

# Snowy 2.0 Biosecurity Risk Management Plan

Annual Report for the Financial Year ended 30 June 2025



## **Executive Summary**

This first Annual Report for the Snowy 2.0 Biosecurity Risk Management Plan (BRMP) covers activities undertaken from the Plan's endorsement on 25th October 2024 to 30th June 2025.

Full compliance with BRMP requirements was achieved during this reporting period.

Key findings include:

Pest Fish Surveillance: The Pest Fish Surveillance Program confirmed the expected distribution of pest fish species (Redfin Perch, Eastern Gambusia, Climbing Galaxias) within the surveillance catchments. An equivocal Environmental DNA (eDNA) result for Redfin in Tantangara Reservoir was re-sampled and confirmed negative, reinforcing the assumption that the reservoir remains Redfin-free. No additional surveillance is required or planned beyond the current program.

Disease Surveillance and Management: Surveillance for Epizootic Haematopoietic Necrosis Virus (EHNV) in Talbingo Reservoir resulted in two positive qPCR detections. As EHNV has now been confirmed in Talbingo Reservoir, the disease surveillance program will shift its focus to Tantangara Reservoir, targeting Rainbow Trout, and has been revised accordingly.

**Tantangara Reservoir Spill Management:** No spills occurred over Tantangara Dam during the reporting period.

**Tantangara Fish Screens:** The next stage of design for the Tantangara Fish Screens is nearing completion.

Tantangara Creek Barrier: Construction of the Tantangara Creek Barrier was completed in May 2025 and the structure is now operational. Pre-clearance surveys for Stocky Galaxias were undertaken, and any captured fish were relocated upstream.

# Acronyms and Definitions

Approval	Infrastructure Approval for Snowy 2.0 Main Works issued under Section 5.19 of the <i>Environmental Planning and Assessment Act 1979</i> (Dated: 20th May 2020) (CSSI 9687)
Biosecurity Act	Biosecurity Act 2015 (NSW)
Biosecurity Regulation	Biosecurity Regulation 2017 (NSW)
BRMP	Biosecurity Risk Management Plan
CSSI	Critical State Significant Infrastructure
Dam	A physical structure to hold back water and raise its level, forming a reservoir of water
DAWE	Department of Agriculture Water and the Environment, now Department of Climate Change, Energy, the Environment and Water (DCCEEW)
DCCEEW	Commonwealth Department of Climate Change, Energy, the Environment and Water (formerly the Department of Agriculture Water and the Environment (DAWE)
DPE	NSW Department of Planning and Environment (formerly Department of Planning, Industry and Environment (DPIE) and now Department of Planning, Housing and Infrastructure)
DPHI	Department of Planning, Housing and Infrastructure (formerly DPIE and DPE)
DPIRD	NSW Department of Primary Industries and Regional Development, formerly NSW Department of Primary Industries (DPI), formerly part of the Department of Planning, Industry and Environment at the time of project consent
DPIE	Department of Planning, Industry and Environment, now known as Department of Planning, Housing and Infrastructure (DPHI) and the Department of Energy the Environment, Climate Change and Water (DEECCW)
eDNA	Environmental DNA
EHNV	Epizootic Haematopoietic Necrosis Virus
EIS	Snowy Hydro's Environmental Impact Statement for Snowy 2.0 Main Works (EMM, 2019)
EP&A Act	Environmental Planning and Assessment Act 1979 (NSW)
EPBC Act	Environment Protection and Biodiversity Conservation Act 1999 (Commonwealth)
FM Act	Fisheries Management Act 1994 (NSW)
KNP	Kosciuszko National Park
M-E Tunnel	Murrumbidgee-Eucumbene Tunnel. A one-way tunnel linking Tantangara Reservoir with the Eucumbene Reservoir
NPWS	NSW National Parks and Wildlife Service

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NSW	New South Wales		
Pre-Connection	The period of time before Talbingo and Tantangara Reservoirs are hydrologically linked in an upstream direction via Snowy 2.0		
PHES	Pumped Hydroelectric Station		
Post-Connection	The period of time after Talbingo and Tantangara Reservoirs are hydrologically linked in an upstream direction via Snowy 2.0		
Reservoir	The impoundment of water behind a dam		
ROW	River Outlet Works		
RtS	Response to Submissions for the Snowy 2.0 Main Works EIS (EMM, 2020)		
Snowy 2.0	A PHES that will link the existing Tantangara and Talbingo reservoirs via a new underground tunnel		
RFMP	Recreational Fishing Management Plan		
TFMP	Threatened Fish Management Plan		

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## 1. Introduction

The objective of the Biosecurity Risk Management Plan (BRMP) is to minimise the aquatic biosecurity risks associated with the development of Snowy 2.0, including the movement and/or spread of pests and pathogens.

This is the first Annual Report prepared under the BRMP for Snowy 2.0 Main Works.

This Report provides an update of all activities associated with the BRMP since the Plan was endorsed by the NSW Department of Primary Industries and Regional Development (DPIRD) Deputy Secretary Agriculture and Biosecurity, Rachel Connell on the 25th October 2024 up to the 30th June 2025.

