrahim, 2020). Many bacterial fish pathogens naturally inhabit freshwater and marine aquatic environments (Lewbart, 2001), however there is no species-specific bacteria for tilapia (Plumb and Hanson, 2011). If water conditions are ideal, tilapia are extremely hardy fish with good resistance to bacterial infections (Plumb and Hanson, 2011). Six major bacteria pathogens associated with mortality events in tilapia have been documented and include the genus of *Streptococcus, Flavobacterium, Aeromonas, Francisella*, *Edwardsiella* and *Pseudomonas* (Bromage et al., 1999, Anshary et al., 2014, Raj et al., 2019, Tartor et al., 2021, Plumb and Hanson, 2011, Ibrahim, 2020). Tilapia's susceptibility to bacterial infections is usually associated with environmental stressors such as over-crowding, water temperature, O_2 levels, pH, and pollution (Ibrahim, 2020) or skin injury and scale loss (Plumb and Hanson, 2011). These bacteria have not only caused natural outbreaks in other freshwater fish (Pękala-Safińska, 2018), but also in marine fish species (Toranzo et al., 2005). Therefore, all are inappropriate as BCA candidates.

Streptococcus

The first recorded occurrence of *Streptococci* in fish was discovered on a trout farm in Japan in 1957; prior to that it occurred mainly in humans, warm-blooded animals or dairy products (Agnew and Barnes, 2007, Perera et al., 1994, Hoshina et al., 1958). Since then, *Streptococcus spp.* infections have been identified in at least 27 marine and freshwater fishes (Creeper and Buller, 2006). *Strepotococcus* seems to have no geographic boundaries, with outbreaks occurring worldwide in various cultured fish species(Bromage et al., 1999, Bromage and Owens, 2002, Agnew and Barnes, 2007). Significant losses in warm water aquaculture have been documented in Israel, Australia, North America, Japan, Indonesia, Bahrain and Europe (Bromage and Owens, 2002, Creeper and Buller, 2006, Nawawi et al., 2008).

Streptococcus agalactiae and *S. iniae* are the most commonly encountered bacterial infections in tilapia, as well as numerous other cultured fish species (Perera et al., 1994, Plumb and Hanson, 2011). *Streptococcus iniae* is found more commonly in freshwater (Plumb and Hanson, 2011) with a temperature range between 25°-28°C (Bromage and Owens, 2009), while *S. agalactiae* occurs mostly in brackish water with outbreaks usually triggered when water temperatures climb above 31°C (Plumb and Hanson, 2011) . Although *Streptococcus spp.* are not species specific to tilapia, they do seem to cause the most serious bacterial infections in cultured tilapia (Plumb and Hanson, 2011). However, if tilapia recover from a *S. iniae* infection, they will actually acquire immunity to any subsequent exposure to the pathogen (Shoemaker et al., 2006).

In Australian aquaculture, *Streptococcus* outbreaks have occurred mainly in rainbow trout (*Oncorhynchus mykiss)*, however yellowtail (*Seriola quinqueradiata*), rabbitfish (*Siganus canaliculatus*) and barramundi (*Lates calcarifera*) have all suffered mass mortality events as well (Bromage et al., 1999). Cultured Barramundi in marine cages suffered detrimental losses due to *S. iniae* outbreaks every summer from 1992 to 2002 (Bromage and Owens, 2002). Losses average between 8-15% of the annual production, with severe outbreaks causing up to 70% mortality (Bromage and Owens, 2002). *Streptococcus* infections can be subacute, with clinical symptoms such as exophthalmia, ascites in the abdominal cavity and erratic swimming (Bromage and Owens, 2002, Nawawi et al., 2008) resulting in about a 15% loss; or acute, which is the most devastating due to limited clinical signs (Bromage and Owens, 2002). During the 1992 outbreak at a Barramundi farm in Queensland, the only factor that tipped off the farmers to a pending outbreak occurred when the rabbitfish co-habitating with the barramundi suffered a mortality event. Within 48 hours, over 40% of the barramundi had died (Bromage and Owens, 2002). Upon further investigation, it was discovered that healthy barramundi can carry the bacterium asymptomatically (Bromage and Owens, 2002). In Australia, Streptococcus *iniae* is not only found in barramundi (*Lates* calcarifera), rainbow trout (*Oncorhynchus mykiss*) and rabbitifish (*Siganus canaliculatus*), it has also been reported in Barramundi cod (*Cromileptes alitivelis*), Gold spot cod (*Epinephalis tauvina*), Puffer fish (*Arothron hispidus*), Silver bream (*Acanthopagrus australis*), Trevally (*Caranx ignobilis*) and coral trout (*Plectropomus leopardus*) (Agnew and Barnes, 2007, Bromage and Owens, 2002). The Kimberly coastline in Western Australia saw roughly 17,000 dead or dying fish in March 2016 including lionfish (*Pterois* volitans), angelfish (*Pomacanthus sp.),* stripet snapper (*Lutjanus carponotatus*), sand bass (*Psammoperca waigiensis*), yellowtail grunter (*Amniataba caudavittata*), damselfish (*Pomacentridae sp.*), as well as flatback sea turtles (*Natator depressus*), and olive (*Aipysurus laevis*)and black-ringed (*Hydrelaps darwiniensis*) sea snakes (Young et al., 2020). *Streptococcus iniae* was determined to be the cause, which was the first time in Australia that *S. iniae* had been associated with a major multi-species wild marine fish kill (Young et al., 2020).

In Toronto, Canada, a cluster of *S. iniae* infections in 9 humans was traced back to the fresh, whole fish they handled from a local farm. Further research concluded that *S. iniae* is zoonotic and can be transferred to humans if they have a skin injury and handle live, or freshly killed tilapia that had been grown in an aquaculture facility (Weinstein et al., 1997). At least 25 cases of *S. iniae* infections in humans caused by their handling of live, or freshly killed fish, have been reported (Facklam et al., 2005, Lau et al., 2006, Agnew and Barnes, 2007, Weinstein et al., 1997).

Aeromonas

Aeromonas spp. are found most commonly in warm freshwater environments and are responsible for causing mass mortality events in aquaculture farms worldwide (Nielsen et al., 2001). *Aeromonas hydrophila, A. sobria, A. cavieae* (Ibrahim, 2020) and *A. veronii* (Raj et al., 2019) have all been identified in cultured tilapia, however *A. hydrophila* is considered to be the most prevalent, and most devastating Gram negative waterborne pathogen responsible for severe economic losses around the world (Tartor et al., 2021, Ibrahim, 2020). Unfavourable environmental conditions can be stressful to tilapia, causing them to be more susceptible to disease and can result in mass mortality. *Aeromonas hydrophila* was responsible for a 95% loss in young-aged tilapia stock at a farm in northern Egypt after the water temperature dropped to 5.2°C (Elgendy et al., 2015, Ibrahim, 2020). Tilapia in shallow water ponds (55cm depth) had a mortality rate of almost 98%, while the tilapia in deeper ponds (more than 100cm) had a mortality rate closer to 30%(Elgendy et al., 2015). Tilapia tend to be more susceptible to *Aeromonas spp.* when they are slightly immunosuppressed from stressful winter conditions (Ibrahim, 2020). Studies in India and Thailand have looked at the effects of *A. veronii* on tilapia. In India, Raj et al. (2019) showed that 100% of tilapia were dead within 120 hours after being infected with *A. veronii.* In Thailand, experimental fish were infected with a high dose of *A. veronii* and 100% of the fish were dead within 24 hours. The challenge dose was then reduced to 10- and 100-fold which resulted in 50% & 10% mortality respectfully (Dong et al., 2017c). The fish that survived the

lowest dose of *A. veronii* were able to resist secondary infection, suggesting they may have an adaptive immune response which can protect them from secondary infection (Dong et al., 2017c).

In Australia, *Aeromonas spp.* have also been found in rainbow trout (*Oncorhynchus mykiss*) and atlantic salmon (*Salmo salar*) hatcheries(Humphrey et al., 1987). In addition to infecting cultured fish, *A. hydrophila* is a zoonotic pathogen and can infect immunocompetent and immunocompromised humans which is a potential risk for anyone coming into contact with the diseased fish (Tartor et al., 2021).

Flavobacterium

Flavobacterium columnare is the causative agent of columnaris disease in farmed and wild freshwater fish (Shoemaker et al., 2018). It has been identified as one of the most challenging pathogens in Finnish freshwater farms, greatly impacting rainbow trout (*O. mykiss)* ((Suomalainen et al., 2005, Bandilla et al., 2006). Generally, healthy, unstressed fish are not at risk of contracting columnaris disease, however, certain factors including water temperatures above 15°C, high stocking density, high levels of ammonia and organic load can lead to an outbreak (Ibrahim, 2020). *Flavobacterium columnare* was responsible for an acute mass mortality event at a tilapia (*O. niloticus*) farm in Egypt (Ibrahim, 2020). It has also been identified by the Western Australia Department of Fisheries as causing low level mortality in aquaculture farms, usually with a seasonal pattern (Creeper and Buller, 2006, Young et al., 2020).

Francisella

Francisella spp. have been found to affect a wide range of animals, including humans (Plumb and Hanson, 2011). It was first detected in Nile tilapia in Taiwan in 1994 (Chen et al., 1994, Plumb and Hanson, 2011). Since then, *F. asiatica* has been found in warm water fish in Hawaii, the continental United States, and Latin America and affects both freshwater and saltwater species (Plumb and Hanson, 2011) . *Francisella spp.* have been identified in at least 3 species of tilapia (*O. mossambicus, O. niloticus, Sarotherodon melanotheron)*, as well as three-line grunt (*Parapristipoma trilineatum*) (Kamaishi et al., 2005) and grouper (*Epinephelus melanoshoma*) (Plumb and Hanson, 2011). It affects all ages and sizes of fishes with mortality rates ranging from 5-80% (Plumb and Hanson, 2011).

Edwardsiella

Edwardsiella tarda has been reported in marine and freshwater environments in at least 25 countries, including Australia (Ibrahim, 2020, Plumb and Hanson, 2011). It has been isolated from numerous freshwater and marine fishes including tilapia (Plumb and Hanson, 2011). The Center for Agriculture and Bioscience International (CABI) listed over 47 susceptible fish species to *E. tarda* as of 2006 (CABI, 2006, Plumb and Hanson, 2011). *Edwardsiella tarda* infections in tilapia has repeatedly led to high mortality and morbidity rates in Egyptian fish farms (Ibrahim, 2020), and it affects more than just fish. It has also been found in many fish-eating birds, mammals, amphibians and reptiles (Plumb and Hanson, 2011). Humans are also susceptible and infection with *E. tarda* can result in gastrointestinal issues (Ibrahim, 2020).

Pseudomonas

Pseudomonas spp. do not seem to greatly impact tilapia, however it was linked to a mass mortality event in fish farms at Qaroun and El Rayan Lakes, Egypt (Ibrahim, 2020). It has been isolated from at least 30 marine and freshwater fish species in Asia and Europe but has not been found in Australia (CABI, 2019).

4.3. Fungi

The most common fungal infection in freshwater fish is Saprolegniosis (El-Deen et al., 2018, Torto-Alalibo et al., 2005), while the fungal disease considered the most detrimental to freshwater, brackish water, wild and farmed fish throughout the world appears to be *Aphanomyces invadans* (Afzali et al., 2015). Interestingly, *O. niloticus* (Afzali et al., 2015) and *O. mossambicus* (Lilley et al., 1998) appear to be resistant to this deadly fungus while other tilapia species including *O. andersoni, O. machrochir, T. rendalli* and *T. sparrmanii* (OIE, 2019b) and at least 94 other fish species have been identified as susceptible to *A. invadans.* Likewise, none of the Saprolegnia and Branchiomyces detected in mass mortalities of tilapia are species-specific to tilapia.

Oomycetes

Water moulds such as *Saprolegnia and Aphanomyces* belong to their own distinct phylogenetic lineage, *oomycetes*, and are successful, widespread parasites infecting many different species of fish around the world (Rezinciuc et al., 2018, Torto-Alalibo et al., 2005). Infections caused by these water moulds in cultured fish are one of the main causes for mass mortalities, usually accompanied by other stress factors such as wounds, ulcers, scraped skin, poor water quality or sudden fluctuations in water temperature (El-Deen et al., 2018). Fungal infections occur in the epithelial layer and can expand into the underlying connective tissue or blood vessels of the fish (Paperna and Smirnov, 1997) or can cause oxidative damage in the gills leading to massive osmoregulatory problems (Ali and Aboyadak, 2018, Hussein et al., 2013).

Saprolegniosis affects many different species of fish in aquaculture facilities around the world, as well as in the wild. *Salmo salar* (Atlantic salmon), *Oncorhynchus mykiss* (Rainbow trout) and *O. tshawtscha* (King salmon) fisheries in Chile suffer a 10% annual loss in their fish stock due to *Saprolegniosis* (Torto-Alalibo et al., 2005). Catfish (*Clarias gariepinus*) farms in Indonesia have reported a 90% infection rate of *Saprolegniosis* resulting in mortality rates ranging between 10-50% (Kusdarwati et al., 2017). An Egyptian facility farming *O.* niloticus suffered from mass outbreaks of *Achlya proliferiodes* and *Saprolegina dicina,* which led to mortality of 60% and 100% respectively of the infected fish (Hussein et al., 2013). During another *Saprolegenia sp.* outbreak in Egypt, two strains of the fungal infection were isolated, ManS22 and ManS33, and found to be the reason behind a mass mortality event of *O. niloticus* in which 88.9% (ManS22) and 95.6% (ManS33) of the infected fish died (Zahran et al., 2017). Many different fish species, including wild carp (*Cyprinus carpio*), living in the Tajo river in Spain suffered a devastating outbreak of *Saprolegniosis* during the spring and summer of 1991. The fungal infection spread, working its way downstream, and making its way into Portugal after only a few months (Muñoz et al., 1994).

Branchiomyces

Branchiomyces is a fungal fish disease, also referred to as “gill rot”, which is difficult to control and often prevails in eutrophic, warm water conditions that provides a favourable environment for this fungus to proliferate (Paperna and Smirnov, 1997). The most commonly infected fish are *Cyprinus carpio* (common carp), *Tinea tinea (*tench) and *Oncorhynchus mykiss* (Bruno and Ellis, 1996). Hybrid tilapia (*O. niloticus* x *O. aureus*) cultured in an earthen pond in Israel suffered a mass mortality event in the summer (July 1985) resulting in an 85% loss in the stock of tilapia due to *Branchiomyces sp.* (Paperna and Smirnov, 1997).

4.4 Parasites

Parasites can be primary pathogens or can open up a portal of entry for other diseases including other parasites, fungi, and bacteria (Buchmann et al., 2009). They can attach themselves to the outside of the fish on the gills or fins (García-Vásquez et al., 2010, Zhang et al., 2019) or can be found internally in locations like the oesophagus, swim bladder or major organs (Jesus et al., 2018). Various factors can determine how quickly parasites spread through these facilities, including water

temperature, salinity, oxygen levels and fish density (Kuperman et al., 2001). In addition to the damage caused by *O. mossambicus* in Australia, it appears that some of their exotic parasites have likely been co-introduced from African rivers and tributaries as four species of parasites - three monogeneans (*Cichlidogyrus tilapiae, C. sclerosus, C. halli)* and one trichodinid (*Trichodinia sp*) - have been reported on both African native and introduced Australian tilapia (Wilson et al., 2019).

A plethora of fish parasites exist which cause mass mortality in cultured tilapia, particularly in young ages. Many of these parasites are a natural occurrence and will not cause problems in low numbers, however an outbreak in an aquaculture facility can result in widespread mortality due to the artificial density and conditions fish are cultured under (Ali and Aboyadak, 2018). In addition, the most serious monogenean parasites in tilapia, *Gyrodactylus sp.,* and the most numerous protozoans, *Trichodina sp.,* are not species-specific to tilapia. A novel Myxosporean parasite, *Myxobolus bejeranoi,* has only been reported in tilapia hybrid (*O. aureus* male x *O. niloticus* female), which is an important aquaculture species in Israel (Lövy et al., 2018). However, the effectiveness of *Myxobolus spp.* in wild fisheries is unknown. In fact, every parasite found in aquaculture facilities are present in wild fish populations but most of them are not associated with disease outbreaks (Valladao et al., 2018).

Monogenean

Monogeneans have been recognised as serious pathogens in fish due to their ability to rapidly reach high levels of infection, as well as infect other phylogenetically related fish species (Soler-Jiménez et al., 2017). *Gyrodactylus cichlidarum* is the dominant species affecting *O. niloticus, O. mossambicus* (García-Vásquez et al., 2011) and *O. aureus* (García-Vásquez et al., 2007) and is mainly found on the skin, fins (García-Vásquez et al., 2010), and gills (Zhang et al., 2019). Mass mortality events linked to *G. cichlidarum* have been recorded in tilapia aquaculture facilities in Mexico (Mendoza Franco et al., 2018), Egypt (Ali and Aboyadak, 2018) and China (Zhang et al., 2019). These events occurred during the warmer summer months (Ali and Aboyadak, 2018, Mendoza Franco et al., 2018, Rubio-Godoy et al., 2012), however one study showed *G. cichlidarum* infections in *O. niloticus* and *O. mossambicus* in Mexico peaked in winter (January) as well as the second hottest summer month (June) (Rubio-Godoy et al., 2012). This spike in infections in winter could be attributed to fish immunity being negatively affected by the cold (Rubio-Godoy, 2010, Rubio-Godoy et al., 2012, Tatner, 1996), while warm summer temperatures promote Gyrodactylid reproduction (Bakke et al., 2007, Rubio-Godoy et al., 2012), possibly accounting for the spike in June.

Gyrodactylus cichlidarum caused a mass mortality event of 20,000 mature fish, at an aquaculture farm in Mexico, when water temperatures reached 32°C (Grano-Maldonado et al., 2018). *Gyrodctylus cichlidarum* is not just limited to *Oreocrhomis sp.,* it has also been recorded in several other tropical freshwater fishes, including *Hemichromis fasciatus, H. bimaculatus* (García-Vásquez et al., 2011, García-Vásquez et al., 2007, Grano-Maldonado et al., 2018, Prikrylová et al., 2009), *Astronatus ocellatus* (Grano-Maldonado et al., 2018, Mousavi et al., 2013), *Poecila Mexicana, Poeciliopsis gracilis, Pseudoxiphophorus bimaculus* (García-Vásquez et al., 2017), *S. galilaeus, S. melanotheron heudelotii, Tilapia zillii, T. guineensis, Haplochromis flaviijosephi* and *Tristamella simonis* (García-Vásquez et al., 2011, García-Vásquez et al., 2007).

Protozoan

Species of the genus *Trichodina* are the most numerous of the protozoans found in freshwater fish (Valladao et al., 2018). Mortality outbreaks associated with *Trichodina* are very common in tilapia farms and are capable of causing significant mortality at high infestation levels, mainly parasitising the skin, fins, and gills (Maciel et al., 2018). Native and non-native fish species in South America, including *O. niloticus, Leporinus macrophalus, Brycon cephalus, Cyprinus carpio* and *Claria gariepinus* have all suffered mass mortality events due to *Trichodina* (Jesus et al., 2018, Maciel et al., 2018). All of these outbreaks occurred over spring and summer, correlated with increasing water temperatures (Maciel et al., 2018).

A highly saline lake in California, Salton Sea, has a wild population of *O. mossambicus* (Kuperman et al., 2001). These wild tilapias, along with *Bairdiella icistia* and *Gillichythys mirabilis* have all been found to be infected by ectoparasites, including three protozoan species: *Amyloodinium ocellatum, Ambiphrya ameiuri,* and *Cryptobia branchialis*, as well as two monogeneans: *Gyrodactylus olsoni* and *G. imperialis* (Kuperman et al., 2001). The greatest infestation by *A. ocellatum* occurred over the summer when temperatures ranged between 29-40°C, whereas the greatest occurrence of *A. ameiuri* arose in spring and autumn when water temperatures ranged between 22-27°C (Kuperman et al., 2001). Although accurate mortality rates were not recorded in these wild populations, high parasite loads caused severe damage to the gills and skin, possibly resulting in depressed respiration and osmoregulation, leading to suffocation and death in many of the infected fish (Kuperman et al., 2001).