Full compliance with the requirements of the BRMP was achieved during the reporting period.

Key components of the report include:

- → Outcomes from the Pest Fish Surveillance Program
- → Outcomes from the Disease Surveillance Program
- → Tantangara Fish Barrier Construction
- → Tantangara Fish Screen Design Update

The BRMP, alongside the associated Threatened Fish Management Plan (TFMP) and Recreational Fishing Management Plan (RFMP), demonstrate Snowy Hydro's commitment to minimising the potential impacts of the project on biosecurity and fish. They will underpin the DPIRD consideration of necessary instruments under the *Biosecurity Act 2015* that will be issued prior to the operation of the Snowy 2.0 Pumped Hydro-electric Power Station (PHES).

#### 1.1 Snowy 2.0 Project Overview

Snowy Hydro owns, manages, and maintains the Snowy Mountains Hydroelectric Scheme (the Scheme). The Scheme currently includes 16 major dams, eight power stations, one pumped power station, 145 km of interconnected tunnels and pipelines, and 80 km of aqueducts. The Scheme, principally located within the Kosciuszko National Park (KNP),

is one of the largest and most complex hydro-electric schemes in the world.

The pumped hydro-electric expansion of the Scheme (Snowy 2.0) will link the existing Tantangara and Talbingo reservoirs via a new underground tunnel and a PHES. Snowy 2.0 will provide an additional 2,200 MW of dispatchable generating capacity, along with approximately 350,000 MWh of large-scale energy storage that will be available on demand as quick-start electricity generation at critical times of peak demand.

For almost 75 years Snowy Hydro has responsibly operated the Snowy Scheme within KNP. Snowy Hydro is committed to avoiding and minimising potential impacts from Snowy 2.0, including biosecurity, as it does for the existing business.

#### 1.2 Snowy 2.0 Project Approval

This project was designated Critical State Significant Infrastructure (CSSI 9687) and assessed under Part 5 of the *Environmental Planning and Assessment Act* 1979 (EP&A Act). Under sections 5.23 and 5.24 of the EP&A Act, certain separate approvals and licences are not required. The project was approved by the NSW Minister for Planning and Public Spaces under Section 5.19 of the EP&A Act on the 20th of May 2020.

A referral (EPBC 2018/8322) was also prepared and lodged with the Federal Minister for the Environment under the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) and the proposal was subsequently determined to be a controlled action under that Act. The project was approved by the Department of Agriculture Water and the Environment (DAWE), now the Department of Climate Change, Energy, the Environment and Water (DCCEEW) under sections 130(1) and 133(1) of the EPBC Act on the 29th June 2020.

Full details of the Project Approval and supporting information can be found here.

# 1.3 Biosecurity Risk Management Plan Background

The BRMP and the Screens and Barrier described within, represent the key measures for Snowy Hydro to minimise the biosecurity risks associated with Snowy 2.0, including the movement and/or spread of weeds, pests and pathogens. In addition to the TFMP and RFMP, it is also a key measure to minimise the impact of the development on threatened fish species and their habitat, particularly the Macquarie Perch (Macquaria australasica), and Stocky Galaxias (Galaxias tantangara) and minimise the impact of the development on recreational fishing in Lake Eucumbene. The three management plans are closely linked and have been designed in concert to avoid and minimise the potential aquatic and biosecurity impacts of Snowy 2.0.

A potential biosecurity impact of Snowy 2.0 identified during the impact assessment for the project included the potential transfer and subsequent establishment of pest fish species, in particular the Redfin Perch (Perca fluviatilis) (Redfin), Eastern Gambusia (Gambusia holbrooki) and Climbing Galaxias (Galaxias brevipinnis), from Talbingo Reservoir to Tantangara Reservoir following hydrologic connection between Talbingo and Tantangara reservoirs to facilitate Snowy 2.0 operation (Cardno, 2019). The potential for these species to be entrained during pumping, survive transfer through the power station and subsequently establish within Tantangara Reservoir is uncertain, but, in the absence of controls, the possibility cannot be excluded.

Should Redfin establish in Tantangara Reservoir, impacts to salmonids could include predation and/ or competition resulting in impacts to the population. Similar, but less likely, impacts could also occur if a population of Eastern Gambusia establish. These impacts could also occur in Lake Eucumbene if Redfin or Eastern Gambusia establish in Tantangara Reservoir and are subsequently transferred and establish in Lake Eucumbene. Should Redfin establish in Tantangara Reservoir, and subsequently be transferred and establish in the mid-Murrumbidgee River downstream of Tantangara Dam, impacts to Macquarie Perch could include predation and/or competition resulting in impacts to the population (Cardno, 2020). If Climbing Galaxias are transferred to Tantangara Reservoir and subsequently migrate and establish in Tantangara Creek, impacts to Stocky Galaxias could include

predation and/or competition resulting in impacts to the population (Cardno, 2019).