Regular reports of double infections can be found in the literature and can be a combination of parasites and other infectious agents. It is difficult to know if one or the other is the primary pathogen, but it appears that the combined assault on the fish will enhance pathogenicity. A mass mortality event occurred at a fry hatchery in Egypt and *Gyrodactylus cichlidarum* was found in combination with *Trichodina*, in the farmed *O. niloticus* (Ali and Aboyadak, 2018, Abd El-Galil and Aboelhadid, 2012). Two hatcheries were affected, the first had a mortality rate of 10% while the second had a mortality rate of 14% (Ali and Aboyadak, 2018). The water temperatures at these hatcheries at the time of the outbreak were 28.5°C and 31°C respectively (Ali and Aboyadak, 2018). Abd El-Galil and Abdoelhadid (2012) showed a 53% mortality rate in fry during their first month of life due to the infection caused by *G. cichlidarum* and *Trichodina.*

Myxozoan

Myxozoans, comprising two classes (Myxosporea and Malacosporea), are cnidarian parasites infecting invertebrate and vertebrate (primary fish) hosts in freshwater and marine environments (Paladini et al., 2017, Fiala et al., 2015). The genus *Myxobolus* is the most diverse group in the phylum Myxozoa, consisting of about 900 species (Eiras et al., 2014). *Myxobolus* infection is widespread in natural environments and in cultured tilapia, with 19 species being reported from Nile tilapia or its hybrid (Lövy et al., 2018). A novel myxosporean parasite *Myxobolus bejeranoi* have been found in the gills of three-month-old hybrid *O. aureus* (male) x *O. niloticus* (female) at a fish farm in Israel (Lövy et al., 2018). Furthermore, the authors reported that the infected fish were caught in the summer months between July and September 2015 with the prevalence of *M. bejeranoi* infection being 50% (35/70) and the affected fish displaying higher mortality rate than those infected with other *Myxobolus spp*.

Table 2. Candidate pathogens reviewed against biocontrol assessment framework

Candidate pathogen	Appropriateness			Effectiveness				Efficiency				
	Species specificity	Socially acceptable	Humane	Virulence in tilapia	Impacts on all ages of tilapia	Effectiveness in wild fisheries	Interactions with other pathogens	Transmission	Persists in the environment	Cost for research & development	Cost for manufacture & distribution	Public and government approval requirements
Tilapia lake virus (TiLV)												
Tilapia parvovirus (TiPV)												
Tilapia larvae encephalitis virus (TLEV)												
Nervous Necrosis Virus (NNV)												
Bohle Iridovirus (BIV)												
Infectious spleen and kidney necrosis virus (ISKNV)												
Streptococcus agalactiae												
Streptococcus iniae												
Aeromonas hydrophila												
Aeromonas veronii												
Flavobacterium columnare												
Francisella sp.												
Edwardsiella tarda												
Pseudomanas sp.												
Aphanomyces invadans												
Saprolegenia sp.												
Branchiomyces												
Gyrodactylus cichlidarum												
Gyrodactylus olsoni												
Gyrodactylus imperialis												
Trichodinia sp.												
Myxobolus bejeranoi												

Key: Positive | Minor concerns | Major concerns

Table 3. Summary of information for the candidate biocontrol agents tentatively worthwhile for further investigation

Candidate virus	Appropriateness			Effectiveness				Efficiency				
	Species specificity	**Socially acceptable**	**Humane**	**Virulence in tilapia**	**Impacts on all age of tilapia**	**Effectiveness in wild fisheries**	**Interactions with other pathogens**	**Transmission**	**Persists in the environment**	**Cost for research & development**	**Cost for manufacture & distribution**	**Public and government approval**
Tilapia lake virus (TiLV)	TiLV causes disease outbreaks and mortalities in farmed and wild tilapia, but not in other fish species co-cultured or sharing waterways with tilapia (Eyngor et al., 2014, Surachetpong et al., 2017). However, wild river barb was found to be TiLV-positive by RT-PCR (Abdullah et al., 2018) and giant gourami was affected by TiLV via IP injection and co-habitation challenges (Jaemwimol et al., 2018).	TiLV is a naturally occurring virus in wild and farmed tilapia (not a GMO). Good management practices (Jansen et al., 2018) and biosecurity measures (OIE, 2018a) are in place and prototype vaccines are available to protect farmed and ornamental tilapia (Zeng et al., 2021).	Acute mortality occurs within a few days post infection (Eyngor et al., 2014). Chronic up to 24 days and sub-clinical infection have also been observed (Jaemwimol et al., 2019, Senapin et al., 2018).	Experimental infection of tilapia with TiLV conducted in geographically different regions resulted in consistently high levels of mortality. However, wide variations in mortality associated with TiLV have been reported in wild and farmed tilapia, ranging from very low mortalities (0.71% in Malaysia and 6.4% in Chinese Taipei) to relatively high mortality (80% in Israel, 20-90% in Thailand, and 80-90% in India).	TiLV has been reported to cause mortality in all age groups of tilapia (Yamkasem et al., 2019).	TiLV causes mortality of wild tilapia, for example, declines in tilapia populations in the Sea of Galilee, Israel (Eyngor et al., 2014). TiLV have also been reported in wild tilapia from Malaysia, Tanzania, Uganda, and Peru.	No antagonistic interactions have been observed during co-infections in tilapia (Abdel-Latif et al., 2020). Co-infections of TiLV and *A. hydrohila* caused 93% mortality of tilapia compared to either TiLV (34%) and *A. hydrophila* (6.7%) alone (Nicholson et al., 2020). No other viruses within the family *Amnoonviridae* have been reported in tilapia (ICTV, 2018).	Epidemiological findings and cohabitation mode of horizontal transmission (Eyngor et al., 2014, Liamnimitr et al., 2018) demonstrates the ability of TiLV to spread by waterborne routes. Vertical transmission has also been observed (Yamkasem et al., 2019).	Most likely but need to determine how long TiLV survives in the water and in dead fish. TiLV RNA has been detected in mucus (Liamnimitr et al., 2018), feces and water tanks containing TiLV-infected fish (Pierezan et al., 2019). Persistent or latent infection has not been reported.	Medium-sized project to test the efficacy (virulence and transmission). Large project to test the safety (susceptibility of non-target species).	TiLV grows in cell cultures and could be transported in freeze dried form or cold at 4°C.	Viral biocontrol agent has never been used or approved for use against invasive fish, and therefore, public and government approval for the viral biocontrol in tilapia is a major concern. However, Australia has very strong legislative mechanisms for approval of biocontrol agent (Biological Control Act 1984) which may facilitate the process.

Tilapia parvovirus (TiPV)	It has only been reported in tilapia in China (Liu et al., 2020) and Thailand (Yamkasem et al., 2021).	Vaccine is not available.	Experimental infection showed TiPV causes 90% mortality in tilapia within 11 days.	TiPV causes 60-70% mortality in cage-farmed tilapia.	TiPV has been reported in all size of adult tilapia.	Unknown	No other parvovirus has been reported in tilapia or any other fish species.	TiPV is contagious, spreading to six cities in three provinces in China.	Unknown	TiPV is a newly emerging virus with only two publications available and therefore little is known about the characteristics of the virus.	TiPV grows in cell culture.	Ditto above.
Tilapia larvae encephalitis virus (TLEV)	It has only been reported in tilapia in Israel (Shlapobersky et al., 2010, Sinyakov et al., 2011).	Vaccine is not available.	The virus affects brain and the disease is characterised by a whirling syndrome (a spiral swimming behaviour).	High mortality rates of up to 96% and 80% in blue and red tilapia larvae, respectively.	TLEV has only been reported in larvae of tilapia.	Unknown	No other herpesvirus has been reported in tilapia.	TLEV is capable of both vertical transmission from the mother to their offspring and horizontal transmission through water from infected fish.	Unknown	Although it was reported a decade ago, only two publications are available and therefore little is known about the characteristics of the virus.	TLEV has not been isolated or cultured in cell lines.	Ditto above.

1. Discussion

A wide range of pathogens associated with disease outbreaks and mortalities in tilapia were assessed for their potential as BCAs for tilapia in Australia. To be safe and effective as a biocontrol agent, a pathogen needs to be species-specific, highly virulent and easily transmissible (Di Giallonardo and Holmes, 2015a). All bacteria, fungi, and parasites associated with mortalities in tilapia are not considered as being host-specific to tilapia (Table 2), except a novel myxosporean parasite, *M. bejeranoi* (Lövy et al., 2018). Although many bacteria (e.g. *Salmonella* spp. for rodents), fungi (chytrid fungus for frogs) and parasites (protozoan for rats) have been proposed and tested as potential BCAs for vertebrates pests, only viruses have demonstrated efficacy and been successfully released (Saunders et al., 2010). To date, there have only been three successful viral biocontrols of vertebrate pests: FPLV (parvovirus) to eliminate cats on Marion Island, and MYXV (poxvirus) and RHDV (calicivirus) to control the feral rabbit population in Australia and New Zealand (Saunders et al., 2010, McColl et al., 2014). The authors also noted that the remarkable success of MYXV and RHDV in the biological control of rabbits in Australia has led to ongoing research into similar solutions for other vertebrate aquatic pests including carp and recently tilapia.

Out of nine viruses detected in tilapia, six viruses (LCDV, IPNV, BIV, VNN, ISKNV and Irido-like viruses which are possibly ISKNV isolates) were first reported in species other than tilapia and therefore are not suitable as BCA candidates. Interestingly, the three viruses originally reported in tilapia (TLEV, TiPV and TiLV) seem to be species-specific to tilapia, and therefore are considered as 'tentatively worthwhile agents for further investigation' and discussed below. Since the first outbreaks of TLEV in tilapia larvae in Israel a decade ago (Shlapobersky et al., 2010, Sinyakov et al., 2011), the virus has never been reported again either in Israel or in other countries, raising a question of whether the virus still persists in the environment. The virus has only been associated with mortalities in tilapia larvae in hatcheries, suggesting that the impact of TLEV in adult tilapia and its effectiveness in wild fisheries may be reduced. TLEV has not been isolated in cell cultures, hindering further characterisation of the virus, and therefore, the cost for research and development as well as manufacture and distribution are major concerns.

A newly emerging virus designated as tilapia parvovirus (TiPV) which caused 60-70% mortality in tilapia but not in other fish species in China (Liu et al., 2020) and very recently in Thailand (Yamkasem et al., 2021) is identified as an agent for possible further investigation in the future. First observed in Hubei province, TiPV has now been reported from six cities in three provinces, suggesting that the virus is rapidly spreading. The virus has also been isolated in tilapia brain cells, allowing further characterisation of the virus including experimental challenge, in which TiPV caused 90% mortality in tilapia within 11 days. TiPV is the first and only parvovirus known to infect fish (ICTV, 2018). Interestingly, FPLV (parvovirus) has been successfully used as a BCA to help eradicate feral cats from sub-Antarctic Marion Island (Howell, 1984).

TiLV was first reported to cause mass die-offs in farmed and wild tilapia in Israel as early as medio 2009 (Eyngor et al., 2014). Since then, it has been reported in 16 countries across four continents, suggesting that the virus is able to survive in different ecological niches and climates. Mathematical modelling estimated the reproductive number (R_0) for Nile tilapia infected with TiLV at 2.6 x 10^5 $TCID_{50}$/fish via cohabitation was 2.59, indicating that the virus was spreading within a tilapia population and the incidence of the disease was increasing under the test conditions (Yang et al., 2018). Furthermore, the authors estimated that the population of Nile tilapia decreased to 12% of the initial population size after 16 dpi. These epidemiological findings suggest that TiLV is contagious and spread through a waterborne route, an important transmission pathway for a potential biocontrol virus of fish.

It is unavoidable for fish to be exposed to a multitude of microorganisms in their environment and while most are a part of the natural biome, some may opportunistically become pathogenic and

influence the outcome of a deliberate infection with a BCA. Naturally occurring and experimentally induced co-infections of TiLV and other pathogens including *A. hydrophila, A. veronii, A. isthiosmia, A. enteropelogenes,* and *S. agalactiae* showed higher rates of mortality in tilapia (Basri et al., 2020, Nicholson et al., 2020), suggesting that multiple infections in tilapia have a synergistic effect. In contrast, some infections may reduce the impact of a BCA, if a previously encountered pathogen is somehow similar, thereby inducing immunological cross-protection against the BCA in question (e.g.TiLV).

TiLV causes disease outbreaks and mortalities in farmed and wild tilapia populations, but not in other fish species co-cultured or sharing waterways with tilapia (Eyngor et al., 2014, Surachetpong et al., 2017, Behera et al., 2018, Fathi et al., 2017), suggesting that TiLV is species-specific to tilapia. Though tilapia and its hybrids are the only species known naturally to be affected by TiLV, viral genomic RNA has also been detected by RT-PCR in healthy wild river barb (Abdullah et al., 2018) and mortality in giant gourami experimentally infected with TiLV has been reported (Jaemwimol et al., 2018). However, only 53.55% (8/15) of giant gourami samples were TiLV-positive by RT-qPCR compared to 100% (15/15) of those of tilapia, suggesting that not all dead giant gourami may have been infected with the virus. The huge difference of mortality rate of giant gourami infected with TiLV by IP injection (100%) and co-habitation (5%) further raises questions if the giant gourami is a true alternative host for TiLV. OIE provides criteria for listing species as susceptible to infection with a specific pathogen including criteria to determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection (OIE, 2019a). The criteria to determine infection includes the pathogenic agent is multiplying in the host, developing stages of the agent are present in the host, viable agent is isolated from the host, infectivity is demonstrated by way of transimission to naive fish, clinical or pathological changes are associated with the infection, and the specific location of the pathogenic agent corresponds with the expected target tissues.

Although TiLV has a good track record regarding species specificity, and although there are no native Australian fish belonging to the families *Cichlidae* (tilapia), *Osphronemidae* (gourami) or *Cyprinidae* (carp and barb) rigorous non-target species testing would likely be required before the use of any viral biocontrol could be considered (Di Giallonardo and Holmes, 2015a). This has been the case with the proposed viral biocontrol agents for carp (McColl et al., 2017) and would be equally applicable for tilapia biocontrol to alleviate concerns around the host-specificity of TiLV and its safety as a BCA for tilapia. For example, being an RNA virus, which is known to have higher mutation rates than those of DNA viruses, often raises concerns that mutations in the TiLV genome may enable the virus to jump to other fish species. High mutation rate, however, does not necessarily equate with increased likelihoods for a species jump. For example, RHDV first emerged in China in 1984 and killed 140 million rabbits and spread over 50,000 km^2 in less than a year, before rapidly spread worldwide and released as a BCA for rabbit in Australia in 1995 (Abrantes et al., 2012). RHDV is a small RNA virus with the genome size of ~7400 kb and one of the highest mutation rates described for viruses (Eden et al., 2015), but there is no evidence of transmission of the virus to animal species other than lagomorphs in Australia. More importantly, there is no evidence of host jumping to non-lagomorphs in Europe, where mammals are abundant and where the vector-mediated mode of transmission will increase exposure to other species (Di Giallonardo and Holmes, 2015a).

All these suggest that when used with care, viral biocontrols can be safely undertaken and be powerful tools for landscape-scale mitigation of invasive species impacts (Di Giallonardo and Holmes, 2015a, Strive and Cox, 2019). The Convention on Biological Diversity recognised classical biocontrol as an effective measure to manage invasive species causing environmental impacts (ISSG, 2018) and viruses have also been successfully used for biocontrol of terrestrial vertebrate pests including cats in Marion Island and rabbits in Australia. These provide a platform for investigating the use of viral biocontrol for invasive fish species. If TiLV or TiPV were considered for further investigation, work would progress following a process similar to approved rabbit biocontrol (IA-CRC, 2014) and currently underway for carp biocontrol (NCCP, 2019). This process broadly consists of the following

components: cost-benefit analysis for tilapia biocontrol, efficacy testing, safety testing, epidemiological modelling, and development of release strategy. Social and ecological risk assessments, bioethical issues and public acceptance will be needed to support an application to release a new BCA against tilapia in Australia. If a new tilapia BCA is approved for release in Australia, a structured collaborative program of release strategies, clean-up, and post-release monitoring will be developed. Further work including the identification of other broad-scale control measure(s) such as genetic control to complement the virus would need to be considered. Australia is currently investing in research to investigate these broadly applicable technologies for managing invasive fish species. Prerequisite for genetic biocontrol approaches is also a thorough assessment of the genetic makeup and diversity of Australian tilapia (population genomics analyses). This is important as there already is significant evidence of hybridisation occurring among wild populations (Ovenden et al., 2014).

2. Conclusions

A plethora of bacteria, fungi, and parasites have been associated with natural disease outbreaks in tilapia. However, none of them are species-specific to tilapia and therefore are rejected as BCA candidates. Nine viruses have been reported in tilapia. Six of them (LCDV, IPNV, BIV, VNN, ISKNV and Irido-like viruses) were first reported in species other than tilapia and therefore are not suitable as BCA candidates. Three viruses originally reported in tilapia (TLEV, TiPV and TiLV) are considered to be species-specific to tilapia and categorised as being tentatively worthwhile for further investigation as potential BCAs. TLEV and in particular TiPV, a newly emerging parvovirus in tilapia, are identified for watching as possible future BCAs. TiLV is considered as the most promising potential BCA candidate and proposed for current further investigation. Safety and efficacy, two major concerns for a successful biocontrol virus, need to be taken into consideration before the use of any exotic biocontrol virus is considered.

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Appendix 2: Ex-ante Cost-Benefit Analysis of Proposed Investment in Tilapia Biocontrol RD&E in Australia

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Executive Summary

This report provides an independent ex-ante cost-benefit analysis (CBA) of proposed investment in research, development and extension (RD&E) to advance biological control (biocontrol) as a practical and effective control method for invasive tilapia in Australian waterways. The assessment was undertaken as part of a CSIRO led Project titled '*Tilapia biocontrol: prospecting and evaluation*' funded by the Centre for Invasive Species Solutions (Project Code P01-B-003).

Based on a study conducted in Queensland published in 2008, it was estimated that the current economic impact costs of tilapia may lie between $1.2 million and $13.6 million per annum (2020/21 dollar terms). If targeted efforts to control tilapia are not undertaken to prevent the future spread of tilapia, the economic costs could increase to over $35.4 million per annum. Further, it is likely that, on a national scale, the impact costs could be significantly higher were tilapia to spread into other key Australian waterways, in particular the Murray-Darling Basin. Without intervention this scenario is considered highly likely.

There is currently no single overall option for the control of tilapia in Australia. Ongoing RD&E is being funded and carried out by various research organisations to refine detection and control methods for tilapia. Biocontrol is thought to be a potentially cost-effective and practical solution for the management for invasive fish species, including tilapia.

The primary objective of the ex-ante CBA was to assess whether the investment (the total costs of the RD&E addressing the advancement of new biocontrol agents (BCAs) to manage tilapia in Australia) would be paid for by the estimated potential benefits of the proposed BCA(s).

Tilapia bioprospecting Project P01-B-003 has, to date, successfully identified three potential tilapia biocontrol candidates categorised as tentatively worthwhile for further investigation. TiLV currently is considered the most promising potential biocontrol candidate and was categorised as 'worthwhile for active further investigation'. CSIRO already have imported the virus and are currently developing the capability to work with TiLV in a laboratory setting.

The CBA was set within a staged risk management framework of investment. The approach included identifying and describing the six stages of RD&E for the proposed tilapia biocontrol investment, RD&E objectives, planned activities, expected outputs and outcomes. Potential impacts associated with the expected outcomes then were identified and categorised as economic, environmental, and social impacts. The primary impact is expected to be a net reduction in the total annual impact costs of tilapia to the Australian community and economy through a reduction in tilapia biomass.

Valuation of the primary impact involved making several uncertain assumptions as a number of key relationships along the pathways to impact were unknown. The total expected RD&E investment was estimated at $18.69 million (present value terms). The investment was estimated to produce total expected net benefits of $52.53 million (present value terms). This gave a net present value of $33.84 million, a benefit cost-ratio of 2.81 to 1, an internal rate of return of 9.3% and a modified internal rate of return of 7.1%.

Care should be taken when interpreting the results of the ex-ante analysis. It is important to note that the expected release and subsequent impact of a new tilapia BCA, such as TiLV, would not occur until approximately 22 years after the first year of investment in Project P01-B-003. Given that the investment criteria became positive between 25 and 30 years after the first year of investment, this

indicates that implementation of a new tilapia BCA would create benefits sufficient to cover the costs of the proposed tilapia biocontrol RD&E investment within five to ten years of release of the BCA.

Further, it is important to remember that the ex-ante analysis was conducted within a risk management framework and that the results are expected values. This means that it is theoretically possible for the total proposed investment in tilapia biocontrol to be made (approximately $45.6 million in nominal dollars) and for there to be no benefits realised. That is, the new agent is released and is unsuccessful in reducing tilapia impact costs. However, the risk of this is very minimal as the proposed tilapia biocontrol RD&E investment has been planned as a staged investment with a number of key stop/ go points that would enable funding partners, researchers and other stakeholders to adjust and/ or redirect the RD&E to alternative and more promising directions. Also, the knowledge generated through Stages 1 to 3 are likely to contribute to increased scientific knowledge and research capacity associated with management of pest tilapia in Australia.

The positive investment criteria suggest that the initial investments (Stages 1 to 5) would be worthwhile given the estimates made of the current and future potential impact and control costs of tilapia in Australia, likely pathways to impact for proposed new BCAs, the RD&E investment and associated timelines required, and the risks involved. Further, the proposed investment can be staged conditionally (stop/go points) so that, as the investment proceeds along a particular pathway, the direction of the RD&E could be changed according to any past success and the availability of any new information available. This may avoid or minimise any potential losses and maximise the chances of significant impacts being delivered.

The successful identification of BCA candidates and the positive ex-ante CBA results from Project P01-B-003 indicate that the proposed investment in tilapia biocontrol RD&E is likely to be worthwhile and should be viewed favourably by the Centre for Invasive Species Solutions, potential funding partners, and other tilapia biocontrol and/or management stakeholders.

However, to strengthen any future analysis of the potential costs and benefits of tilapia biocontrol in Australia, it is strongly recommended that any future RD&E include:

1. Identification and estimation of the current and likely future impact and control costs associated with tilapia in Australia.
2. Work that demonstrates and quantifies the relationship between tilapia and the biophysical impacts to which tilapia are assumed to contribute.
3. Quantification of the potential relationship between reductions in tilapia biomass and the drivers of key medium- and long-term impacts of biocontrol.

1. Introduction

This report provides an independent ex-ante cost-benefit analysis (CBA) of proposed investment in research, development and extension (RD&E) to advance biological control (biocontrol) as a practical and effective control method for invasive tilapia in Australian waterways. The assessment was undertaken as part of a CSIRO led Project titled '*Tilapia biocontrol: prospecting and evaluation*' funded by the Centre for Invasive Species Solutions (CISS, Project Code P01-B-003).

The purpose of the ex-ante analysis was to support the business case for further investment to deliver improved control of tilapia through biocontrol. In September 2020, Agtrans Research contracted to carry out the CBA.

2. Terms of Reference

The specific terms of reference (ToR) for the ex-ante CBA are described below.

1. Complete a desktop review of available information on tilapia, its distribution, and its economic, social and environmental impacts in Australia.
2. Review past and current measures undertaken that address tilapia control.
3. Review the likely future spread and impact of tilapia on Australian waterways given current control measures.
4. Value the expected net economic benefits of the proposed biocontrol investment, taking into account the projected investment costs (provided as part of the Business Case development, see ToR 6 above), timelines and risk factors including the projected reduction in impacts of tilapia due to successful biocontrol of the species.
5. Estimate a set of investment criteria including present value of benefits, present value of costs, net present value, benefit-cost ratio, internal rate of return and modified internal rate of return.
6. Carry out sensitivity analyses that demonstrate the change in investment criteria with changes in key assumptions including current and future tilapia costs with and without successful biocontrol, and changes in levels of potential risk factors.
7. Provide discussion and conclusions on the economic merits of the RD&E investment.

3. Background

Tilapia were first introduced to Australia in the 1970s as an ornamental fish (Queensland Government, 2021). Tilapia are listed in the top 100 of the world's worst introduced species. All tilapia species are considered pest species in Australia and pose a significant threat to native fish and Australian ecosystems.

There is currently no single overall option for the control of tilapia in Australia. Ongoing RD&E is being funded and carried out by various research organisations to refine detection and control methods for tilapia. Biocontrol is thought to be a potentially cost-effective and practical solution for the management for invasive fish species, including tilapia. For example, significant RD&E investment already has been made to advance cyprinid herpes virus 3 (CyHV-3) as a biocontrol agent (BCA) for the control of invasive European carp in Australian waterways. CyHV-3 currently is under review by the Australian Government for approval for use in Australia. For more information, see: https://carp.gov.au/.

Australia previously has had success using biocontrol to reduce populations of invasive European rabbits through the release of the myxoma virus (1950) and variants of rabbit haemorrhagic disease virus (RHDV) (1995 and 2017). Rabbit biocontrol had significant positive impacts on Australian ecosystems and agricultural industries, providing estimated economic benefits of $70 billion over the last 60 years (Cooke, Chudleigh, Simpson, & Saunders, 2013).

A current RD&E project, led by CSIRO, titled '*Tilapia biocontrol: prospecting and evaluation*', was funded by the Centre for Invasive Species Solutions (CISS) to conduct a review of tilapia pathogens

and assess their potential as BCAs (a process known as bioprospecting). As part of this review project, CISS required an ex-ante CBA to assess the potential benefits of investment in the proposed tilapia BCAs. The findings of the CBA then would support a business case for further RD&E investment to deliver improved control of tilapia through biocontrol.

4. Method

The primary objective of the ex-ante analysis was to assess whether the investment (the total costs of the RD&E addressing the advancement of new BCAs to manage tilapia in Australia) would be paid for by the estimated potential benefits of the proposed BCA(s). The primary benefit is expected to be a net reduction in the total annual impact costs of tilapia to the Australian community and economy.

The ex-ante CBA followed general economic evaluation guidelines that are now well entrenched within the Australian primary industry research sector including Research and Development Corporations, Cooperative Research Centres (CRCs), State Departments of Agriculture, and some universities. The approach includes both qualitative and quantitative descriptions that are in accord with the impact assessment guidelines of the Council of Rural Research and Development Corporations (CRRDC) (CRRDC, 2018).

The CBA was set within a staged risk management framework of investment. The approach included identifying and describing the current and proposed tilapia biocontrol investment and its objectives, planned activities, expected outputs and outcomes. Potential impacts associated with the expected outcomes then were identified and categorised as economic, environmental, and social impacts.

It is axiomatic that successful biocontrol RD&E typically requires significant and long-term investment and can be considered somewhat risky. One of the principal considerations in analysing and valuing impacts from biocontrol RD&E is how the counterfactual is defined, that is, what would most likely occur without the proposed investment. A second principal consideration that has to be accommodated in the analysis is how risk is represented. All key data and assumptions used in the valuation of impacts are described and reported.

It is anticipated that the total tilapia biocontrol RD&E investment costs will be staged (go/no go decisions at particular stages of the investment, depending on progress and findings) in order to minimise investment risk. Also, risk factors were built into the analysis to ensure output, outcome and impact risks are taken into account so that the likely benefits from the investment are realistic and not overestimated.

The CBA focused on identifying and valuing economic impacts with some consideration given to identifying and qualitatively describing any environmental and social impacts.

5. Desktop Economic Literature Review

5.1 Overview of Potential Tilapia Impacts

There have been three species of tilapia introduced to Australia, the Mozambique tilapia (*Oreochromis mossambicus*), the black mangrove cichlid (*Pelmatolapia mariae,* formerly *Tilapia mariae*), and the redbelly tilapia (*Coptodon zillii*) (Native Fish Australia, n.d.). Characteristics that allow tilapia to establish in new areas include (NSW Department of Industry, n.d.):

- Highly efficient breeding strategies including mouth brooding,
- Simple food requirements (feeding on a wide variety of plant and animal matter), and
- Flexible habitat preferences (including the ability to breed in both fresh and brackish water).

Redbelly tilapia were reported near Perth in Western Australia (WA) in 1975 but were subsequently eradicated by the state fisheries department (NSW Department of Industry, n.d.). However, feral populations of *O. mossambicus* and *T. mariae* spread and now are widely distributed in tropical north-eastern Queensland (QLD), with *O. mossambicus* also occurring in south-eastern QLD and river systems of WA (Russell et al., 2010) and a population of *T. mariae* persisting in Hazelwood in Victoria (Centre for Invasive Species Solutions (CISS), 2021).

Populations of feral tilapia in Australia have continued to increase and, in 2014, the first established population of tilapia (*O. mossambicus*) in New South Wales (NSW) was confirmed at Cudgen Lake on the state's far north coast. Populations of tilapia in southern QLD also have been reported as little as three kilometres from the Murray Darling Basin (MDB) (NSW Department of Industry, n.d.).

Tilapia impacts have been recorded and reported in a number of locations in Australia and overseas. The key impacts recorded include major declines in commercial and traditional fisheries, fish extinctions, destruction of beds of macrophytes (large aquatic plants) and declines in water quality. Some of the direct impacts of tilapia in Australian waterways include (Hutchison, Sarac, & Norris, 2011):

- Impacts on native fish and other biota by:
    - direct predation by tilapia
    - competition for resources (food, habitat)
    - destruction of macrophytes and other aquatic plants used as breeding or nursery habitat by native species
    - habitat disturbance
    - transmission of diseases and parasites
    - competitive exclusion of native fish from favourable habitat by tilapia's aggressive behaviour
- Reduction in water quality, including potable water supplies, through:
    - increase of blue-green algal blooms (through resuspension of nutrients)
    - winter die-offs of tilapia (polluting waterways)
    - undermining riverbanks due to destruction of river plants and nesting behaviour.

The direct impacts of tilapia are largely environmental; however, these direct environmental impacts contribute to a number of indirect or secondary economic and social impacts, including potentially (CISS, 2021c):

- Increased water treatment/ water management and infrastructure costs [economic]
- Reduced amenity for recreational fishers [economic and/or social]
- Reduced productivity/ profitability for commercial fisheries that rely on species and/or ecosystems negatively affected by tilapia (e.g. barramundi) [economic]
- Reduced public amenity of tilapia affected waterways [social and/or economic]
- Increased management and control costs, likely incurred by landholders, land management groups, and/or Government, to mitigate tilapia impacts [economic].

As well as the likely negative impacts associated with tilapia in Australia, it is important to recognise there may be some positive impacts associated with the presence of tilapia in Australia. For example, tilapia are valued as an ornamental fish species and can be caught by recreational fishers and/or used for human or animal consumption. However, the potential positive impacts are likely to be minor relative to the recognised negative impacts of invasive species of tilapia.

5.2 Existing Current Control Methods

To minimise the potential environmental, social and economic impacts of introduced tilapia, current management principles include early detection of new populations, minimising the ability of the species to establish and/or spread to new environments, protecting native biodiversity, and conserving natural resources and associated recreational and commercial fisheries.

There are a range of control measures currently available for use on tilapia, but most are situation specific. Management tools include containment and/or exclusion, physical removal through netting, electrofishing, and angling/ line fishing, draining of waterbodies, and chemical removal (poisons) (CISS, 2021a). However, in the majority of situations and in the absence of effective ongoing management, unless the entire population and any possible source of reintroduction are removed, the highly flexible reproductive capacity of tilapia will see the population quickly return to original numbers (CISS, 2021a). There is currently no single overall option for the control of tilapia.

Targeted education campaigns, run by Government departments, research organisation, and other natural resources/environmental groups, actively highlight the potential damage caused by introduced tilapia to the natural environment and work to educate the public on what the fish look like and what to do if one is found. Community involvement in protecting and conserving local waterways is, to-date, the most effective control method in stopping the further spread of the two species of tilapia currently established in Australia (CISS, 2021b).

5.2 Estimated Impact Costs of Tilapia in Australia

Total annual impact costs of tilapia in Australian waterways include both (a) costs associated with tilapia species' negative impacts on native fish and ecosystems, including potential costs or losses to industries that rely on those ecosystems such as tourism and recreational fishing, and (b) tilapia management and control costs.

There have been several past studies that have attempted to identify and estimate, in monetary terms, the impact costs of a number of different invasive species in Australia including:

- Bomford and Hart (2002): the study concluded that the agricultural costs due to the major introduced vertebrate pests in Australia were difficult to accurately estimate due to a shortage of reliable data but totalled at least $420 million/year for direct short-term losses. Longer-term losses are also likely to be large. Further, landholders and governments in Australia spend over $60 million/year controlling introduced vertebrate pests. In addition to the resources spent on control an additional cost is the value of lost opportunities from alternative investment of this expenditure. Governments also spend around $20 million/year on research to control vertebrate pest species.
- McLeod (2004): the study investigated the impact costs for 11 major introduced vertebrate pests of Australian agricultural industries and the environment. The 2004 study reported total estimated annual impact costs of $720 million for Australia for the 11 invasive species included.
- Gong, Sinden, Braysher and Jones (2009): the study used an economic welfare framework and estimated that the total annual economic impact, comprising agricultural losses and expenditures on management, administration and research, at $743.5 million.
- McLeod (2016): this second study by McLeod was funded to update the impact costs estimates reported in Gong et al. (2009). The updated study reported estimated annual

Australia pest animal impact costs (including production losses and invasive species management costs) of between $416.2 and $797.3 million (average of $596.6 million).

- Hoffmann and Broadhurst (2016): the 2016 study, published by CSIRO titled '*The economic cost of managing invasive species in Australia*') included vertebrate, invertebrate and weed pest species. The study estimated that, in 2001–02, total national expenditure on invasive species was $2.31 billion ($3.03 billion adjusted to 2012 values), rising to $3.77 billion in 2011–12. For 2001–02 and 2011–12, these total expenditure figures equated to $123 and $197 per person per year respectively, as well as 0.32 and 0.29% of Gross Domestic Product (GDP) respectively.
- Bradshaw et al. (2021): this was a detailed analysis of reported costs associated with invasive species in Australia since the 1960s based on a recently published database known as *InvaCost*. Bradshaw et al. (2021) analysed 2,078 unique cost entries and supplementary information and found that, since 1960, Australia has spent or incurred losses of a total of at least US$298.58 billion (2017-dollar terms, all cost data) or approximately AUD$390 billion from invasive species (2017 average exchange rate).

However, none of these Australian studies included an estimate of the impact costs of tilapia. Only one study was found that attempted to estimate the likely impact costs of tilapia in Australia. Greiner & Gregg (2008) undertook a study for the Australian Centre for Tropical Freshwater Research at James Cook Univeristy in Townsville. The purpose of their report was to provide an attempt at estimating the conomic impact of tilapia in north Queensland and was based on a desktop review of existing information and a limited empirical investigation involving key waterway managers and recreational fishers.

Greiner & Gregg (2008) reported that The direct costs associated with monitoring, management and prevention of tilapia amounted to nearly $900,000 during 2006/07. Further, based on a total economic benefit framework and a series of assumptions, Greiner & Gregg reported some "least cost" and "highest cost" cost estimates for various tilapia impact cost items. The "highest cost" estimate was based on the assumption that tilapia become widespread, that there is an intensified effort to control tilapia, and that key commercial fisheries are severly impacted/obliterated by feral tilapia.

Figure B1 shows the current and future economic cost estimates for tilapia in northern Queensland reproduced from Greiner & Gregg (2008).

Costs/Uses/Values	Current	Hypothetical Future Economic Impact	
	Observed ($ per year--2006/07)	Least Cost ($ per year)	Highest Cost ($ per year)
Direct costs			
Monitoring	$237,939	$0 [4]	>$1 million [5]
Management	$90,000	$0 [4]	>$1 million [5]
Prevention	$561,227	$0 [4]	>$1 million [5]
Cost to direct use value of waterways			
Human consumption of water	$0	$0 [6]	$10 million ++ [7]
Irrigation water	+ [1]	$0 [6]	+ [8]
Recreational fishing	+ [2]	$0 [6]	$3 million [9]
Other recreation	+ [2]	$0 [6]	$3 million [10]
Amenity	+ [2]	$0 [6]	+ [11]
Cost to indirect use values of waterways			
Commercial fishing	$0	$0 [12]	$16 million [3]
Loss of non-use values associated with waterways (ecology, water quality)	no evidence	$0 [13]	$ 1 million ++ [14]

Figure B1: Estimated economic impact of tilapia in the context of northern Queensland (Source: pg. 38 Greiner & Gregg (2008))

Based on the current and hypothetical future economic impact of tilapia, the assumptions made in the Greiner & Gregg (2008) study, and adjusting to 2020/21 dollar terms[2], it is possible that the economic impact costs of tilapia in QLD currently lie between $1.2 million and $13.6 million per annum (taking into account only annualised costs and excluding the estimated future commercial fishing costs where the hypothesised negative impacts have not yet occurred). Further, if targeted efforts to control tilapia are not undertaken and key commercial QLD fisheries are essentially destroyed by the future spread of tilapia, the economic costs of tilapia could increase to over $35.4 million per annum in 2020/21 dollar terms.

Further, it is likely that these data underestimate or exclude values for a number of environmental and social impact costs associated with tilapia. Also, the costs were estimated for QLD only. On a national scale, the impact costs could be significantly higher if tilapia are allowed to spread into other key Australian waterways, particulalry the Murray-Darling Basin that stretches across five Australian states/territories from QLD to South Australia.

6. Logical Framework for the Proposed RD&E Investment

A logical framework for analysing the current and proposed tilapia biocontrol RD&E investment was developed. This required the evaluation team to understand the current (Project P01-B-003) and intended future investments and their likely outputs, outcomes and impacts. The logical framework was developed with input from the CSIRO P01-B-003 project team, including the draft review of candidate tilapia biocontrol pathogens.

Tilapia lake virus (TiLV) was identified as the primary candidate for further research under bioprospecting Project P01-B-003. However, a second biocontrol candidate, tilapia parvovirus (TiPV) also was considered tentatively worthwhile for further investigation.

It was understood that the required RD&E to advance either BCA candidate in Australia would likely be similar but independent (that is, separate investment would need to be made to progress each

[2] Cost data in 2006/07 dollar terms adjusted using the Australian implicit price deflator for gross domestic product, multiplier of x1.3605 (Australian Bureau of Statistics (ABS), 2021)

candidate agent). Further, the proposed tilapia biocontrol RD&E investment, including the initial bioprospecting investment (Project P01-B-003) would be undertaken in six overall stages with stop/go decision points based on the success of various stages. The following briefly describes each of the likely RD&E stages required to progress candidate tilapia BCAs (e.g. TiLV or TiPV).

- Stage 1: Bioprospecting and Evaluation

CISS Project P01-B-003: *Tilapia biocontrol: prospecting and evaluation* represents Stage 1 of the RD&E investment required to identify and advance the selection of a new BCA for tilapia in Australia. To progress any tilapia BCA candidates identified as worthwhile for further investigation, it will be essential to formally and thoroughly evaluate the agent.

As of August 2021, TiLV had been selected for active further investigation and additional research already had commenced (see Stage 2A below).