Redfin (and, to a lesser extent Rainbow Trout (Oncorhynchus mykiss)) are known hosts of the fish disease Epizootic Haematopoietic Necrosis Virus (EHNV). There is potential that water transfer through Snowy 2.0 could increase the range of EHNV. Several native species, including Macquarie Perch, are susceptible to EHNV under laboratory conditions, although natural disease events caused by EHNV have never been detected in species other than Redfin and Rainbow Trout (Hick et al., 2019; Cardno, 2019).

Snowy Hydro comprehensively assessed the likelihood of these potential impacts and options to avoid transfer of pest fish through Snowy 2.0 as well as options to minimise potential impacts. This information is provided with the Snowy 2.0 Environmental Impact Statement (EIS) and Response to Submissions (RtS) (EMM, 2019; 2020). This assessment found that the most reasonably practicable way to minimise biosecurity risks and impacts to threatened species was to install structures to minimise the potential for pest fish to enter the habitat of threatened species (Tantangara Creek and the mid-Murrumbidgee River below Tantangara Dam), and Lake Eucumbene. As a result, Snowy Hydro committed to designing and constructing:

- → A 'Galaxiid barrier' at the downstream extent of the Stocky Galaxias habitat (subsequently imposed as Condition 21(a) of the Infrastructure Approval)
- → Fish screens at Tantangara Dam and the inlet to the Murrumbidgee to Eucumbene tunnel (M-E Tunnel) to prevent the transfer of all life stages of fish so far as is reasonably practicable from Tantangara Reservoir through the Dam to the mid-Murrumbidgee River and to Lake Eucumbene (subsequently imposed as Condition 21(b) of the Infrastructure Approval).

Once these controls are in place, the likelihood of Climbing Galaxias being transferred to Tantangara Reservoir from Talbingo Reservoir, establishing in the upper Murrumbidgee catchment then migrating upstream of the planned barrier and establishing in upper Tantangara Creek leading to competition with and extinction of Stocky Galaxias was assessed by Cardno (2019) as rare. Similarly, the likelihood of a reservoir spill or a failure of the proposed screens leading to transfer and establishment of Redfin in

the mid-Murrumbidgee River downstream of Tantangara Reservoir leading to competition with and impacts to the population of Macquarie Perch or Transfer of Redfin to Lake Eucumbene was also assessed as rare (Cardno, 2019).

The Critical State Infrastructure Assessment Report for the project found that the controls proposed by the project, being the screens at Tantangara Dam and the barrier on Tantangara Creek, were expected to minimise any adverse biosecurity impacts of the project as far as is reasonably practicable. The three management plans, being the BRMP, TFMP and RFMP, are intended to collectively complement these measures to further minimise any adverse biosecurity impacts, should the controls fail (NSW DPIE, 2020).

Measures described in the TFMP are intended to assist NSW DPIRD Fisheries and DCCEEW in protecting these endangered fish species. Such measures may improve the resilience of Stocky Galaxias and Macquarie Perch to withstand competition from Climbing Galaxias and Redfin respectively, should the controls fail at some point in the future. Similarly, measures described in the RFMP are intended to ensure that any impacts to recreationally important salmonids in both Tantangara Reservoir and Lake Eucumbene can be minimised via the targeted fish stocking of these reservoirs if and as required.

Tantangara Reservoir represents the potential initial pest fish incursion location associated with the operation of Snowy 2.0 due to the establishment of the hydrological connection with Talbingo Reservoir. It is assumed that all other catchments may only be impacted from the operation of Snowy 2.0, if transfer of pest fish from Talbingo Reservoir and establishment of pest fish populations in Tantangara Reservoir occurs.

#### 1.4 BRMP Implementation

Snowy Hydro is responsible for the implementation of activities to be undertaken as part of the BRMP for the life of the development. Under the EPBC approval, the BRMP must be implemented until the end date of the approval (31 December 2140) unless otherwise agreed by the Commonwealth Minister<sup>1</sup> in writing.

Works to be undertaken as part of the BRMP will be informed by the outcomes of previous studies and based upon the requirements to successfully achieve the objectives of the BRMP.

#### 1.5 BRMP Annual Report Requirements

Each year, by 31 August, or as otherwise advised to NSW DPIRD in writing, Snowy Hydro will publish an Annual Report detailing the monitoring data (excluding sensitive ecological data) and outcomes from surveillance and management Activities undertaken as part of this Plan.

Where relevant, the Annual Report will also include planned deviations from the surveillance activities detailed in Appendix E and F for subsequent years as well as any deviations that occurred in the previous year.

Once finalised, this report will be publicly available via Snowy Hydro's website (<a href="https://www.snowyhydro.com.au">www.snowyhydro.com.au</a>).

<sup>&</sup>lt;sup>1</sup>Minister means the Australian Government Minister administering the EPBC Act including any delegate thereof.

## 2. Surveillance Catchments

The pest fish and disease surveillance activities and management measures referred to in this BRMP Annual Report have occurred within the following catchments (surveillance catchments) (Figure 1):

- 1. Tantangara Reservoir
- Upper Murrumbidgee Catchment (including Tantangara Creek)
- 3. Mid-Murrumbidgee catchment
- 4. Lake Eucumbene
- 5. Talbingo Reservoir.

## 3. Aquatic Assets

The key aim of the pest fish and disease surveillance programs in the BRMP are to identify if a pest fish or disease incursion occurs within an area of habitat of threatened species or recreationally important salmonids, where they are not already present.

For the program, the key aquatic assets include:

- → Stocky Galaxias
- → Macquarie Perch
- → Salmonids.<sup>2</sup>

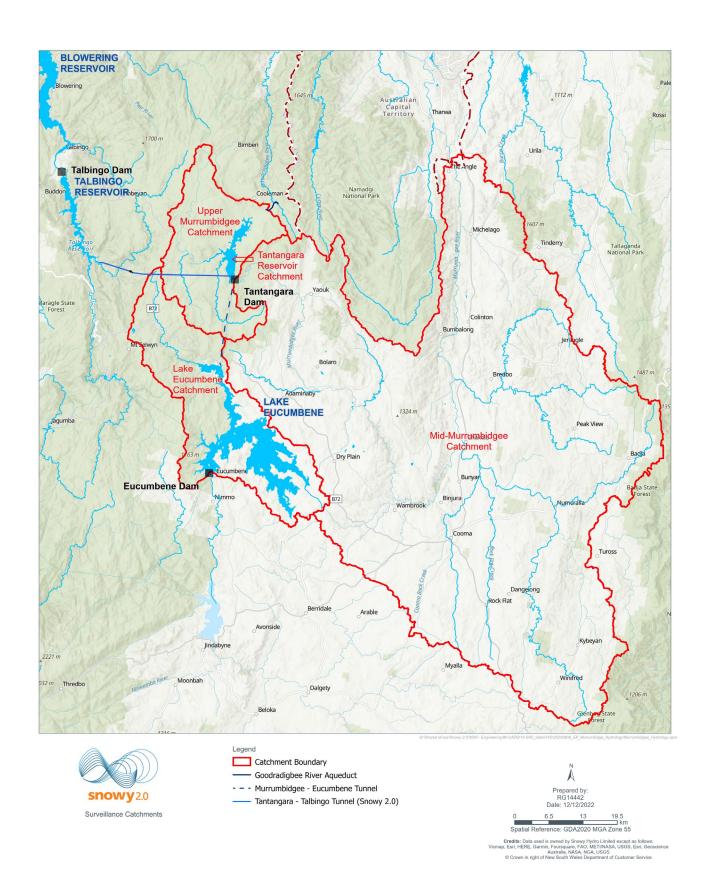
The known distribution of the aquatic assets within the surveillance catchments as of February 2024 is provided in Table 1. A table of the distribution of all fish species in the surveillance catchments and surrounding areas as at 2019 was provided in the EIS and is replicated in Appendix D of the BRMP (Cardno, 2019).

Table 1: Known distribution of aquatic assets within the surveillance catchments

	Talbingo Reservoir	Tantangara Reservoir	Upper Murrumbidgee catchment	Upper Tantangara Creek	Mid- Murrumbidgee River	Lake Eucumbene
Stocky Galaxias	-	-	-	Present*	-	-
Macquarie Perch	-	-	-	-	Present	-
Salmonids	Present	Present	Present	-	Present	Present

<sup>\*</sup>Stocky Galaxias are currently only known to occur in Upper Tantangara Creek (part of the Upper Murrumbidgee catchment), above the Tantangara Creek Waterfall where salmonids are absent and in Sallys Flat Creek, a tributary of the Goodradigbee River (Lintermans and Allan, 2025).

<sup>-</sup> Not detected.



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Figure 1: Overview of surveillance catchments

## 4. Pest Fish Surveillance

All pest fish surveillance was undertaken as required by the BRMP Pest Fish Surveillance Program set out in Appendix E of the BRMP. This surveillance program is based on advice provided by Dr Tarmo Raadik and Associate Professor Mark Lintermans (Raadik and Lintermans, 2022).

#### 4.1 Surveillance Objective

As Snowy 2.0 is not yet operational, the objective of the activities during the reporting period was to ensure the pre-connection distribution of pest fish in the surveillance catchments is well known.

#### 4.1.1 Target Pest Species

The target pest fish species for surveillance under the BRMP include:

→ Redfin Perch (Perca fluviatilis) (Redfin), exotic, notifiable under Schedule 1 of the NSW Biosecurity Regulation 2017

- → Eastern Gambusia (Gambusia holbrooki), exotic
- → Climbing Galaxias (Galaxias brevipinnis), native to the Snowy River catchment (including Lake Eucumbene) but considered translocated outside of its natural range into the Murray and Tumut River catchments.

#### 4.1.2 Expected Distribution

The expected distribution of the target pest species within the surveillance catchments is provided in Table 2. A table of the distribution of all fish species, including pest fish, at the time of the Snowy 2.0 EIS preparation is provided in Appendix D of the BRMP.