- Stage 2A: Efficacy Testing

Where, based on the findings of Stage 1, the EIC and individual jurisdictions (States and Territory) support additional investment to progress tilapia biocontrol RD&E, the next stage of the project (Stage 2) would be testing the efficacy (virus virulence and transmission). Further, alongside the efficacy testing, RD&E is required to systematically assess the possibility of interfering endemic viruses and also the possibility of reassortments (Chaput et al., 2020). This would involve, for example, meta-transcriptomic analyses (Turnbull et al., 2020), of other viruses in Australian tilapia populations.

CSIRO already have imported TiLV and are developing the capability to work with it in a laboratory setting. The project team will commence testing of TiLV's susceptibility in tilapia sourced from QLD waters in January 2022. Additional and independent investment in similar RD&E would be required should stakeholders choose also to progress TiPV as a potential tilapia BCA.

- Stage 2B: RD&E on Complementary Tilapia Control Methods

Further work including the identification of other broad-scale control measures, such as genetic control, to complement the virus would need to be considered. A number of genetic technology options for broad-scale control may be applicable for tilapia, and some are currently under investigation. These include genetic biocontrol such as 'gene drives' and/or self-stocking incompatible male systems (CISS Project P01-B-005). Australia is currently investing in RD&E to investigate these broadly applicable technologies for managing invasive fish species. A prerequisite for genetic biocontrol approaches is also a thorough assessment of the genetic makeup and diversity of Australian tilapia (population genomics analyses). This is important as there already is significant evidence of hybridisation occurring among wild populations.

It would be beneficial if the findings of any successful RD&E into complementary control measures could be built into Stage 4 (if available) and Stage 6 to ensure the greatest control can be achieved (i.e. optimal and maximum reduction of tilapia biomass in Australian waterways).

- Stage 3: Safety Testing

Stage 3 would involve testing the safety (susceptibility of non-target species) of TiLV as a BCA. If successful, the data generated from the efficacy (Stage 2) and safety (Stage 3) trials on the virus then will provide input to development of an epidemiological model for TiLV.

The findings from the Stage 2 and 3 RD&E investment represent an important stop/go decision point for any future investment to further advance a new BCA toward release as a practical tool for tilapia control in Australia.

Note: if resources committed to tilapia biocontrol RD&E in the future permitted, it would be possible that Stage 3 could be undertaken concurrently with Stage 2 (A and B). This would reduce the overall timeframe for the proposed tilapia biocontrol RD&E.

- Stage 4: Planning and Modelling Optimal Release

If the findings from Stage 2 and 3 RD&E indicate that the proposed BCA (e.g. TiLV) could be used as a safe and effective tilapia BCA in Australia, the epidemiological model then will be used as a key part of further RD&E required to determine the optimal release strategy, or strategies, for the virus (Stage 4). Understanding and optimising potential release strategies will provide critical input for planning, coordinating, and costing any actual future release of a new BCA, pending necessary approvals. It is likely that work conducted in Stage 4 would be predominantly QLD-centric and would be modelled on work associated with European Carp undertaken as part of the recent National Carp Control Program (NCCP).

- Stage 5: Other Assessments and Regulatory Approvals

Social and ecological risk assessments will be needed to support an application to release a new BCA against tilapia in Australia. Application for release of any such tilapia BCA would be made through the Commonwealth Department of Agriculture, Water, and the Environment (DAWE) and Australian Pesticide and Veterinary Medicines Authority (APVMA).

Applications for regulatory approval to release a new tilapia BCA in Australia would rely heavily on data and information generated through investment in Stages 1 to 4. Further, the outcomes of the required applications (e.g. approval, approval with conditions, approval in principle with additional information/ data required, and/or non-approval) represent another important stop/go point for further investment in tilapia biocontrol.

- Stage 6: Nationally Coordinated Release and Clean-up

If a new tilapia BCA is approved for release in Australia, a structured collaborative program of release strategies and planning and coordination of any clean-up will be developed. This stage of investment (Stage 6) also will address bioethical issues and public acceptance of viral biocontrol of tilapia. This stage also will need to include investment for activities to support effective and efficient biocontrol release such as post-release monitoring and additional RD&E focused on the development of new virus variants in subsequent years as fish develop natural immunity/ resistance to the BCA.

To date, a viral BCA has never been used or approved for use against aquatic invasive species in Australia[3]. Therefore, public and government approval is considered a major concern. Australia has very strong legislative mechanisms for approval of BCA including the Commonwealth Biological Control Act 1984 along with Acts in the States and Territories as well as numerous international conventions. The legislation requires procedures to demonstrate that:

1) There is an urgent need to control the pest,
2) The BCA will likely reduce the impacts caused by the invasive species, and
3) The release of the BCA will not negatively affect the environment and the non-target species sharing the waterways.

[3] It is worth noting that cyprinid herpes virus 3 (CyHV-3) as a BCA to control feral European carp in Australian waterways currently is under review by the Australian Government following the National Carp Control Program (NCCP) undertaken between 2016 and 2021. More information can be found at: https://carp.gov.au/

7. Proposed Investment in Tilapia Biocontrol RD&E

The initial RD&E investments, including current Project P01-B-003, required to advance a tilapia BCA (either TiLV or TiPV) are shown in Table B1 (below) according to the RD&E Stages described in Section 6.

Table B1: Current and Proposed Investment in RD&E Stages 1-5 for Tilapia Biocontrol in Australia

Year ended 30 June	Tilapia Biocontrol RD&E Stage			Totals
	Stage 1[(a)]	Stage 2-4[(b)]	Stage 5[(c)]	
2021	302,444	0	0	302,444
2022	302,444	0	0	302,444
2023	0	3,000,000	0	3,000,000
2024	0	3,000,000	0	3,000,000
2025	0	3,000,000	0	3,000,000
2026	0	3,000,000	0	3,000,000
2027	0	3,000,000	0	3,000,000
2028	0	3,000,000	0	3,000,000
2029	0	3,000,000	0	3,000,000
2030	0	3,000,000	0	3,000,000
2031	0	3,000,000	0	3,000,000
2032	0	3,000,000	0	3,000,000
2033	0	0	3,000,000	3,000,000
2034	0	0	3,000,000	3,000,000
2035	0	0	3,000,000	3,000,000
2036	0	0	3,000,000	3,000,000
2037	0	0	3,000,000	3,000,000
Totals	604,888	30,000,000	15,000,000	45,604,888

(a) Based on total annual RD&E costs (cash and in-kind) for tilapia bioprospecting project P01-B-003 funded by CISS.
(b) Estimated RD&E cost of $3 million per annum over ten years for Stages 2-4 based on the CISS bioprospecting project and RD&E conducted for the NCCP.
(c) Based on funding of $15 million over five years for the NCCP.

For the purpose of the current analysis, investment for Stage 6 (nationally coordinated BCA release and clean up) has been excluded. The potential costs associated with Stage 6 are highly uncertain and dependent on a wide range of factors including:

- Efficacy of the proposed BCA
- Regulatory requirements
- Future spread and density of feral tilapia
- Optimal/ viable release strategies

As a result, the total RD&E investment costs presented in Table B1 are likely to somewhat underestimate the total investment costs required to advance and implement a new tilapia BCA in Australia.

8. Potential Impacts of Tilapia Biocontrol

8.1 Triple Bottom Line Impacts

The overarching goal of any tilapia BCA would be to reduce the total biomass of tilapia in Australian waterways. This, in turn, is expected to result in a net reduction in tilapia impact and control costs (for a description of tilapia impacts see Section 5.1).

Table B2 summarises the broad potential economic, environmental and social impacts (triple bottom line impact types) that may be achieved through future investment in tilapia biocontrol RD&E.

Table B2: Potential Triple Bottom Line Impact Types for Investment in Tilapia Biocontrol

Economic	Environmental	Social
Reduced and/or avoided costs associated with water treatment/ water management/ water infrastructure Increased expenditure by recreational fishers Maintained or enhanced productivity/ profitability for commercial fisheries in tilapia affected regions Reduced and/or avoided tilapia control/ management costs Increased net income for some regional Australian tourism sectors	Improved biodiversity through maintained or enhanced populations of native fish and other biota Improved health and wellbeing for native fish species through reduced competition, predation and disease risks Improved ecosystem health through reduced/ avoided risk of algal blooms, tilapia die-offs and riverbank damage	Improved amenity for recreational fishers Improved amenity for public users of tilapia affected waterways Increased scientific knowledge and research capacity with respect to biocontrol of invasive fish in Australia

8.2 Pathways to Impacts

A simplified description of the pathways to impact for the proposed investment in tilapia biocontrol RD&E is shown in Figure B2.

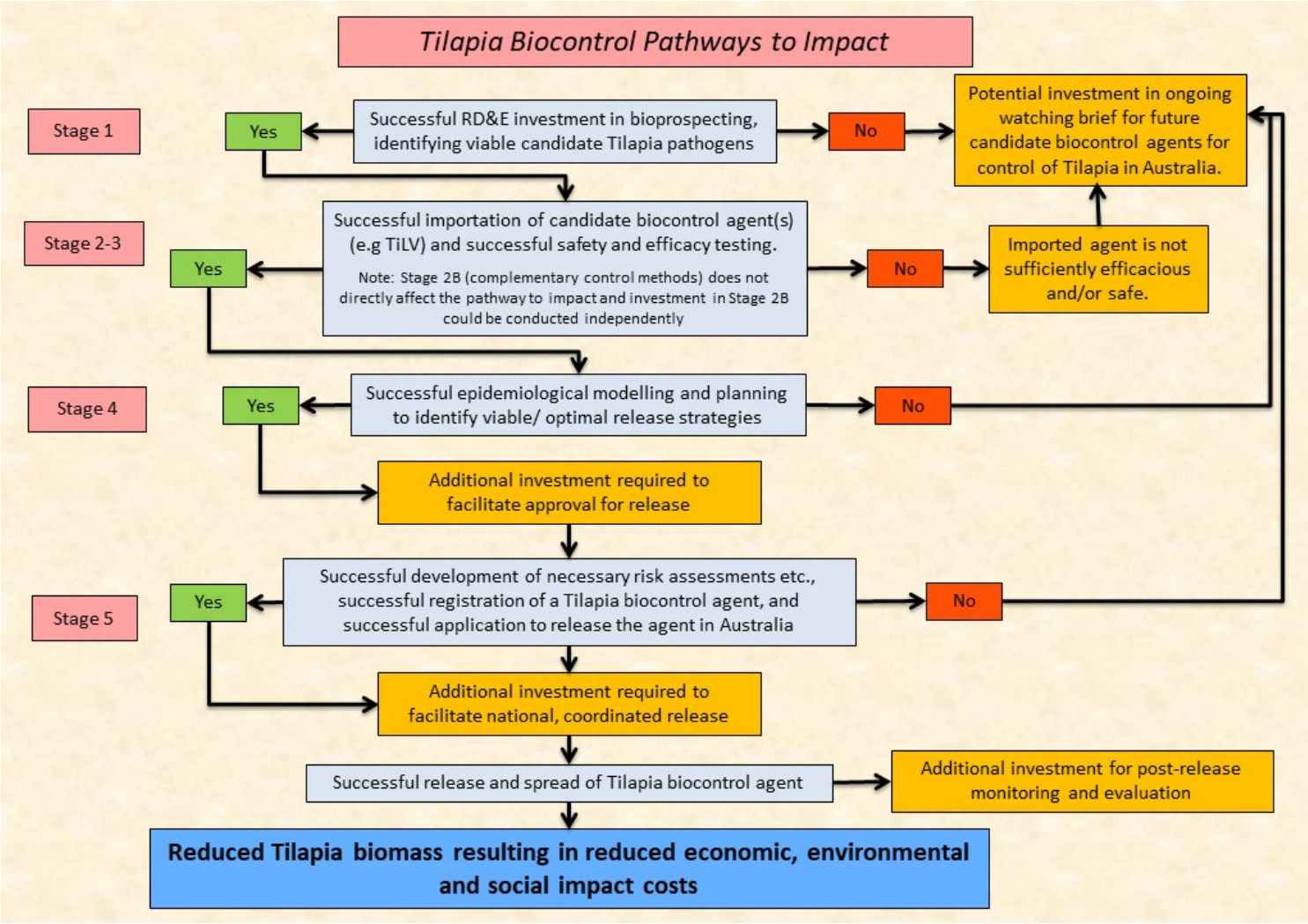

Figure B2: Likely pathways to impact for tilapia biocontrol RD&E

9. Valuation of Potential Impacts

9.1 Impacts Not Valued

Not all of the potential impacts of the proposed investment in tilapia biocontrol RD&E identified in Table B2 could be valued within the scope of the current ex-ante analysis. In particular, environmental and social impacts can be difficult to estimate in monetary terms and often require the application of complex and resource intensive non-market economic valuation methods.

The following impacts were not valued because of a lack of data/ evidence on which to base credible assumptions, uncertainty about the linkages between the initial investment and the expected impacts, available time/ resources for the current analysis and/or the impact was considered to be minor relative to the impact(s) valued.

Environmental impacts not valued included:

- Improved biodiversity through maintained or enhanced populations of native fish and other biota.
- Improved health and wellbeing for native fish species through reduced competition, predation and disease risks.
- Improved ecosystem health through reduced/ avoided risk of algal blooms, tilapia die-offs and riverbank damage.

Social impacts not valued included:

- Improved amenity for recreational fishers[4].
- Improved amenity for public users of tilapia affected waterways.
- Increased scientific knowledge and research capacity with respect to biocontrol of invasive fish in Australia.

9.2 Impacts Valued

The primary economic impact valued was the potential net reduction in tilapia impact and controls costs associated with reduced tilapia biomass from implementation of a new tilapia BCA.

Valuation of the impact involved making several uncertain assumptions as a number of key relationships/ variables along the pathways to impact were unknown. Specifically, the following relationships/ variables are currently unknown:

a) The potential reduction in tilapia biomass through use of a given candidate BCA,
b) The reduction in tilapia biomass through implementation of a new BCA, and the resulting change in tilapia impact and control costs in Australia, and
c) The probability of success of each stage of the proposed tilapia biocontrol RD&E investment.

The impact was valued for TiLV as the selected BCA. This was because TiLV was identified as the most promising tilapia biocontrol candidate and already has been imported to Australia for initial efficacy testing. However, a similar valuation framework also would apply to TiPV. Other than the RD&E timeframes, it is considered likely that the total RD&E investment costs for TIPV would be similar to those for TiLV.

[4] The value of improved amenity for recreational fishers may be partially captured through valuation of a related, economic impact: increased expenditure by recreational fishers.

9.3 Summary of Assumptions

The specific assumptions used to value the primary economic impact, a net reduction in tilapia impact and control costs, are described in Table B3.

Table B3: Impact Valuation Assumptions

Variable	Assumption	Source/ Notes
WITHOUT investment in tilapia biocontrol RD&E (Counterfactual)		
Estimated total average annual tilapia impact and control costs	$13.6 million p.a. in 2020/21	2020/21 dollar terms Based on Greiner & Gregg (2008) – see Section 5.2
Maximum potential annual impact and control costs for tilapia	$88.5 million p.a. Based on maximum annual impact and control costs in QLD of $35.4 million in 2020/21 dollar terms based on Greiner & Gregg (2008). The maximum total QLD costs then were multiplied by a factor of x2.5	The cost multiplier (x2.5) was applied to accommodate the likely future spread tilapia to the Murray-Darling Basin without intervention, resulting in a significant increase in tilapia impact and control costs in Australia
Change in estimated total average annual impact and control costs over time without significant intervention	Increasing linearly from initial value of $13.6 million p.a. to $88.5 million p.a. over the next 20 years	Analyst assumption
WITH tilapia biocontrol RD&E investment – TiLV		
Stage 1 total RD&E costs	$302,444 p.a. for two years (2020/21 and 2021/22)	See Table B1
Probability of Stage 1 success	100%	Based on identification of TiLV and TiPV as potential candidate BCAs through the review conducted under P01-B-003
Stage 2-4 total RD&E costs	$3 million p.a. over 10-years (2022/23 to 2031/32)	See Table B1
Probability of Stage 2-4 funding and RD&E success (agent found to be safe and efficacious)	70%	Analyst assumption – based on CSIRO importing TiLV for further safety and efficacy testing
Stage 5 total RD&E costs	$15 million over 5-years (2032/33 to 2036/37)	See Table B1
Probability of Stage 5 funding and success (given successful Stage 1-4 RD&E)	50%	Analyst assumption
Estimated reduction in tilapia biomass from nationally coordinated release of TiLV	50%	Based on an experimental challenge with TiLV in Thailand, in which mortalities of 48.89% and 77.78% were observed in *O. mossambicus* (Agus Sunarto, CSIRO, pers. comm., 2021)
Estimated reduction in total annual impact and control	50%	Analyst assumption – assumes a one-to-one relationship between

costs from reduced tilapia biomass after release of TiLV		tilapia biomass and tilapia impact and control costs
Year of TiLV release and first year of impact (reduction in tilapia biomass)	2042/43	Analyst assumption based on an additional 5-years after the initial Stage 1-5 RD&E investment for Government approvals, planning and national coordination and release. Also, mathematical modelling estimated that the population of Nile tilapia decreased to 12% of the initial population size of 30 fish after 16 days post TiLV infection (Agus Sunarto, CSIRO, pers. comm., 2021)
Period of maximum impact	10 years, after which tilapia biomass and associated impacts will increase to 70% of pre-release levels over a period of 20 years because of development of host resistance	Analyst assumption – it is unknown whether tilapia populations are susceptible or resistant to the virus; however, for invasive carp, it was predicted that the development of host resistance might take decades to impact on CyHV-3 field effectiveness (Agus Sunarto, CSIRO, pers. comm., 2021)
Probability of impact occurring	80%	Allows for uncertainty regarding the field efficacy of TiLV and exogenous factors that may affect realisation of impact (e.g. climate)

10. Results

All benefit and cost cash flows were expressed in 2020/21 dollar terms using the Implicit Price Deflator for Gross Domestic Product (ABS, 2021) and were discounted to 2020/21 using a discount rate of 5% as required by the CRRDC guidelines (CRRDC, 2018).

To accommodate the relatively long timeframes associated with biocontrol RD&E in Australia, the ex-ante analysis ran for the length of the proposed RD&E investment plus 50 years from the first year of investment in Project P01-B-003 (2020/21) (Stage 1 of the overall proposed investment in tilapia biocontrol RD&E).

Given the inclusion of risk factors associated with the investment and success of the proposed future stages of tilapia biocontrol RD&E, the results reported are expected values.

10.1 Investment Criteria

Table B4 shows the investment criteria estimated for different periods of expected benefits for the total expected (risk adjusted) investment in tilapia biocontrol.

Table B4: Investment Criteria for Total Proposed Investment in Tilapia Biocontrol

Investment Criteria	Years after First Year of Investment (2020/2021)										
	0	5	10	15	20	25	30	35	40	45	50
Present Value of Benefits ($m)	0.00	0.00	0.00	0.00	0.00	11.26	22.58	31.82	39.82	46.68	52.53
Present Value of Costs ($m)	0.30	7.68	14.81	18.21	18.69	18.69	18.69	18.69	18.69	18.69	18.69
Net Present Value ($m)	-0.30	-7.68	-14.81	-18.21	-18.69	-7.43	3.89	13.12	21.12	27.99	33.84
Benefit-Cost Ratio	0.00	0.00	0.00	0.00	0.00	0.60	1.21	1.70	2.13	2.50	2.81
Internal Rate of Return (%)	negative	negative	negative	negative	negative	1.76	6.07	7.73	8.58	9.06	9.34
Modified Internal Rate of Return (%)	negative	negative	negative	negative	negative	2.89	5.66	6.40	6.85	7.03	7.09

The annual undiscounted total estimated expected benefit and cost cash flows for the total RD&E investment plus 50 years from the first year of investment in Project P01-B-003 are shown in Figure B3.

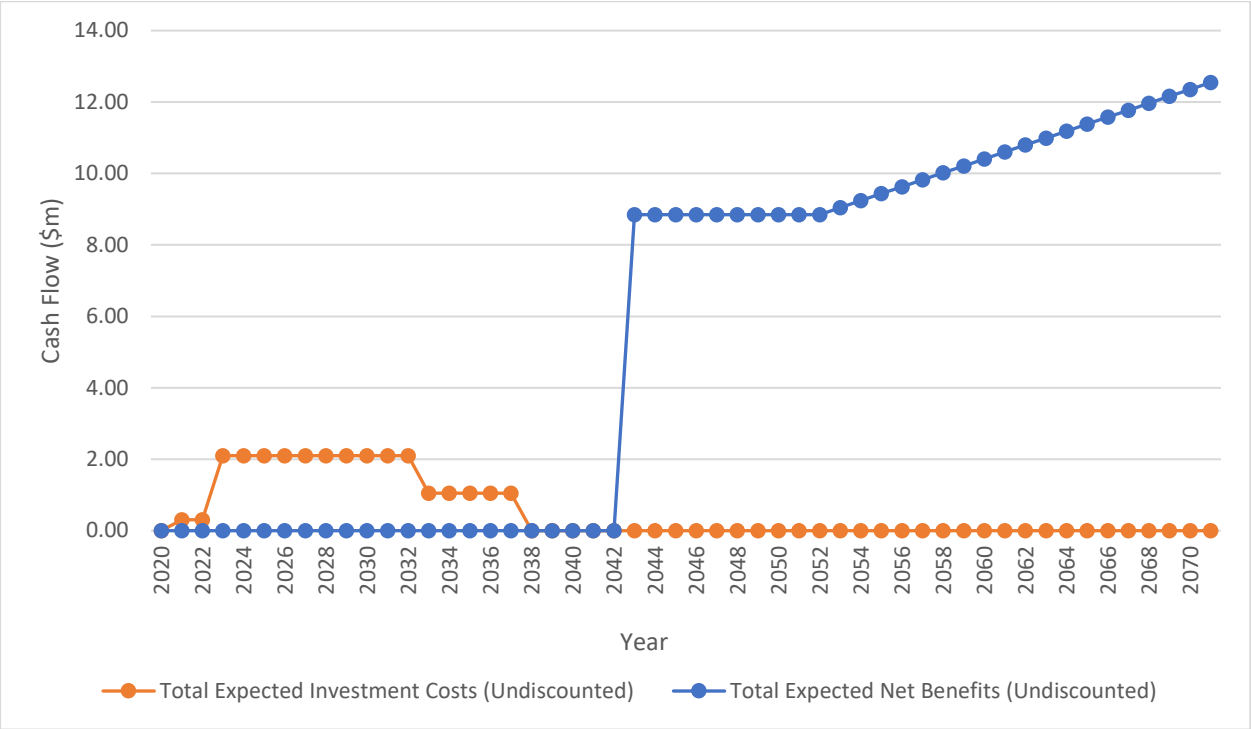

Figure B3: Annual Undiscounted Total Expected Investment Cost and Total Expected Benefit Cash Flows

10.2 Sensitivity Analyses

Sensitivity analyses were carried out on variables that were considered key drivers of the investment criteria and/or were particularly uncertain. All analyses were performed for the total investment with benefits taken over the life of the investment plus 50 years from the first year of investment in Project P01-B-003 (Stage 1 RD&E: tilapia bioprospecting). All other parameters were held at their base values.

A sensitivity analysis was carried out on the discount rate. Table B5 presents the results. The results showed a high sensitivity to the discount rate, this was largely due to the fact that the benefit cash flows that start after the end of the tilapia biocontrol RD&E investment (a period of 22 years). This means that the benefit cash flows were subjected to relatively greater discounting than the cost cash flows.

Table B5: Sensitivity of Investment Criteria to the Discount Rate (Total investment, 50 years)

Investment Criteria	Discount Rate		
	0%	5% (base)	10%
Present Value of Benefits ($m)	293.64	52.53	11.82
Present Value of Costs ($m)	26.85	18.69	13.70
Net Present Value ($m)	266.79	33.84	-1.88
Benefit-Cost Ratio	10.93	2.81	0.86

A sensitivity analysis then was carried out on the assumption regarding the maximum potential annual impact and control costs for tilapia. The estimated current and potential future impact and control

costs of tilapia in Australia are a critical assumption and underpin both the valuation of impacts and the counterfactual. The sensitivity analysis was carried out based on the multiplier used to estimate the hypothetical maximum impact and control costs if tilapia continue to spread, including to the Murray-Darling Basin, without intervention.

The results, presented in Table B6, show a moderate sensitivity to the maximum potential impact and control costs of tilapia. A break-even analysis indicated that the investment criteria were positive when the multiplier used to estimate the maximum potential impact costs was x1.046. This means that, with all other variables at their base values, the proposed investment in tilapia biocontrol RD&E would be a worthwhile investment if the total average annual impact and control costs of tilapia in Australia increase to $37.02 million. Further, this demonstrates that the estimated annual impact costs of tilapia are likely to be a key driver of any potential benefits of tilapia biocontrol.

Table B6: Sensitivity of Investment Criteria to the Maximum Potential Impact and Control Costs of Tilapia in Australia (Total investment, 5% discount rate, 50 years)

Investment Criteria	**Maximum Potential Impact and Control Costs of Tilapia in Australia - Multiplier**		
	$36.4m x1.0	**$36.4m x2.5 (base)**	**$36.4m x5.0**
Present Value of Benefits ($m)	17.63	52.53	110.72
Present Value of Costs ($m)	18.69	18.69	18.69
Net Present Value ($m)	-1.07	33.84	92.02
Benefit-Cost Ratio	0.94	2.81	5.92

A sensitivity analysis then was carried out on the assumption regarding the expected reduction in tilapia impact and control costs associated with a reduction in tilapia biomass caused by release of a new tilapia BCA. Table B7 presents the results. The investment criteria showed a moderate to low sensitivity to the assumed reduction in tilapia impact and control costs. A break-even analysis suggested that, with all other assumptions at their base values, the proposed investment in tilapia biocontrol RD&E would give positive results if the expected reduction in tilapia impact and control costs was as low as 5.49% (noting that this assumes that, without intervention, tilapia impact costs will increase significantly in the future).

Table B7: Sensitivity of Investment Criteria to the Expected Reduction in Tilapia Impact and Control Costs (Total investment, 5% discount rate, 50 years)

Investment Criteria	**Expected Reduction in Tilapia Impact and Control Costs**		
	30%	**50% (base)**	**80%**
Present Value of Benefits ($m)	37.33	52.53	75.34
Present Value of Costs ($m)	18.69	18.69	18.69
Net Present Value ($m)	18.64	33.84	56.65
Benefit-Cost Ratio	2.00	2.81	4.03

A final break-even analysis then was conducted jointly on the two key variables tested previously (Table B6 and B7). This analysis tested what combination of maximum potential impact and control costs and what expected reduction in impact and control costs because of a new tilapia BCA would result in positive investment criteria. The analysis found that, with all other variables held at their base values, the investment criteria for tilapia biocontrol RD&E were positive when the maximum potential annual impact costs were $51.1 million (multiplier of x1.44) and the reduction in tilapia impact costs

from biocontrol was 28.9%. This combined break-even analysis indicates that the investment criteria are relatively robust to the key assumptions made.

11. Key Findings: Summary and Discussion

Tilapia bioprospecting Project P01-B-003 has, to date, successfully identified three potential tilapia biocontrol candidates categorised as tentatively worthwhile for further investigation.

TLEV was categorised under a 'watching brief'. This means that TLEV was not selected for further investigation right now but will be watched as possible future BCA through the international literature and scientific networks.

TiPV was categorised as 'tentatively worthwhile' for further investigation. TiPV is the first and only parvovirus known to infect fish. The virus also has been isolated in cell cultures, allowing future testing of the virus including experimental challenge.

TiLV was considered the most promising potential BCA candidates and was categorised as 'worthwhile for active further investigation'. CSIRO already have imported the virus and are currently developing the capability to work with TiLV in a laboratory setting. The project team currently plans to test TiLV's susceptibility in tilapia sourced from QLD waters in January 2022.

The primary objective of the preceding ex-ante analysis was to assess whether the investment (the total costs of the RD&E addressing the advancement of new BCAs to manage tilapia in Australia) would be paid for by the estimated potential benefits of the proposed BCA(s).

The CBA was set within a staged risk management framework of investment. The approach included identifying and describing the six stages of RD&E for the proposed tilapia biocontrol investment, RD&E objectives, planned activities, expected outputs and outcomes. Potential impacts associated with the expected outcomes then were identified and categorised as economic, environmental, and social impacts. The primary impact is expected to be a net reduction in the total annual impact costs of tilapia to the Australian community and economy through a reduction in tilapia biomass.

Valuation of the primary impact involved making several uncertain assumptions as a number of key relationships/ variables along the pathways to impact were unknown. Specifically, the following relationships/ variables are currently unknown:

a) The potential reduction in tilapia biomass through use of a given candidate BCA,
b) The reduction in tilapia biomass and the resulting change in tilapia impact and control costs in Australia, and
c) The probability of success of each stage of the proposed tilapia biocontrol RD&E investment.

The impact was valued for TiLV as the selected BCA. This was because TiLV was identified as the most promising tilapia biocontrol candidate and already has been imported to Australia for initial efficacy testing. However, a similar valuation framework also would apply to TiPV. Other than the RD&E timeframes, it is considered likely that the total RD&E investment costs for TIPV would be similar to those for TiLV.

To accommodate the relatively long timeframes associated with biocontrol RD&E in Australia, the ex-ante analysis ran for the length of the proposed RD&E investment period plus 50 years from the first year of investment in Project P01-B-003 (2020/21) (Stage 1 of the overall proposed investment in tilapia biocontrol RD&E). Based on the assumptions made, the total expected RD&E investment was estimated at $18.69 million (present value terms). The investment was estimated to produce total expected net benefits of $52.53 million (present value terms). This gave a net present value of $33.84 million, a benefit cost-ratio of 2.81 to 1, an internal rate of return of 9.3% and a modified internal rate of return of 7.1%.

Care should be taken when interpreting the results of the ex-ante analysis. It is important to note that the expected release and subsequent impact of a new tilapia BCA, such as TiLV, would not occur until approximately 22 years after the first year of investment in Project P01-B-003. Given that the

investment criteria became positive between 25 and 30 years after the first year of investment, this indicates that implementation of a new tilapia BCA would create benefits sufficient to cover the costs of the proposed tilapia biocontrol RD&E investment within five to ten years of release of the BCA.

Further, it is important to remember that the ex-ante analysis was conducted within a risk management framework and that the results are expected values. This means that it is theoretically possible for the total proposed investment in tilapia biocontrol to be made (approximately $45.6 million in nominal dollars) and for there to be no benefits realised. That is, the new agent is released and is unsuccessful in reducing tilapia impact costs. However, the risk of this is very minimal as the proposed tilapia biocontrol RD&E investment has been planned as a staged investment with a number of key stop/ go points that would enable funding partners, researchers and other stakeholders to adjust and/ or redirect the RD&E to alternative and more promising directions. Also, the knowledge generated through Stages 1 to 3 are likely to contribute to increased scientific knowledge and research capacity associated with management of pest tilapia in Australia.

Sensitivity analyses of key variables in the CBA showed that the current and expected total average annual impact and control costs attributable to tilapia in Australia are a critical assumption when considering the estimated benefits of proposed biocontrol. Currently, the current and likely future impact and controls costs of tilapia without intervention are highly uncertain.

However, a break-even analysis conducted on what combination of maximum potential tilapia impact and control costs and the expected reduction in impact and control costs because of a new tilapia BCA found that the investment criteria for tilapia biocontrol RD&E were positive when the maximum potential annual impact costs were $51.1 million (multiplier of x1.44) and the reduction in tilapia impact costs from biocontrol was 28.9%. This combined break-even analysis indicated that the investment criteria are relatively robust to the key assumptions made.

12. Conclusions and Recommendations

The investment criteria estimated from the base set of assumptions for the proposed investment in tilapia biocontrol (including Project P01-B-003) are all positive from a period of 30 years after the first year of investment (2020/21). The positive investment criteria suggest that the initial investments (Stages 1 to 5) would be worthwhile given the estimates made of the current and future potential impact and control costs of tilapia in Australia, likely pathways to impact for proposed new BCAs, the RD&E investment and associated timelines required, and the risks involved.

The proposed investment can be staged conditionally so that, as the investment proceeds along a particular pathway, the direction of the RD&E could be changed according to any past success and any new information available. This may avoid or minimise any potential losses and maximise the chances of significant impacts being delivered.

The successful identification of BCA candidates and the positive ex-ante CBA results from Project P01-B-003 indicate that the proposed investment in tilapia biocontrol RD&E is likely to be worthwhile and should be viewed favourably by the Centre for Invasive Species Solutions, potential funding partners, and other tilapia biocontrol and/or management stakeholders.

However, to strengthen any future analysis of the potential costs and benefits of tilapia biocontrol in Australia, it is strongly recommended that any future RD&E include:

1. Identification and estimation of the current and likely future impact and control costs associated with tilapia in Australia. In particular, the non-market values associated with tilapia impacts are likely to be the most significant in terms of tilapia impact costs and the potential benefits of any future tilapia biocontrol. Further, the likely future increase in tilapia impact costs without intervention is a key driver of any potential benefits of implementation of any future tilapia BCAs.

2. Work that demonstrates and quantifies the relationship between tilapia and the biophysical impacts to which tilapia are assumed to contribute taking into account different levels of existing tilapia biomass in different types of habitats/ ecosystems in different regions.
3. Quantification of the potential relationship between reductions in tilapia biomass and the drivers of key medium- and long-term impacts of biocontrol including biodiversity/ ecosystem health outcomes.

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- Tanja Strive, Team Leader, Biosecurity Flagship, CSIRO
- Tim Lucas, Biosecurity Queensland
- Waltraud Attia, Contract Support Office, CSIRO

Abbreviations and Acronyms

ABS	Australian Bureau of Statistics
APVMA	Australian Pesticide and Veterinary Medicines Authority
BCA	Biocontrol Agent
Biocontrol	Biological Control
CBA	Cost-Benefit Analysis
CISS	Centre for Invasive Species Solutions
CRRDC	Council of Rural Research and Development Corporations
CRC	Cooperative Research Centre
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAWE	Department of Agriculture, Water and the Environment (Commonwealth)
MDB	Murray-Darling Basin
NCCP	National Carp Control Program
RD&E	Research, Development and Extension
TiLV	Tilapia Lake Virus
TiPV	Tilapia Parvovirus
ToR	Terms of Reference

Glossary of Economics Terms

Cost–benefit analysis (CBA):	An economic analysis technique for assessing the economic merit of a proposed initiative by assessing the benefits, costs, and net benefits to society of the initiative. Aims to value benefits and costs in monetary terms wherever possible and provide a summary indication of the net benefit.
Benefit-cost ratio (BCR):	Ratio of the present value of economic benefits to the present value of economic costs of a proposed initiative. Indicator of the economic merit of a proposed initiative at the completion of cost–benefit analysis. Commonly used to aid comparison of initiatives competing for limited funds.
Discounting:	The process of converting money values that occur in different years to a common year. This is done to convert the dollars in each year to present value terms.
Implicit price deflator for gross domestic product (GDP)	The implicit price deflator for GDP is a price index for all final goods and services produced and is calculated as the ratio of nominal GDP to real GDP. The GDP deflator expresses the extent of price level changes, or inflation, within an economy. The implicit price deflator for GDP is used to convert past, nominal dollar terms to current, real dollar terms in a cash flow analysis.
Internal rate of return (IRR):	The discount rate that makes the net present value equal to zero. Internal rate of return must be greater than or equal to the discount rate for an initiative to be economically justified. The discount rate is also known as the hurdle rate.
Investment criteria:	A set of parameters used by decision-makers to assess or compare initiatives. Investment criteria may include the benefit-cost ratio, net present value, and internal rate of return.
Net present value (NPV):	The combined discounted present value of one or more streams of benefits and costs over the appraisal period. The term ‘net’ denotes that the net present value is calculated as present value of benefits minus the present value of costs.
Nominal dollars	Dollars not adjusted for inflation
Present value of benefits (PVB):	The sum of the discounted benefit streams (cash flows) over the appraisal period.
Present value of costs (PVC):	The sum of the discounted cost streams (cash flows) over the appraisal period.

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GENERAL DISCUSSION AND CONCLUSION

Mozambique tilapia is listed in the top 100 of the world's worst introduced species. All tilapia species are considered pest species in Australia and pose a significant threat to native fish and Australian ecosystems. There is currently no single overall option for the control of tilapia. Eradication attempts using a combination of electrofishing and poison are rarely successful in open waterways and, given their invasive nature, there is a lack of demonstrated broadscale effective control mechanisms for tilapia. Biocontrol, where it is feasible, can be a cost-effective, safe (species-specific) and practical solution for managing invasive species.

The objectives of this project were to review tilapia pathogens and assess their potential as biological control agents (BCAs), to determine the susceptibility of the two tilapia species present in Australian waterways (*O. mossambicus* and *P. mariae*) to tilapia lake virus (TiLV), to demonstrate the efficacy of TiLV as a potential BCA for tilapia in Australia, and to develop a business case to advance the selection and testing of new tilapia biocontrol agents in Australia. The main research question is: are the two tilapia species present in Australian waterways susceptible to TiLV?

The bioprospecting processes, described in Chapter 1, have successfully identified TiLV as the most promising BCA for invasive tilapia in Australia. The susceptibility testing described in Chapter 2 has successfully addressed the main research questions and provided a proof-of-principle demonstration of the susceptibility of two tilapia species presents in Australian waterways to TiLV. These findings have been used to inform the cost-benefit analysis (CBA) and developed a business case to advance the selection and testing of new tilapia biocontrol agents in Australia. The positive benefit-cost ratio of 2.81 to 1 from this project indicates that the proposed investment in tilapia biocontrol research, development, and extension (RD&E) is likely to be worthwhile.

When this project started in 2020, no tilapia pathogens were characterised at that time as potential BCAs, nor was it known if the two species of tilapia present in Australian waterways were susceptible any potential BCAs. The benefit-cost ratio for investment and a business case for tilapia biocontrol RD&E in Australia, was also unknown. As a direct result of this project, we have filled these knowledges gaps, and biocontrol tools (TiLV) and strategies (business case) have been identified for long-term tilapia management. As this was a first- stage research project the outcomes are not yet ready for deployment in the field. However, the business case developed for this project provides a logical framework for proposed investment strategies in tilapia biocontrol RD&E and the pathways to impact. The proposed tilapia biocontrol RD&E investment would be undertaken in six overall stages with stop/go decision points based on the success of various stages to maximise the potential to yield significant impacts. The investment would be realised through the development of a biocontrol program that reduces tilapia biomass and reduces the associated economic, environmental, and social impacts and costs.

The project has coordinated collaboration with Macquarie University (MU) through Project P01-B-005: Proof-of-concept for genetic biocontrol in a vertebrate, which has validated components of the self-stocking incompatible male system (SSIMS) technology in zebra fish model. It has also coordinated with a unique national capability at James Cook University (JCU) for the successful breeding of two species of invasive tilapia, providing a critical supply of healthy fish for the current Project P01-B-003. This collaboration and coordination has also created a key opportunity to develop genetic biocontrol of tilapia (Stage 2B). Specifically, our collaboration with both MU and JCU presents the opportunity for translation of SSIMS technology from the zebra fish model to tilapia as a target species. Integrated viral and genetic biocontrol has been identified by Thresher et al. (2014) as critical for lasting impact, indicating this is an important strategy for tilapia. Built on findings from Project P01-B-005, CSIRO is currently supporting for a Masters student from Deakin University who is characterising genes associated with SSIMS technology in tilapia. This support is to ensure continuity of the project workflow from proof-of-concept of genetic biocontrol in the zebra fish model to translation to tilapia target species. The project has also engaged Dr Nick Whiterod, Nature Glenelg Trust, and Professor Claus Wedekind from University of Lausanne, Switzerland, on Trojan Y chromosome biocontrol approach for tilapia. Along with SSIMS, gene drive, and female lethality, Trojan Y chromosome has

been identified as a genetic biocontrol option for aquatic invasive species (McColl and Sunarto 2020; Wedekind 2019); although this approach would need further investigation since tilapia have both XY and ZW sex chromosomes (Tao et al. 2021).