Table 2: Expected distribution of target pest fish species within the surveillance catchments

	Talbingo Reservoir	Tantangara Reservoir	Upper Murrumbidgee catchment	Mid- Murrumbidgee River	Lake Eucumbene
Redfin	Present	-	-	-	-
Eastern Gambusia	Present	-	-	Present	-
Climbing Galaxias	Present*	-	-	-	Present (endemic)

\*Climbing Galaxias has been detected within the Yarrangobilly River and Tumut River, hydrologically connected tributaries. The species has not been recorded in Talbingo Reservoir using traditional physical sampling and eDNA sampling techniques (Cardno, 2019; Griffiths et al., 2024).

<sup>-</sup> Not detected.

#### 4.2 Surveillance Overview

Surveillance during the reporting period was a combination of physical surveys using a variety of sampling methods and environmental DNA (eDNA) analysis of water samples.

Austral Research and Consulting was engaged to undertake physical sampling in Talbingo Reservoir, Tantangara Reservoir, Lake Eucumbene and the Upper Murrumbidgee catchment while EnviroDNA undertook eDNA analysis of samples collected by staff from Austral Research and Consulting, EnviroDNA and Snowy Hydro from all five surveillance catchments. Full details of the methods and level of effort are detailed in Appendix A – Snowy 2.0 Pest Fish Surveillance Program Report and Appendix B – Snowy 2.0 Environmental DNA Surveillance Report.

In addition to the required surveillance, other surveys in the catchments provided additional strength to the

findings. Additional eDNA and physical surveys occurred in the Mid-Murrumbidgee catchment for the Macquarie Perch population and catchment surveys (Lintermans, 2025 and Duncan and Sarwer, 2025) and in Tantangara and Sallys Flat Creeks for population surveys for Stocky Galaxias (Lintermans and Allan, 2025) undertaken for the TFMP. These reports are available in the Snowy 2.0 Threatened Fish Management Plan, 2024/25 Annual Report (in preparation). The pre-clearance survey for Stocky Galaxias at the Tantangara Barrier construction site also provided additional data in this location (Section 8; Austral Research and Consulting, 2025b).

#### 4.3 Surveillance Results

Surveillance activities during the reporting period confirmed the expected distribution of pest fish within the surveillance catchments (Table 3). The full results of the physical surveys are provided in Appendix A and the eDNA analysis in Appendix B.

Table 3: Summary of Pest Fish Sampling Results from the reporting period

	Talbingo Reservoir	Tantangara Reservoir	Upper Murrumbidgee catchment	Mid- Murrumbidgee River	Lake Eucumbene
Redfin	Detected (Physical & eDNA)	Not Detected (Physical) & Equivocal (eDNA)#	Not Detected (Physical & eDNA)	Not Detected (Physical & eDNA)	Not Detected (Physical & eDNA)
Eastern Gambusia	Detected (Physical & eDNA)	Not Detected (Physical & eDNA)	Not Detected (Physical & eDNA)	Detected (Physical & eDNA)	Not Detected (Physical & eDNA)
Climbing Galaxias	Not Detected (Physical & eDNA) NB. Assumed Present*	Not Detected (Physical & eDNA)	Not Detected (Physical & eDNA)	Not Detected (Physical & eDNA)	Detected (Physical & eDNA)  NB. Species is endemic to this catchment

<sup>\*</sup>Climbing Galaxias has previously been detected within the Yarrangobilly and Tumut Rivers, hydrologically connected tributaries (Cardno, 2019; Griffiths et al. 2024).

<sup>\*</sup>Resampling in the same location returned a negative result. See Appendix B for more detail.

Results from physical and eDNA sampling confirmed the presence of Redfin and Eastern Gambusia within Talbingo Reservoir. Climbing Galaxias was not detected in either the physical or eDNA surveys (Austral Research and Consulting, 2025a and Griffiths et al., 2025). This is not unexpected as the species has only previously been detected in tributaries upstream of the reservoir (Cardno, 2019). A positive eDNA detection for Climbing Galaxias from 2024 in the Tumut River, upstream of Talbingo Reservoir, confirms the ongoing presence of this species within this catchment (Griffiths et al., 2024).

An equivocal result for Redfin was returned for Tantangara Reservoir from the eDNA sampling however, re-sampling returned negative results and no Redfin were detected during physical sampling. As such, this species is still assumed absent from this catchment and the Upper Murrumbidgee, as is the Eastern Gambusia and Climbing Galaxias (Austral Research and Consulting, 2025a and Griffiths et al., 2025).

Redfin DNA was detected in the Gudgenby River, a tributary of the Murrumbidgee River, downstream of Angle Crossing where the species is known to occur. Multiple eDNA surveys in the Mid-Murrumbidgee River upstream of Angle Crossing did not detect any Redfin, nor did the physical sampling undertaken for the Macquarie Perch population and habitat surveys

(Griffiths et al., 2025, Lintermans, 2025 and Duncan and Sarwer, 2025). Climbing Galaxias was also not detected in any of these surveys. Eastern Gambusia is known to occur in this catchment and was detected as far upstream as Adaminaby in the eDNA sampling and Macquarie Perch population surveys (Griffiths et al., 2025, Lintermans, 2025).

Salmonids were also confirmed to be absent in the habitat of the Stocky Galaxias through both physical and eDNA surveys (Austral Research and Consulting, 2025a, Griffiths et al., 2025, Austral Research and Consulting, 2025b, Lintermans and Allan, 2025).

These results are all in accordance with previous sampling undertaken in these catchments for the Snowy 2.0 project (Cardno, 2019; Griffiths et al., 2017; Griffiths et al., 2020; 2021; 2022; 2023; 2024; Robinson et al. 2019; Weeks et al. 2019)

#### 4.4 Outcomes/future plan

As the sampling did not detect any target species in any locations where it is not currently expected, no additional surveillance beyond the program in the BRMP is required and there is no need to review the current program.

As physical sampling is only required once prior to connection, this requirement has now been fulfilled.

## 5. Disease Surveillance and Management

Disease surveillance was undertaken as required by the BRMP Pest Fish Surveillance Program set out in Appendix F of the BRMP. This surveillance program is based on advice and reports provided by Dr Ben Diggles (Diggles, 2022a; Diggles, 2022b) and Dr Paul Hick at the Elizabeth Macarthur Agricultural Institute (Hick et al., 2019).

#### 5.1 Surveillance Objective

The primary objective for the disease surveillance activities for the BRMP is to determine whether Epizootic Haematopoietic Necrosis Virus (EHNV) is present in the Talbingo Reservoir Redfin population prior to the connection of Talbingo and Tantangara reservoirs by Snowy 2.0.

A secondary objective is to establish the distribution of *Lernaea cyprinacea* (Lernaea), also known as anchorworm, in the surveillance catchments.

#### 5.1.1 Target Diseases

The target diseases for the BRMP Disease Surveillance Program are EHNV and Lernaea.

At the time of the Snowy 2.0 EIS and BRMP preparation, previous outbreaks of EHNV had been detected in nearby Blowering Reservoir, however, an outbreak or evidence of infection has never been reported within Talbingo Reservoir, including following limited surveillance undertaken for the Snowy 2.0 EIS (Cardno, 2019; Hick et al., 2019; Diggles, 2022a).

Given that EHNV is predominantly a disease of Redfin, which are only known to occur within Talbingo Reservoir, the priority for the disease surveillance program was to identify whether EHNV disease is currently present within the Talbingo Reservoir Redfin population.

#### 5.2 Surveillance Overview

Austral Research and Consulting was engaged to undertake the collection of whole fish and blood samples which were then shipped to the Elizabeth Macarthur Agricultural Institute (EMAI) for EHNV analysis. Details on the fish collection methods and full results of all fish observed during sampling is provided in Appendix A – Snowy 2.0 Pest Fish Surveillance

Program Report. The sample collection protocol and preparation details are provided in Appendix C – 2025 EHNV Sample Collection Protocol.

#### **5.3 Surveillance Results**

Surveillance for EHNV in Talbingo Reservoir involved the collection of 204 adults and 51 young of year (YOY) whole fish samples which were sent for analysis using quantitative polymerase chain reaction (qPCR) analysis. Where practicable, blood samples were also collected for ELISA analysis.

All fish were observed to be in good condition with no external evidence of disease or infection with Lernaea (see Appendix A).

The qPCR analysis returned two positive results for EHNV. All 131 serology samples were negative. The full table of results is provided in Appendix D – EHNV and Lernaea Results. The two positive results for EHNV were sent to the Australian Centre for Disease Preparedness (ACDP) at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Geelong who undertook further sampling to verify the positive detections. A copy of this report is provided in Appendix E - CSIRO Positive Detection Verification Report.

#### 5.3.1 Response to Positive Detection

Notification to DPIRD in response to the positive detection was undertaken in accordance with legislative requirements.

As EHNV has now been confirmed to occur within Talbingo Reservoir, the focus of the BRMP Disease Surveillance program will shift to determining the status of Tantangara Reservoir which is considered to be Redfin free.

The Surveillance Program has been revised with the new Program detailed in *Appendix F – Revised BRMP* 

Disease Surveillance Program. The target species for this Program will be Rainbow Trout as this is the only other species that has historically been infected by EHNV in a non-experimental setting (Hick et al., 2019). As Snowy 2.0 is not yet operational, the Pest Fish and Disease Response and Control Plan (Appendix C of the BRMP) is not activated by this detection.

# 6. Tantangara Reservoir Spill Management

Condition 22(b) requires that spills over Tantangara Dam are prevented so far as is reasonably practicable. There were no spills over Tantangara Dam during the reporting period.

## 7. Tantangara Fish Screens

As part of the EIS, Snowy Hydro made a commitment to construct fish screens at Tantangara Dam (Figure 2) to prevent, so far as is reasonably practicable, the movement of pest fish (in all its forms: eggs, larvae, juveniles and adults) and spread of disease from Tantangara Reservoir to the mid-Murrumbidgee River and Lake Eucumbene. This commitment was subsequently imposed as Condition 21(b) of the Infrastructure Approval.