The successful identification of TiLV as a potentially effective BCA for invasive tilapia, the demonstrated susceptibility of two tilapia species present in Australian waterways to TiLV, the positive CBA of this project, and the business case to advance the selection and testing of new tilapia biocontrol agents in Australia all combine to make a compelling case for investment by key stakeholders at the state and federal levels. The outcomes of this project warrant further discussion to secure coordinated investment to build on these tools and strategies for the integrated viral and genetic biocontrol of invasive tilapia in Australia.

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APPENDICES

APPENDIX 1: BIOPROSPECTING FOR BIOLOGICAL CONTROL AGENTS FOR INVASIVE TILAPIA IN AUSTRALIA*

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APPENDIX 2: EX-ANTE COST-BENEFIT ANALYSIS OF PROPOSED INVESTMENT IN TILAPIA BIOCONTROL RD&E IN AUSTRALIA

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EXECUTIVE SUMMARY

This report provides an independent ex-ante cost-benefit analysis (CBA) of proposed investment in research, development, and extension (RD&E) to advance biological control (biocontrol) as a practical and effective control method for invasive tilapia in Australian waterways. The assessment was undertaken as part of a CSIRO led project titled '*Tilapia biocontrol: prospecting and evaluation*' funded by the Centre for Invasive Species Solutions (project Code P01-B-003).

Based on a study conducted in Queensland published in 2008, it was estimated that the current economic impact costs of tilapia may lie between $1.2 million and $13.6 million per annum (2020/21 dollar terms). If targeted efforts to control tilapia are not undertaken to prevent the future spread of tilapia, the economic costs could increase to over $35.4 million per annum. Further, it is likely that, on a national scale, the impact costs could be significantly higher were tilapia to spread into other key Australian waterways, in particular the Murray-Darling Basin. Without intervention this scenario is considered highly likely.

There is currently no single overall option for the control of tilapia in Australia. Ongoing RD&E is being funded and carried out by various research organisations to refine detection and control methods for tilapia. Biocontrol is thought to be a potentially cost-effective and practical solution for the management for invasive fish species, including tilapia.

The primary objective of the ex-ante CBA was to assess whether the investment (the total costs of the RD&E addressing the advancement of new biocontrol agents (BCAs) to manage tilapia in Australia) would be paid for by the estimated potential benefits of the proposed BCA(s).

Tilapia bioprospecting Project P01-B-003 has, to date, successfully identified three potential tilapia biocontrol candidates categorised as tentatively worthwhile for further investigation. TiLV currently is considered the most promising potential biocontrol candidate and was categorised as 'worthwhile for active further investigation'. CSIRO already have imported the virus and are currently developing the capability to work with TiLV in a laboratory setting.

The CBA was set within a staged risk management framework of investment. The approach included identifying and describing the six stages of RD&E for the proposed tilapia biocontrol investment, RD&E objectives, planned activities, expected outputs and outcomes. Potential impacts associated with the expected outcomes then were identified and categorised as economic, environmental, and

social impacts. The primary impact is expected to be a net reduction in the total annual impact costs of tilapia to the Australian community and economy through a reduction in tilapia biomass.

Valuation of the primary impact involved making several uncertain assumptions as a number of key relationships along the pathways to impact were unknown. The total expected RD&E investment was estimated at $18.69 million (present value terms). The investment was estimated to produce total expected net benefits of $52.53 million (present value terms). This gave a net present value of $33.84 million, a benefit cost-ratio of 2.81 to 1, an internal rate of return of 9.3% and a modified internal rate of return of 7.1%.

Care should be taken when interpreting the results of the ex-ante analysis. It is important to note that the expected release and subsequent impact of a new tilapia BCA, such as TiLV, would not occur until approximately 22 years after the first year of investment in Project P01-B-003. Given that the investment criteria became positive between 25 and 30 years after the first year of investment, this indicates that implementation of a new tilapia BCA would create benefits sufficient to cover the costs of the proposed tilapia biocontrol RD&E investment within five to ten years of release of the BCA.

Further, it is important to remember that the ex-ante analysis was conducted within a risk management framework and that the results are expected values. This means that it is theoretically possible for the total proposed investment in tilapia biocontrol to be made (approximately $45.6 million in nominal dollars) and for there to be no benefits realised. That is, the new agent is released and is unsuccessful in reducing tilapia impact costs. However, the risk of this is very minimal as the proposed tilapia biocontrol RD&E investment has been planned as a staged investment with a number of key stop/ go points that would enable funding partners, researchers and other stakeholders to adjust and/ or redirect the RD&E to alternative and more promising directions. Also, the knowledge generated through Stages 1 to 3 are likely to contribute to increased scientific knowledge and research capacity associated with management of pest tilapia in Australia.

The positive investment criteria suggest that the initial investments (Stages 1 to 5) would be worthwhile given the estimates made of the current and future potential impact and control costs of tilapia in Australia, likely pathways to impact for proposed new BCAs, the RD&E investment and associated timelines required, and the risks involved. Further, the proposed investment can be staged conditionally (stop/go points) so that, as the investment proceeds along a particular pathway, the direction of the RD&E could be changed according to any past success and the availability of any new information available. This may avoid or minimise any potential losses and maximise the chances of significant impacts being delivered.

The successful identification of BCA candidates and the positive ex-ante CBA results from Project P01-B-003 indicate that the proposed investment in tilapia biocontrol RD&E is likely to be worthwhile and should be viewed favourably by the Centre for Invasive Species Solutions, potential funding partners, and other tilapia biocontrol and/or management stakeholders.

However, to strengthen any future analysis of the potential costs and benefits of tilapia biocontrol in Australia, it is strongly recommended that any future RD&E include:

1. identification and estimation of the current and likely future impact and control costs associated with tilapia in Australia
2. work that demonstrates and quantifies the relationship between tilapia and the biophysical impacts to which tilapia are assumed to contribute
3. quantification of the potential relationship between reductions in tilapia biomass and the drivers of key medium- and long-term impacts of biocontrol.

1. INTRODUCTION

This report provides an independent ex-ante cost-benefit analysis (CBA) of proposed investment in research, development and extension (RD&E) to advance biological control (biocontrol) as a practical and effective control method for invasive tilapia in Australian waterways. The assessment was undertaken as part of a CSIRO led project titled '*Tilapia biocontrol: prospecting and evaluation*' funded by the Centre for Invasive Species Solutions (CISS, project Code P01-B-003).

The purpose of the ex-ante analysis was to support the business case for further investment to deliver improved control of tilapia through biocontrol. In September 2020, Agtrans Research contracted to carry out the CBA.

2. TERMS OF REFERENCE

The specific terms of reference (ToR) for the ex-ante CBA are described below.

4. Complete a desktop review of available information on tilapia, its distribution, and its economic, social and environmental impacts in Australia.
5. Review past and current measures undertaken that address tilapia control.
6. Review the likely future spread and impact of tilapia on Australian waterways given current control measures.
7. Value the expected net economic benefits of the proposed biocontrol investment, taking into account the projected investment costs (provided as part of the Business Case development, see ToR 6 above), timelines and risk factors including the projected reduction in impacts of tilapia due to successful biocontrol of the species.
8. Estimate a set of investment criteria including present value of benefits, present value of costs, net present value, benefit-cost ratio, internal rate of return and modified internal rate of return.
9. Carry out sensitivity analyses that demonstrate the change in investment criteria with changes in key assumptions including current and future tilapia costs with and without successful biocontrol, and changes in levels of potential risk factors.
10. Provide discussion and conclusions on the economic merits of the RD&E investment.

3. BACKGROUND

Tilapia were first introduced to Australia in the 1970s as an ornamental fish (Queensland Government, 2021). Tilapia are listed in the top 100 of the world's worst introduced species. All tilapia species are considered pest species in Australia and pose a significant threat to native fish and Australian ecosystems.

There is currently no single overall option for the control of tilapia in Australia. Ongoing RD&E is being funded and carried out by various research organisations to refine detection and control methods for tilapia. Biocontrol is thought to be a potentially cost-effective and practical solution for the management for invasive fish species, including tilapia. For example, significant RD&E investment already has been made to advance cyprinid herpes virus 3 (CyHV-3) as a biocontrol agent (BCA) for the control of invasive European carp in Australian waterways. CyHV-3 currently is under review by the Australian Government for approval for use in Australia. For more information, see: https://carp.gov.au/.

Australia previously has had success using biocontrol to reduce populations of invasive European rabbits through the release of the myxoma virus (1950) and variants of rabbit haemorrhagic disease virus (RHDV) (1995 and 2017). Rabbit biocontrol had significant positive impacts on Australian ecosystems and agricultural industries, providing estimated economic benefits of $70 billion over the last 60 years (Cooke, Chudleigh, Simpson, & Saunders, 2013).

A current RD&E project, led by CSIRO, titled '*Tilapia biocontrol: prospecting and evaluation*', was funded by the Centre for Invasive Species Solutions (CISS) to conduct a review of tilapia pathogens and assess their potential as BCAs (a process known as bioprospecting). As part of this review project, CISS required an ex-ante CBA to assess the potential benefits of investment in the proposed tilapia BCAs. The findings of the CBA then would support a business case for further RD&E investment to deliver improved control of tilapia through biocontrol.

4. METHOD

The primary objective of the ex-ante analysis was to assess whether the investment (the total costs of the RD&E addressing the advancement of new BCAs to manage tilapia in Australia) would be paid for by the estimated potential benefits of the proposed BCA(s). The primary benefit is expected to be a net reduction in the total annual impact costs of tilapia to the Australian community and economy.

The ex-ante CBA followed general economic evaluation guidelines that are now well entrenched within the Australian primary industry research sector including Research and Development Corporations, Cooperative Research Centres (CRCs), State Departments of Agriculture, and some universities. The approach includes both qualitative and quantitative descriptions that are in accord with the impact assessment guidelines of the Council of Rural Research and Development Corporations (CRRDC) (CRRDC, 2018).

The CBA was set within a staged risk management framework of investment. The approach included identifying and describing the current and proposed tilapia biocontrol investment and its objectives, planned activities, expected outputs and outcomes. Potential impacts associated with the expected outcomes then were identified and categorised as economic, environmental, and social impacts.

It is axiomatic that successful biocontrol RD&E typically requires significant and long-term investment and can be considered somewhat risky. One of the principal considerations in analysing and valuing impacts from biocontrol RD&E is how the counterfactual is defined, that is, what would most likely occur without the proposed investment. A second principal consideration that has to be accommodated in the analysis is how risk is represented. All key data and assumptions used in the valuation of impacts are described and reported.

It is anticipated that the total tilapia biocontrol RD&E investment costs will be staged (go/no go decisions at particular stages of the investment, depending on progress and findings) in order to minimise investment risk. Also, risk factors were built into the analysis to ensure output, outcome and impact risks are taken into account so that the likely benefits from the investment are realistic and not overestimated.

The CBA focused on identifying and valuing economic impacts with some consideration given to identifying and qualitatively describing any environmental and social impacts.

5. DESKTOP ECONOMIC LITERATURE REVIEW

5.1 OVERVIEW OF POTENTIAL TILAPIA IMPACTS

There have been three species of tilapia introduced to Australia, the Mozambique tilapia (*Oreochromis mossambicus*), the black mangrove cichlid (*Pelmatolapia mariae,* formerly *Tilapia mariae*), and the redbelly tilapia (*Coptodon zillii*) (Native Fish Australia, n.d.). Characteristics that allow tilapia to establish in new areas include (NSW Department of Industry, n.d.):

- highly efficient breeding strategies including mouth brooding
- simple food requirements (feeding on a wide variety of plant and animal matter)
- flexible habitat preferences (including the ability to breed in both fresh and brackish water).

Redbelly tilapia were reported near Perth in Western Australia (WA) in 1975 but were subsequently eradicated by the state fisheries department (NSW Department of Industry, n.d.). However, feral populations of *O. mossambicus* and *T. mariae* spread and now are widely distributed in tropical north-eastern Queensland (QLD), with *O. mossambicus* also occurring in south-eastern QLD and river

systems of WA (Russell et al., 2010) and a population of *T. mariae* persisting in Hazelwood in Victoria (Centre for Invasive Species Solutions (CISS), 2021).

Populations of feral tilapia in Australia have continued to increase and, in 2014, the first established population of tilapia (*O. mossambicus*) in New South Wales (NSW) was confirmed at Cudgen Lake on the state's far north coast. Populations of tilapia in southern QLD also have been reported as little as three kilometres from the Murray Darling Basin (MDB) (NSW Department of Industry, n.d.).

Tilapia impacts have been recorded and reported in a number of locations in Australia and overseas. The key impacts recorded include major declines in commercial and traditional fisheries, fish extinctions, destruction of beds of macrophytes (large aquatic plants) and declines in water quality. Some of the direct impacts of tilapia in Australian waterways include (Hutchison, Sarac, & Norris, 2011):

- Impacts on native fish and other biota by:
    a. direct predation by tilapia
    b. competition for resources (food, habitat)
    c. destruction of macrophytes and other aquatic plants used as breeding or nursery habitat by native species
    d. habitat disturbance
    e. transmission of diseases and parasites
    f. competitive exclusion of native fish from favourable habitat by tilapia's aggressive behaviour
- Reduction in water quality, including potable water supplies, through:
    a. increase of blue-green algal blooms (through resuspension of nutrients)
    b. winter die-offs of tilapia (polluting waterways)
    c. undermining riverbanks due to destruction of river plants and nesting behaviour.

The direct impacts of tilapia are largely environmental; however, these direct environmental impacts contribute to a number of indirect or secondary economic and social impacts, including potentially (CISS, 2021c):

- Increased water treatment/ water management and infrastructure costs [economic]
- Reduced amenity for recreational fishers [economic and/or social]
- Reduced productivity/ profitability for commercial fisheries that rely on species and/or ecosystems negatively affected by tilapia (e.g. barramundi) [economic]
- Reduced public amenity of tilapia affected waterways [social and/or economic]
- Increased management and control costs, likely incurred by landholders, land management groups, and/or government, to mitigate tilapia impacts [economic].

As well as the likely negative impacts associated with tilapia in Australia, it is important to recognise there may be some positive impacts associated with the presence of tilapia in Australia. For example, tilapia are valued as an ornamental fish species and can be caught by recreational fishers and/or used for human or animal consumption. However, the potential positive impacts are likely to be minor relative to the recognised negative impacts of invasive species of tilapia.

5.2 EXISTING CURRENT CONTROL METHODS

To minimise the potential environmental, social and economic impacts of introduced tilapia, current management principles include early detection of new populations, minimising the ability of the species to establish and/or spread to new environments, protecting native biodiversity, and conserving natural resources and associated recreational and commercial fisheries.

There are a range of control measures currently available for use on tilapia, but most are situation specific. Management tools include containment and/or exclusion, physical removal through netting, electrofishing, and angling/ line fishing, draining of waterbodies, and chemical removal (poisons) (CISS, 2021a). However, in the majority of situations and in the absence of effective ongoing management, unless the entire population and any possible source of reintroduction are removed, the highly flexible reproductive capacity of tilapia will see the population quickly return to original numbers (CISS, 2021a). There is currently no single overall option for the control of tilapia.

Targeted education campaigns, run by government departments, research organisation, and other natural resources/environmental groups, actively highlight the potential damage caused by introduced tilapia to the natural environment and work to educate the public on what the fish look like and what to do if one is found. Community involvement in protecting and conserving local waterways is, to-date, the most effective control method in stopping the further spread of the two species of tilapia currently established in Australia (CISS, 2021b).

5.2 ESTIMATED IMPACT COSTS OF TILAPIA IN AUSTRALIA

Total annual impact costs of tilapia in Australian waterways include both (a) costs associated with tilapia species' negative impacts on native fish and ecosystems, including potential costs or losses to industries that rely on those ecosystems such as tourism and recreational fishing, and (b) tilapia management and control costs.

There have been several past studies that have attempted to identify and estimate, in monetary terms, the impact costs of a number of different invasive species in Australia including:

- Bomford and Hart (2002): the study concluded that the agricultural costs due to the major introduced vertebrate pests in Australia were difficult to accurately estimate due to a shortage of reliable data but totalled at least $420 million/year for direct short-term losses. Longer-term losses are also likely to be large. Further, landholders and governments in Australia spend over $60 million/year controlling introduced vertebrate pests. In addition to the resources spent on control an additional cost is the value of lost opportunities from alternative investment of this expenditure. Governments also spend around $20 million/year on research to control vertebrate pest species.

- McLeod (2004): the study investigated the impact costs for 11 major introduced vertebrate pests of Australian agricultural industries and the environment. The 2004 study reported total estimated annual impact costs of $720 million for Australia for the 11 invasive species included.

- Gong, Sinden, Braysher and Jones (2009): the study used an economic welfare framework and estimated that the total annual economic impact, comprising agricultural losses and expenditures on management, administration and research, at $743.5 million.

- McLeod (2016): this second study by McLeod was funded to update the impact costs estimates reported in Gong et al. (2009). The updated study reported estimated annual Australia pest animal impact costs (including production losses and invasive species management costs) of between $416.2 and $797.3 million (average of $596.6 million).

- Hoffmann and Broadhurst (2016): the 2016 study, published by CSIRO titled '*The economic cost of managing invasive species in Australia*') included vertebrate, invertebrate and weed pest species. The study estimated that, in 2001–02, total national expenditure on invasive species was $2.31 billion ($3.03 billion adjusted to 2012 values), rising to $3.77 billion in

2011–12. For 2001–02 and 2011–12, these total expenditure figures equated to $123 and $197 per person per year respectively, as well as 0.32 and 0.29% of Gross Domestic Product (GDP) respectively.

- Bradshaw et al. (2021): this was a detailed analysis of reported costs associated with invasive species in Australia since the 1960s based on a recently published database known as *InvaCost*. Bradshaw et al. (2021) analysed 2,078 unique cost entries and supplementary information and found that, since 1960, Australia has spent or incurred losses of a total of at least US$298.58 billion (2017-dollar terms, all cost data) or approximately AUD$390 billion from invasive species (2017 average exchange rate).

However, none of these Australian studies included an estimate of the impact costs of tilapia. Only one study was found that attempted to estimate the likely impact costs of tilapia in Australia. Greiner & Gregg (2008) undertook a study for the Australian Centre for Tropical Freshwater Research at James Cook Univeristy in Townsville. The purpose of their report was to provide an attempt at estimating the conomic impact of tilapia in north Queensland and was based on a desktop review of existing information and a limited empirical investigation involving key waterway managers and recreational fishers.

Greiner & Gregg (2008) reported that The direct costs associated with monitoring, management and prevention of tilapia amounted to nearly $900,000 during 2006/07. Further, based on a total economic benefit framework and a series of assumptions, Greiner & Gregg reported some “least cost” and “highest cost” cost estimates for various tilapia impact cost items. The “highest cost” estimate was based on the assumption that tilapia become widespread, that there is an intensified effort to control tilapia, and that key commercial fisheries are severly impacted/obliterated by feral tilapia.

Figure B1 shows the current and future economic cost estimates for tilapia in northern Queensland reproduced from Greiner & Gregg (2008).