The screens will filter water released from environmental flows through the Tantangara Dam River Outlet Works (ROW) to the Murrumbidgee River and water entering the existing Murrumbidgee-Eucumbene tunnel to Lake Eucumbene (Figure 3).

#### 7.1 Activity during the reporting period

Since approval of the BRMP, fish screen optioneering has been completed, and two contracts, for the integrated fish screen system design and the design and supply of the fish screens, have been tendered. Negotiation and contract executions were completed

early January 2025 with Aurecon awarded the hydraulic, civil, structural and integrated fish screen system design, and AWMA the design and supply of the fish screens, collector pipes and associated equipment.

As at the end of June 2025, Aurecon and AWMA have completed the first stage of design and the next stage of 'Issue for Tender (IFT)' design is in progress. Snowy Hydro will continue to keep DPIRD updated as the design progresses.

Once further details are available, Stage 2 of the BRMP will be submitted to DPIRD and other agencies.

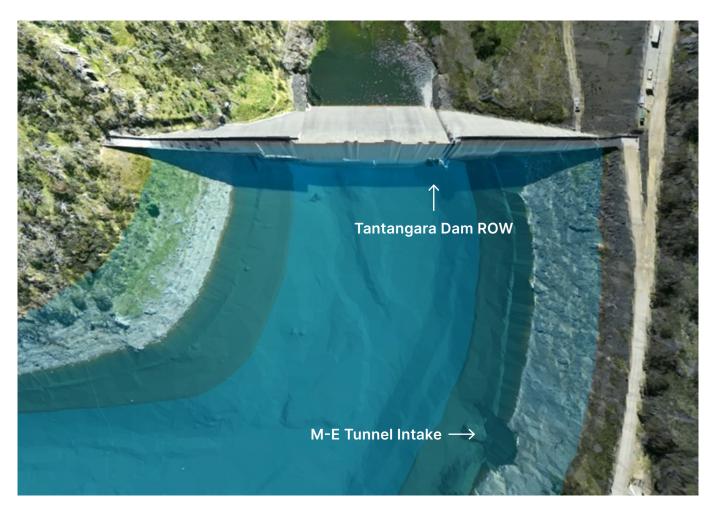


Figure 2: Tantangara Dam, Spillway and ROW and M-E Tunnel Intake

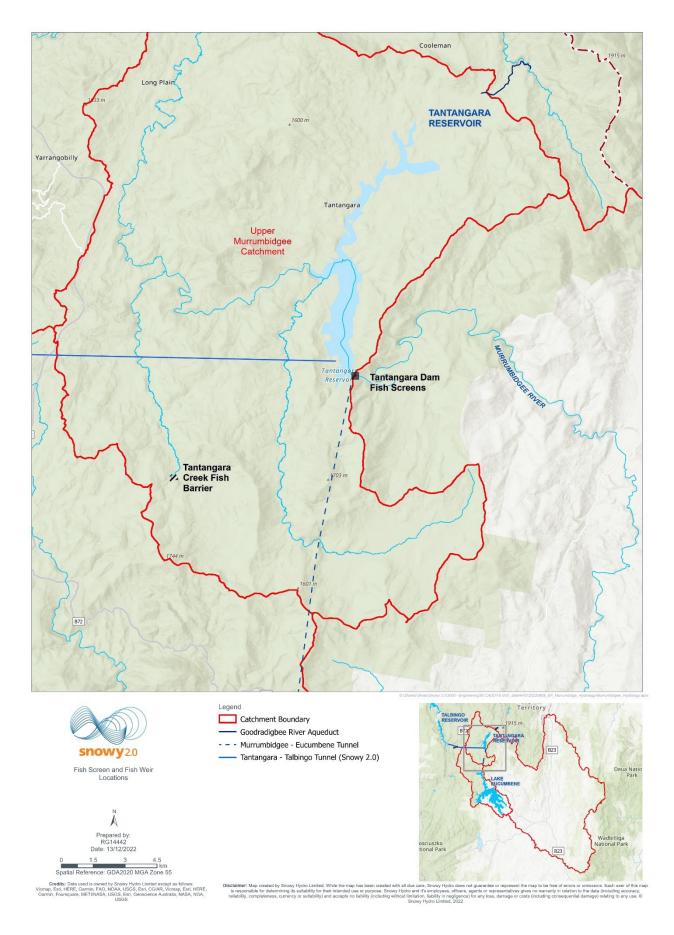


Figure 3: Locality map showing the location of the Tantangara Fish Screens and the Tantangara Creek Fish Barrier

## 8. Tantangara Creek Barrier

As part of the EIS, Snowy Hydro made a commitment to construct a fish barrier on Tantangara Creek. This commitment was subsequently imposed as Condition 21(a) of the Infrastructure Approval. The objective of the Tantangara Creek Barrier (the Barrier) is to prevent so far as is reasonably practicable, Climbing Galaxias reaching the existing population of Stocky Galaxias in the upper reaches of Tantangara Creek.