Costs/Uses/Values	Current	Hypothetical Future Economic Impact	
	Observed ($ per year--2006/07)	Least Cost ($ per year)	Highest Cost ($ per year)
Direct costs			
Monitoring	$237,939	$0 [4]	>$1 million [5]
Management	$90,000	$0 [4]	>$1 million [5]
Prevention	$561,227	$0 [4]	>$1 million [5]
Cost to direct use value of waterways			
Human consumption of water	$0	$0 [6]	$10 million ++ [7]
Irrigation water	+ [1]	$0 [6]	+ [8]
Recreational fishing	+ [2]	$0 [6]	$3 million [9]
Other recreation	+ [2]	$0 [6]	$3 million [10]
Amenity	+ [2]	$0 [6]	+ [11]
Cost to indirect use values of waterways			
Commercial fishing	$0	$0 [12]	$16 million [3]
Loss of non-use values associated with waterways (ecology, water quality)	no evidence	$0 [13]	$ 1 million ++ [14]

Figure B1: Estimated economic impact of tilapia in the context of northern Queensland (Source: pg. 38 Greiner & Gregg (2008))

Based on the current and hypothetical future economic impact of tilapia, the assumptions made in the Greiner & Gregg (2008) study, and adjusting to 2020/21 dollar terms[5], it is possible that the economic impact costs of tilapia in QLD currently lie between $1.2 million and $13.6 million per annum (taking into account only annualised costs and excluding the estimated future commercial fishing costs where the hypothesised negative impacts have not yet occurred). Further, if targeted efforts to control tilapia are not undertaken and key commercial QLD fisheries are essentially destroyed by the future spread of tilapia, the economic costs of tilapia could increase to over $35.4 million per annum in 2020/21 dollar terms.

Further, it is likely that these data underestimate or exclude values for a number of environmental and social impact costs associated with tilapia. Also, the costs were estimated for QLD only. On a national scale, the impact costs could be significantly higher if tilapia are allowed to spread into other key Australian waterways, particulalry the Murray-Darling Basin that stretches across five Australian states/territories from QLD to South Australia.

6. LOGICAL FRAMEWORK FOR THE PROPOSED RD&E INVESTMENT

A logical framework for analysing the current and proposed tilapia biocontrol RD&E investment was developed. This required the evaluation team to understand the current (Project P01-B-003) and intended future investments and their likely outputs, outcomes and impacts. The logical framework was developed with input from the CSIRO P01-B-003 project team, including the draft review of candidate tilapia biocontrol pathogens.

Tilapia lake virus (TiLV) was identified as the primary candidate for further research under bioprospecting Project P01-B-003. However, a second biocontrol candidate, tilapia parvovirus (TiPV) also was considered tentatively worthwhile for further investigation.

It was understood that the required RD&E to advance either BCA candidate in Australia would likely be similar but independent (that is, separate investment would need to be made to progress each candidate agent). Further, the proposed tilapia biocontrol RD&E investment, including the initial bioprospecting investment (Project P01-B-003) would be undertaken in six overall stages with stop/go decision points based on the success of various stages. The following briefly describes each of the likely RD&E stages required to progress candidate tilapia BCAs (e.g. TiLV or TiPV).

- Stage 1: Bioprospecting and Evaluation

CISS Project P01-B-003: *Tilapia biocontrol: prospecting and evaluation* represents Stage 1 of the RD&E investment required to identify and advance the selection of a new BCA for tilapia in Australia. To progress any tilapia BCA candidates identified as worthwhile for further investigation, it will be essential to formally and thoroughly evaluate the agent.

As of August 2021, TiLV had been selected for active further investigation and additional research already had commenced (see Stage 2A below).

- Stage 2A: Efficacy Testing

Where, based on the findings of Stage 1, the EIC and individual jurisdictions (States and Territory) support additional investment to progress tilapia biocontrol RD&E, the next stage of the project (Stage 2) would be testing the efficacy (virus virulence and transmission). Further, alongside the efficacy testing, RD&E is required to systematically assess the possibility of interfering endemic viruses and also the possibility of reassortments (Chaput et al., 2020). This would involve, for example, meta-transcriptomic analyses (Turnbull et al., 2020), of other viruses in Australian tilapia populations.

CSIRO already have imported TiLV and are developing the capability to work with it in a laboratory setting. The project team will commence testing of TiLV's susceptibility in tilapia sourced from QLD

[5] Cost data in 2006/07 dollar terms adjusted using the Australian implicit price deflator for gross domestic product, multiplier of x1.3605 (Australian Bureau of Statistics (ABS), 2021)

waters in January 2022. Additional and independent investment in similar RD&E would be required should stakeholders choose also to progress TiPV as a potential tilapia BCA.

- Stage 2B: RD&E on Complementary Tilapia Control Methods

Further work including the identification of other broad-scale control measures, such as genetic control, to complement the virus would need to be considered. A number of genetic technology options for broad-scale control may be applicable for tilapia, and some are currently under investigation. These include genetic biocontrol such as 'gene drives' and/or self-stocking incompatible male systems (CISS Project P01-B-005). Australia is currently investing in RD&E to investigate these broadly applicable technologies for managing invasive fish species. A prerequisite for genetic biocontrol approaches is also a thorough assessment of the genetic makeup and diversity of Australian tilapia (population genomics analyses). This is important as there already is significant evidence of hybridisation occurring among wild populations.

It would be beneficial if the findings of any successful RD&E into complementary control measures could be built into Stage 4 (if available) and Stage 6 to ensure the greatest control can be achieved (i.e. optimal and maximum reduction of tilapia biomass in Australian waterways).

- Stage 3: Safety Testing

Stage 3 would involve testing the safety (susceptibility of non-target species) of TiLV as a BCA. If successful, the data generated from the efficacy (Stage 2) and safety (Stage 3) trials on the virus then will provide input to development of an epidemiological model for TiLV.

The findings from the Stage 2 and 3 RD&E investment represent an important stop/go decision point for any future investment to further advance a new BCA toward release as a practical tool for tilapia control in Australia.

Note: if resources committed to tilapia biocontrol RD&E in the future permitted, it would be possible that Stage 3 could be undertaken concurrently with Stage 2 (A and B). This would reduce the overall timeframe for the proposed tilapia biocontrol RD&E.

- Stage 4: Planning and Modelling Optimal Release

If the findings from Stage 2 and 3 RD&E indicate that the proposed BCA (e.g. TiLV) could be used as a safe and effective tilapia BCA in Australia, the epidemiological model then will be used as a key part of further RD&E required to determine the optimal release strategy, or strategies, for the virus (Stage 4). Understanding and optimising potential release strategies will provide critical input for planning, coordinating, and costing any actual future release of a new BCA, pending necessary approvals. It is likely that work conducted in Stage 4 would be predominantly QLD-centric and would be modelled on work associated with European carp undertaken as part of the recent National Carp Control Program (NCCP).

- Stage 5: Other Assessments and Regulatory Approvals

Social and ecological risk assessments will be needed to support an application to release a new BCA against tilapia in Australia. Application for release of any such tilapia BCA would be made through the Commonwealth Department of Agriculture, Water, and the Environment (DAWE) and Australian Pesticide and Veterinary Medicines Authority (APVMA).

Applications for regulatory approval to release a new tilapia BCA in Australia would rely heavily on data and information generated through investment in Stages 1 to 4. Further, the outcomes of the required applications (e.g. approval, approval with conditions, approval in principle with additional information/ data required, and/or non-approval) represent another important stop/go point for further investment in tilapia biocontrol.

- Stage 6: Nationally Coordinated Release and Clean-up

If a new tilapia BCA is approved for release in Australia, a structured collaborative program of release strategies and planning and coordination of any clean-up will be developed. This stage of investment (Stage 6) also will address bioethical issues and public acceptance of viral biocontrol of tilapia. This stage also will need to include investment for activities to support effective and efficient biocontrol release such as post-release monitoring and additional RD&E focused on the development of new virus variants in subsequent years as fish develop natural immunity/ resistance to the BCA.

To date, a viral BCA has never been used or approved for use against aquatic invasive species in Australia[6]. Therefore, public and government approval is considered a major concern. Australia has very strong legislative mechanisms for approval of BCA including the Commonwealth Biological Control Act 1984 along with Acts in the states and territories as well as numerous international conventions. The legislation requires procedures to demonstrate that:

11. There is an urgent need to control the pest
12. The BCA will likely reduce the impacts caused by the invasive species
13. The release of the BCA will not negatively affect the environment and the non-target species sharing the waterways.

7. PROPOSED INVESTMENT IN TILAPIA BIOCONTROL RD&E

The initial RD&E investments, including current Project P01-B-003, required to advance a tilapia BCA (either TiLV or TiPV) are shown in Table B1 (below) according to the RD&E stages described in Section 6.

Table B1: Current and Proposed Investment in RD&E stages 1–5 for Tilapia Biocontrol in Australia

Year ended 30 June	Tilapia Biocontrol RD&E Stage			Totals
	Stage 1[(a)]	Stage 2–4[(b)]	Stage 5[(c)]	
2021	302,444	0	0	302,444
2022	302,444	0	0	302,444
2023	0	3,000,000	0	3,000,000
2024	0	3,000,000	0	3,000,000
2025	0	3,000,000	0	3,000,000
2026	0	3,000,000	0	3,000,000
2027	0	3,000,000	0	3,000,000
2028	0	3,000,000	0	3,000,000
2029	0	3,000,000	0	3,000,000
2030	0	3,000,000	0	3,000,000
2031	0	3,000,000	0	3,000,000
2032	0	3,000,000	0	3,000,000
2033	0	0	3,000,000	3,000,000
2034	0	0	3,000,000	3,000,000
2035	0	0	3,000,000	3,000,000
2036	0	0	3,000,000	3,000,000
2037	0	0	3,000,000	3,000,000
Totals	604,888	30,000,000	15,000,000	45,604,888

(a) Based on total annual RD&E costs (cash and in-kind) for tilapia bioprospecting project P01-B-003 funded by CISS

(b) Estimated RD&E cost of $3 million per annum over 10 years for Stages 2–4 based on the CISS bioprospecting project and RD&E conducted for the NCCP

(c) Based on funding of $15 million over five years for the NCCP

[6] It is worth noting that cyprinid herpes virus 3 (CyHV-3) as a BCA to control feral European carp in Australian waterways currently is under review by the Australian Government following the National Carp Control Program (NCCP) undertaken between 2016 and 2021. More information can be found at: https://carp.gov.au/

For the purpose of the current analysis, investment for Stage 6 (nationally coordinated BCA release and clean-up) has been excluded. The potential costs associated with Stage 6 are highly uncertain and dependent on a wide range of factors including:

- efficacy of the proposed BCA
- regulatory requirements
- future spread and density of feral tilapia
- optimal/viable release strategies.

As a result, the total RD&E investment costs presented in Table B1 are likely to somewhat underestimate the total investment costs required to advance and implement a new tilapia BCA in Australia.

8. POTENTIAL IMPACTS OF TILAPIA BIOCONTROL

8.1 TRIPLE BOTTOM LINE IMPACTS

The overarching goal of any tilapia BCA would be to reduce the total biomass of tilapia in Australian waterways. This, in turn, is expected to result in a net reduction in tilapia impact and control costs (for a description of tilapia impacts see Section 5.1).

Table B2 summarises the broad potential economic, environmental and social impacts (triple bottom line impact types) that may be achieved through future investment in tilapia biocontrol RD&E.

Table B2: Potential Triple Bottom Line Impact Types for Investment in Tilapia Biocontrol

Economic	**Environmental**	**Social**
Reduced and/or avoided costs associated with water treatment/water management/water infrastructure Increased expenditure by recreational fishers Maintained or enhanced productivity/profitability for commercial fisheries in tilapia affected regions Reduced and/or avoided tilapia control/management costs Increased net income for some regional Australian tourism sectors	Improved biodiversity through maintained or enhanced populations of native fish and other biota Improved health and wellbeing for native fish species through reduced competition, predation and disease risks Improved ecosystem health through reduced/avoided risk of algal blooms, tilapia die-offs and riverbank damage	Improved amenity for recreational fishers Improved amenity for public users of tilapia affected waterways Increased scientific knowledge and research capacity with respect to biocontrol of invasive fish in Australia

8.2 PATHWAYS TO IMPACTS

A simplified description of the pathways to impact for the proposed investment in tilapia biocontrol RD&E is shown in Figure B2.

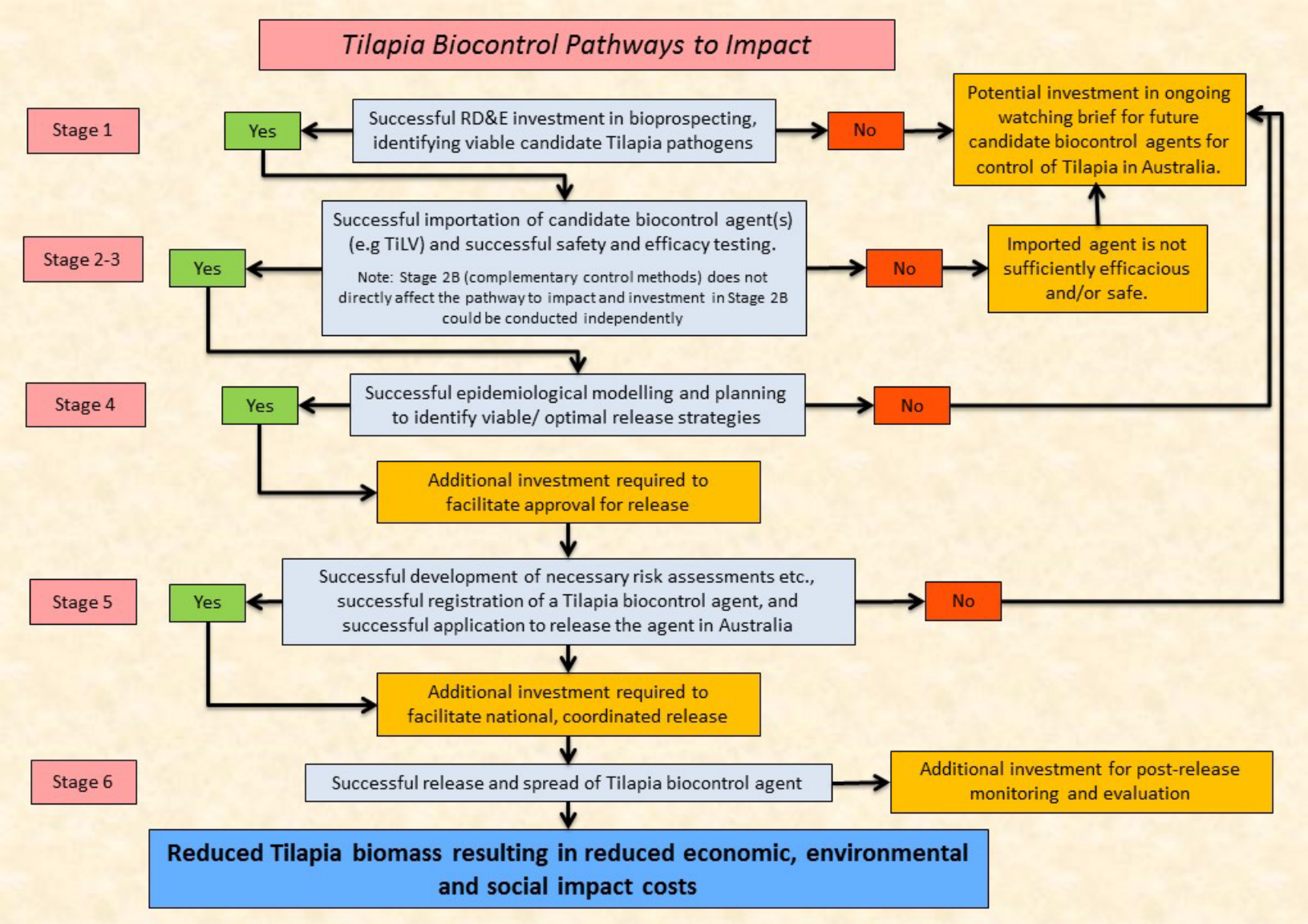

Figure B2: Likely pathways to impact for tilapia biocontrol RD&E

9. VALUATION OF POTENTIAL IMPACTS

9.1 IMPACTS NOT VALUED

Not all of the potential impacts of the proposed investment in tilapia biocontrol RD&E identified in Table B2 could be valued within the scope of the current ex-ante analysis. In particular, environmental and social impacts can be difficult to estimate in monetary terms and often require the application of complex and resource intensive non-market economic valuation methods.

The following impacts were not valued because of a lack of data/evidence on which to base credible assumptions, uncertainty about the linkages between the initial investment and the expected impacts, available time/resources for the current analysis and/or the impact was considered to be minor relative to the impact(s) valued.

Environmental impacts not valued included:

- Improved biodiversity through maintained or enhanced populations of native fish and other biota
- Improved health and wellbeing for native fish species through reduced competition, predation and disease risks
- Improved ecosystem health through reduced/avoided risk of algal blooms, tilapia die-offs and riverbank damage.

Social impacts not valued included:

- improved amenity for recreational fishers[7]
- improved amenity for public users of tilapia affected waterways
- increased scientific knowledge and research capacity with respect to biocontrol of invasive fish in Australia.

9.2 IMPACTS VALUED

The primary economic impact valued was the potential net reduction in tilapia impact and controls costs associated with reduced tilapia biomass from implementation of a new tilapia BCA.

Valuation of the impact involved making several uncertain assumptions as a number of key relationships/variables along the pathways to impact were unknown. Specifically, the following relationships/variables are currently unknown:

1. The potential reduction in tilapia biomass through use of a given candidate BCA
2. The reduction in tilapia biomass through implementation of a new BCA, and the resulting change in tilapia impact and control costs in Australia
3. The probability of success of each stage of the proposed tilapia biocontrol RD&E investment.

The impact was valued for TiLV as the selected BCA. This was because TiLV was identified as the most promising tilapia biocontrol candidate and already has been imported to Australia for initial efficacy testing. However, a similar valuation framework also would apply to TiPV. Other than the RD&E time frames, it is considered likely that the total RD&E investment costs for TiPV would be similar to those for TiLV.

[7] The value of improved amenity for recreational fishers may be partially captured through valuation of a related, economic impact: increased expenditure by recreational fishers.

9.3 SUMMARY OF ASSUMPTIONS

The specific assumptions used to value the primary economic impact, a net reduction in tilapia impact and control costs, are described in Table B3.

Table B3: Impact Valuation Assumptions

Variable	Assumption	Source/Notes
WITHOUT investment in tilapia biocontrol RD&E (Counterfactual)		
Estimated total average annual tilapia impact and control costs	$13.6 million p.a. in 2020/21	2020/21 dollar terms Based on Greiner and Gregg (2008) – see Section 5.2
Maximum potential annual impact and control costs for tilapia	$88.5 million p.a. Based on maximum annual impact and control costs in Qld of $35.4 million in 2020/21 dollar terms based on Greiner and Gregg (2008). The maximum total Qld costs then were multiplied by a factor of x2.5	The cost multiplier (x2.5) was applied to accommodate the likely future spread tilapia to the Murray-Darling Basin without intervention, resulting in a significant increase in tilapia impact and control costs in Australia
Change in estimated total average annual impact and control costs over time without significant intervention	Increasing linearly from initial value of $13.6 million p.a. to $88.5 million p.a. over the next 20 years	Analyst assumption
WITH tilapia biocontrol RD&E investment – TiLV		
Stage 1 total RD&E costs	$302,444 p.a. for two years (2020/21 and 2021/22)	See Table B1
Probability of Stage 1 success	100%	Based on identification of TiLV and TiPV as potential candidate BCAs through the review conducted under P01-B-003
Stage 2–4 total RD&E costs	$3 million p.a. over 10-years (2022/23 to 2031/32)	See Table B1
Probability of Stage 2–4 funding and RD&E success (agent found to be safe and efficacious)	70%	Analyst assumption – based on CSIRO importing TiLV for further safety and efficacy testing
Stage 5 total RD&E costs	$15 million over 5-years (2032/33 to 2036/37)	See Table B1
Probability of Stage 5 funding and success (given successful Stage 1–4 RD&E)	50%	Analyst assumption
Estimated reduction in tilapia biomass from nationally coordinated release of TiLV	50%	Based on an experimental challenge with TiLV in Thailand, in which mortalities of 48.89% and 77.78% were observed in *O. mossambicus* (Agus Sunarto, CSIRO, pers. comm., 2021)

Variable	Assumption	Source/Notes
Estimated reduction in total annual impact and control costs from reduced tilapia biomass after release of TiLV	50%	Analyst assumption – assumes a one-to-one relationship between tilapia biomass and tilapia impact and control costs
Year of TiLV release and first year of impact (reduction in tilapia biomass)	2042/43	Analyst assumption based on an additional 5-years after the initial Stage 1–5 RD&E investment for government approvals, planning and national coordination and release. Also, mathematical modelling estimated that the population of Nile tilapia decreased to 12% of the initial population size of 30 fish after 16 days post TiLV infection (Agus Sunarto, CSIRO, pers. comm., 2021)
Period of maximum impact	10 years, after which tilapia biomass and associated impacts will increase to 70% of pre-release levels over a period of 20 years because of development of host resistance	Analyst assumption – it is unknown whether tilapia populations are susceptible or resistant to the virus; however, for invasive carp, it was predicted that the development of host resistance might take decades to impact on CyHV-3 field effectiveness (Agus Sunarto, CSIRO, pers. comm., 2021)
Probability of impact occurring	80%	Allows for uncertainty regarding the field efficacy of TiLV and exogenous factors that may affect realisation of impact (e.g. climate)

10. RESULTS

All benefit and cost cash flows were expressed in 2020/21 dollar terms using the Implicit Price Deflator for Gross Domestic Product (ABS, 2021) and were discounted to 2020/21 using a discount rate of 5% as required by the CRRDC guidelines (CRRDC, 2018).

To accommodate the relatively long time frames associated with biocontrol RD&E in Australia, the ex-ante analysis ran for the length of the proposed RD&E investment plus 50 years from the first year of investment in project P01-B-003 (2020/21) (Stage 1 of the overall proposed investment in tilapia biocontrol RD&E).

Given the inclusion of risk factors associated with the investment and success of the proposed future stages of tilapia biocontrol RD&E, the results reported are expected values.

10.1 INVESTMENT CRITERIA

Table B4 shows the investment criteria estimated for different periods of expected benefits for the total expected (risk adjusted) investment in tilapia biocontrol.

Table B4: Investment Criteria for Total Proposed Investment in Tilapia Biocontrol

Investment criteria	**Years after First Year of Investment (2020/2021)**										
	0	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**
Present Value of Benefits ($m)	0.00	0.00	0.00	0.00	0.00	11.26	22.58	31.82	39.82	46.68	52.53
Present Value of Costs ($m)	0.30	7.68	14.81	18.21	18.69	18.69	18.69	18.69	18.69	18.69	18.69
Net Present Value ($m)	-0.30	-7.68	-14.81	-18.21	-18.69	-7.43	3.89	13.12	21.12	27.99	33.84
Benefit-Cost Ratio	0.00	0.00	0.00	0.00	0.00	0.60	1.21	1.70	2.13	2.50	2.81
Internal Rate of Return (%)	negative	negative	negative	negative	negative	1.76	6.07	7.73	8.58	9.06	9.34
Modified Internal Rate of Return (%)	negative	negative	negative	negative	negative	2.89	5.66	6.40	6.85	7.03	7.09

The annual undiscounted total estimated expected benefit and cost cash flows for the total RD&E investment plus 50 years from the first year of investment in Project P01-B-003 are shown in Figure B3.

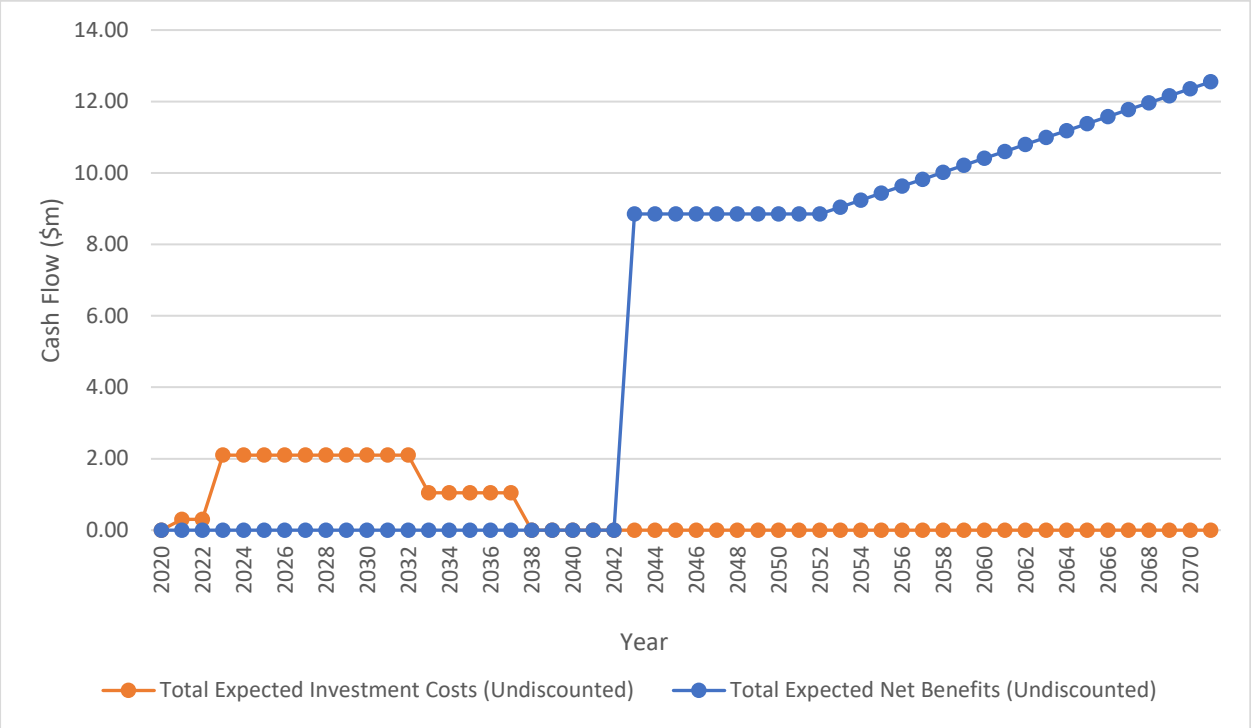

Figure B3: Annual Undiscounted Total Expected Investment Cost and Total Expected Benefit Cash Flows

10.2 SENSITIVITY ANALYSES

Sensitivity analyses were carried out on variables that were considered key drivers of the investment criteria and/or were particularly uncertain. All analyses were performed for the total investment with benefits taken over the life of the investment plus 50 years from the first year of investment in Project P01-B-003 (Stage 1 RD&E: tilapia bioprospecting). All other parameters were held at their base values.

A sensitivity analysis was carried out on the discount rate. Table B5 presents the results. The results showed a high sensitivity to the discount rate, this was largely due to the fact that the benefit cash flows that start after the end of the tilapia biocontrol RD&E investment (a period of 22 years). This means that the benefit cash flows were subjected to relatively greater discounting than the cost cash flows.

Table B5: Sensitivity of Investment Criteria to the Discount Rate (Total investment, 50 years)

Investment Criteria	**Discount Rate**		
	0%	**5% (base)**	**10%**
Present Value of Benefits ($m)	293.64	52.53	11.82
Present Value of Costs ($m)	26.85	18.69	13.70
Net Present Value ($m)	266.79	33.84	-1.88
Benefit-Cost Ratio	10.93	2.81	0.86

A sensitivity analysis then was carried out on the assumption regarding the maximum potential annual impact and control costs for tilapia. The estimated current and potential future impact and control costs of tilapia in Australia are a critical assumption and underpin both the valuation of impacts and the counterfactual. The sensitivity analysis was carried out based on the multiplier used to estimate

the hypothetical maximum impact and control costs if tilapia continue to spread, including to the Murray-Darling Basin, without intervention.

The results, presented in Table B6, show a moderate sensitivity to the maximum potential impact and control costs of tilapia. A break-even analysis indicated that the investment criteria were positive when the multiplier used to estimate the maximum potential impact costs was x1.046. This means that, with all other variables at their base values, the proposed investment in tilapia biocontrol RD&E would be a worthwhile investment if the total average annual impact and control costs of tilapia in Australia increase to $37.02 million. Further, this demonstrates that the estimated annual impact costs of tilapia are likely to be a key driver of any potential benefits of tilapia biocontrol.

Table B6: Sensitivity of Investment Criteria to the Maximum Potential Impact and Control Costs of Tilapia in Australia (Total investment, 5% discount rate, 50 years)

Investment Criteria	**Maximum Potential Impact and Control Costs of Tilapia in Australia – Multiplier**		
	$36.4m x1.0	**$36.4m x2.5 (base)**	**$36.4m x5.0**
Present Value of Benefits ($m)	17.63	52.53	110.72
Present Value of Costs ($m)	18.69	18.69	18.69
Net Present Value ($m)	-1.07	33.84	92.02
Benefit-Cost Ratio	0.94	2.81	5.92

A sensitivity analysis then was carried out on the assumption regarding the expected reduction in tilapia impact and control costs associated with a reduction in tilapia biomass caused by release of a new tilapia BCA. Table B7 presents the results. The investment criteria showed a moderate to low sensitivity to the assumed reduction in tilapia impact and control costs. A break-even analysis suggested that, with all other assumptions at their base values, the proposed investment in tilapia biocontrol RD&E would give positive results if the expected reduction in tilapia impact and control costs was as low as 5.49% (noting that this assumes that, without intervention, tilapia impact costs will increase significantly in the future).

Table B7: Sensitivity of Investment Criteria to the Expected Reduction in Tilapia Impact and Control Costs (Total investment, 5% discount rate, 50 years)

Investment Criteria	**Expected Reduction in Tilapia Impact and Control Costs**		
	30%	**50% (base)**	**80%**
Present Value of Benefits ($m)	37.33	52.53	75.34
Present Value of Costs ($m)	18.69	18.69	18.69
Net Present Value ($m)	18.64	33.84	56.65
Benefit-Cost Ratio	2.00	2.81	4.03

A final break-even analysis then was conducted jointly on the two key variables tested previously (Table B6 and B7). This analysis tested what combination of maximum potential impact and control costs and what expected reduction in impact and control costs because of a new tilapia BCA would result in positive investment criteria. The analysis found that, with all other variables held at their base values, the investment criteria for tilapia biocontrol RD&E were positive when the maximum potential annual impact costs were $51.1 million (multiplier of x1.44) and the reduction in tilapia impact costs from biocontrol was 28.9%. This combined break-even analysis indicates that the investment criteria are relatively robust to the key assumptions made.

11. KEY FINDINGS: SUMMARY AND DISCUSSION

Tilapia bioprospecting Project P01-B-003 has, to date, successfully identified three potential tilapia biocontrol candidates categorised as tentatively worthwhile for further investigation.

1. TLEV was categorised under a 'watching brief'. This means that TLEV was not selected for further investigation right now but will be watched as possible future BCA through the international literature and scientific networks.

2. TiPV was categorised as 'tentatively worthwhile' for further investigation. TiPV is the first and only parvovirus known to infect fish. The virus also has been isolated in cell cultures, allowing future testing of the virus including experimental challenge.

3. TiLV was considered the most promising potential BCA candidates and was categorised as 'worthwhile for active further investigation'. CSIRO already have imported the virus and are currently developing the capability to work with TiLV in a laboratory setting. The project team currently plans to test TiLV's susceptibility in tilapia sourced from Qld waters in January 2022.

The primary objective of the preceding ex-ante analysis was to assess whether the investment (the total costs of the RD&E addressing the advancement of new BCAs to manage tilapia in Australia) would be paid for by the estimated potential benefits of the proposed BCA(s).

The CBA was set within a staged risk management framework of investment. The approach included identifying and describing the six stages of RD&E for the proposed tilapia biocontrol investment, RD&E objectives, planned activities, expected outputs and outcomes. Potential impacts associated with the expected outcomes then were identified and categorised as economic, environmental, and social impacts. The primary impact is expected to be a net reduction in the total annual impact costs of tilapia to the Australian community and economy through a reduction in tilapia biomass.

Valuation of the primary impact involved making several uncertain assumptions as a number of key relationships/variables along the pathways to impact were unknown. Specifically, the following relationships/variables are currently unknown:

- potential reduction in tilapia biomass through use of a given candidate BCA
- reduction in tilapia biomass and the resulting change in tilapia impact and control costs in Australia
- probability of success of each stage of the proposed tilapia biocontrol RD&E investment.

The impact was valued for TiLV as the selected BCA. This was because TiLV was identified as the most promising tilapia biocontrol candidate and already has been imported to Australia for initial efficacy testing. However, a similar valuation framework also would apply to TiPV. Other than the RD&E time frames, it is considered likely that the total RD&E investment costs for TiPV would be similar to those for TiLV.

To accommodate the relatively long time frames associated with biocontrol RD&E in Australia, the ex-ante analysis ran for the length of the proposed RD&E investment period plus 50 years from the first year of investment in project P01-B-003 (2020/21) (Stage 1 of the overall proposed investment in tilapia biocontrol RD&E). Based on the assumptions made, the total expected RD&E investment was estimated at $18.69 million (present value terms). The investment was estimated to produce total expected net benefits of $52.53 million (present value terms). This gave a net present value of $33.84 million, a benefit-cost ratio of 2.81 to 1, an internal rate of return of 9.3% and a modified internal rate of return of 7.1%.

Care should be taken when interpreting the results of the ex-ante analysis. It is important to note that the expected release and subsequent impact of a new tilapia BCA, such as TiLV, would not occur until approximately 22 years after the first year of investment in Project P01-B-003. Given that the investment criteria became positive between 25 and 30 years after the first year of investment, this indicates that implementation of a new tilapia BCA would create benefits sufficient to cover the costs of the proposed tilapia biocontrol RD&E investment within five to 10 years of release of the BCA.

Further, it is important to remember that the ex-ante analysis was conducted within a risk management framework and that the results are expected values. This means that it is theoretically possible for the total proposed investment in tilapia biocontrol to be made (approximately $45.6 million in nominal dollars) and for there to be no benefits realised. That is, the new agent is released and is unsuccessful in reducing tilapia impact costs. However, the risk of this is very minimal as the proposed tilapia biocontrol RD&E investment has been planned as a staged investment with a number of key stop/go points that would enable funding partners, researchers and other stakeholders to adjust and/or redirect the RD&E to alternative and more promising directions. Also, the knowledge generated through Stages 1 to 3 are likely to contribute to increased scientific knowledge and research capacity associated with management of pest tilapia in Australia.

Sensitivity analyses of key variables in the CBA showed that the current and expected total average annual impact and control costs attributable to tilapia in Australia are a critical assumption when considering the estimated benefits of proposed biocontrol. Currently, the current and likely future impact and controls costs of tilapia without intervention are highly uncertain.

However, a break-even analysis conducted on what combination of maximum potential tilapia impact and control costs and the expected reduction in impact and control costs because of a new tilapia BCA found that the investment criteria for tilapia biocontrol RD&E were positive when the maximum potential annual impact costs were $51.1 million (multiplier of x1.44) and the reduction in tilapia impact costs from biocontrol was 28.9%. This combined break-even analysis indicated that the investment criteria are relatively robust to the key assumptions made.

12. CONCLUSIONS AND RECOMMENDATIONS

The investment criteria estimated from the base set of assumptions for the proposed investment in tilapia biocontrol (including Project P01-B-003) are all positive from a period of 30 years after the first year of investment (2020/21). The positive investment criteria suggest that the initial investments (Stages 1 to 5) would be worthwhile given the estimates made of the current and future potential impact and control costs of tilapia in Australia, likely pathways to impact for proposed new BCAs, the RD&E investment and associated timelines required, and the risks involved.

The proposed investment can be staged conditionally so that, as the investment proceeds along a particular pathway, the direction of the RD&E could be changed according to any past success and any new information available. This may avoid or minimise any potential losses and maximise the chances of significant impacts being delivered.

The successful identification of BCA candidates and the positive ex-ante CBA results from Project P01-B-003 indicate that the proposed investment in tilapia biocontrol RD&E is likely to be worthwhile and should be viewed favourably by the Centre for Invasive Species Solutions, potential funding partners, and other tilapia biocontrol and/or management stakeholders.

However, to strengthen any future analysis of the potential costs and benefits of tilapia biocontrol in Australia, it is strongly recommended that any future RD&E include:

1. Identification and estimation of the current and likely future impact and control costs associated with tilapia in Australia. In particular, the non-market values associated with tilapia impacts are likely to be the most significant in terms of tilapia impact costs and the potential benefits of any future tilapia biocontrol. Further, the likely future increase in tilapia impact costs without intervention is a key driver of any potential benefits of implementation of any future tilapia BCAs.

2. Work that demonstrates and quantifies the relationship between tilapia and the biophysical impacts to which tilapia are assumed to contribute taking into account different levels of existing tilapia biomass in different types of habitats/ecosystems in different regions.

3. Quantification of the potential relationship between reductions in tilapia biomass and the drivers of key medium- and long-term impacts of biocontrol including biodiversity/ecosystem health outcomes.

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ABBREVIATIONS AND ACRONYMS

ABS	Australian Bureau of Statistics
APVMA	Australian Pesticide and Veterinary Medicines Authority
BCA	Biocontrol Agent
Biocontrol	Biological Control
CBA	Cost-Benefit Analysis
CISS	Centre for Invasive Species Solutions
CRRDC	Council of Rural Research and Development Corporations
CRC	Cooperative Research Centre
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAWE	Department of Agriculture, Water and the Environment (Commonwealth)
MDB	Murray-Darling Basin
NCCP	National Carp Control Program
RD&E	Research, Development and Extension
TiLV	Tilapia Lake Virus
TiPV	Tilapia Parvovirus
ToR	Terms of Reference

GLOSSARY OF ECONOMICS TERMS

Cost–benefit analysis (CBA):	An economic analysis technique for assessing the economic merit of a proposed initiative by assessing the benefits, costs, and net benefits to society of the initiative. Aims to value benefits and costs in monetary terms wherever possible and provide a summary indication of the net benefit.
Benefit-cost ratio (BCR):	Ratio of the present value of economic benefits to the present value of economic costs of a proposed initiative. Indicator of the economic merit of a proposed initiative at the completion of cost–benefit analysis. Commonly used to aid comparison of initiatives competing for limited funds.

Discounting:	The process of converting money values that occur in different years to a common year. This is done to convert the dollars in each year to present value terms.
Implicit price deflator for gross domestic product (GDP)	The implicit price deflator for GDP is a price index for all final goods and services produced and is calculated as the ratio of nominal GDP to real GDP. The GDP deflator expresses the extent of price level changes, or inflation, within an economy. The implicit price deflator for GDP is used to convert past, nominal dollar terms to current, real dollar terms in a cash flow analysis.
Internal rate of return (IRR):	The discount rate that makes the net present value equal to zero. Internal rate of return must be greater than or equal to the discount rate for an initiative to be economically justified. The discount rate is also known as the hurdle rate.
Investment criteria:	A set of parameters used by decision-makers to assess or compare initiatives. Investment criteria may include the benefit-cost ratio, net present value, and internal rate of return.
Net present value (NPV):	The combined discounted present value of one or more streams of benefits and costs over the appraisal period. The term 'net' denotes that the net present value is calculated as present value of benefits minus the present value of costs.
Nominal dollars	Dollars not adjusted for inflation
Present value of benefits (PVB):	The sum of the discounted benefit streams (cash flows) over the appraisal period.
Present value of costs (PVC):	The sum of the discounted cost streams (cash flows) over the appraisal period.

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