#### 8.1 Activity during the reporting period

Construction of the Tantangara Creek Barrier commenced and was completed in the reporting period and the structure is now operational (Figure 4). Construction was completed by principal contractor Leed. In late 2024 Leed mobilised to site and completed track upgrades to Nungar Creek Trail and Alpine Creek Trail required to facilitate construction at the barrier site. In February 2025, works commenced on the construction of the barrier through diversion of Tantangara Creek, completion of bulk earthworks, completion of detailed ground preparation, formwork and steel installation, concrete pouring, and final hardware fixing, concluding in May 2025. Following this, rehabilitation and demobilisation from the site occurred.

Initial verification of the structures function has occurred with inspections and verification scheduled on an ongoing basis. The works took place in close consultation with relevant environmental regulators without any environmental incidents reported.

Prior to the commencement of construction, the area to be affected by the water diversion around the site was surveyed for Stocky Galaxias using techniques and a level of effort endorsed by NSW DPIRD. Any captured Stocky Galaxias were relocated upstream outside of the disturbance boundary for the works. A report on this work is provided in Appendix G – Tantangara Barrier Pre-clearance report.





Figure 4: The completed Tantangara Creek Barrier

## 9. Additional Barriers

To minimise potential impacts of pest fish movement on Stocky Galaxias and Macquarie Perch, the EPBC Approval for the project includes a requirement to investigate reasonable measures, including the installation of secondary fish barriers, to protect tributaries identified as priority receiving sites for the establishment of stocking insurance populations of the Macquarie Perch and Stocky Galaxias (Condition 13).

The identification of potential locations for the establishment of additional populations of Stocky Galaxias and Macquarie Perch is an activity to occur as part of the Habitat Surveys for each of these species

under the TFMP. Detailed habitat surveys for both species occurred under the TFMP during the 2024/25 activity period, however, specific re-introduction sites have not yet been identified.

## 10. Conclusion

This inaugural Annual Report for the BRMP details the comprehensive activities undertaken from the Plan's endorsement on 25th October 2024 to 30th June 2025. Demonstrating full compliance with all BRMP requirements, the reporting period has seen significant progress in meeting biosecurity obligations for the Snowy 2.0 project.

The Pest Fish Surveillance Program confirmed the expected distribution of pest fish species. The Disease Surveillance Program detected Epizootic Haematopoietic Necrosis Virus (EHNV) in Talbingo Reservoir. This Program will now be adapted to shift its focus to Tantangara Reservoir.

No spills occurred over Tantangara Dam, the design for the Tantangara Fish Screens is ongoing and the Tantangara Creek Barrier has been successfully constructed and is now operational, with pre-clearance surveys and fish relocation conducted as planned. The successful implementation of these measures, alongside the ongoing commitment to the TFMP and RFMP, underscores Snowy Hydro's dedication to minimising potential impacts on biosecurity and native fish populations. The findings of this report will continue to inform and refine future biosecurity management strategies as the Snowy 2.0 project progresses towards full operation.

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# Appendix A

# Snowy 2.0 Pest Fish Surveillance Report

## research and consulting

# Snowy 2.0 Pest Fish Surveillance Program Report 2025



- Final
- August 2025

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## research and consulting

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## 1. Introduction

Austral Research and Consulting (Austral) were contracted by Snowy Hydro to undertake the physical sampling component of the Pest Fish Surveillance Program as outlined in *Appendix E* of the *Snowy 2.0 Biosecurity Risk Management Plan* (Snowy Hydro, 2024)). This program is based on specific advice provided by Raadik and Lintermans (2022).

This surveillance program aims to ensure the distribution of pest fish in the surveillance catchments is well known ahead of the operation of the Snowy 2.0 project and once the project is operating, detect the presence of target pest fish species as early as possible if an incursion occurs (Snowy Hydro, 2024). The sites sampled across the program are shown in the Figure 1 below.

Pest species targeted in the program include Redfin (*Perca fluviatilis*), Eastern gambusia (*Gambusia holbrooki*) and Climbing galaxias (*Galaxias brevipinnis*). The program also aimed to confirm the continued absence of salmonids in the habitat of the Stocky galaxias (*Galaxias tantangara*).

This report details the methods used to undertake the surveillance program and details the results of the surveys.

Austral was also contracted to collect fish for the Snowy 2.0 Disease Surveillance Program (*Appendix F* of the *Snowy 2.0 Biosecurity Risk Management Plan*) which involved an intensive program of sampling in Talbingo reservoir targeting the collection of Redfin for disease analysis. Fish catch and observation data from surveys undertaken for the Snowy 2.0 Disease Surveillance Program are also reported here.

All surveys and activities were conducted under NSW Animal Research Authority – RVF21/3375 (Animal Ethics), NSW DPI fisheries permit Section 37 Research Permit – FP24/89 and Animal Research Licence number – 82073.

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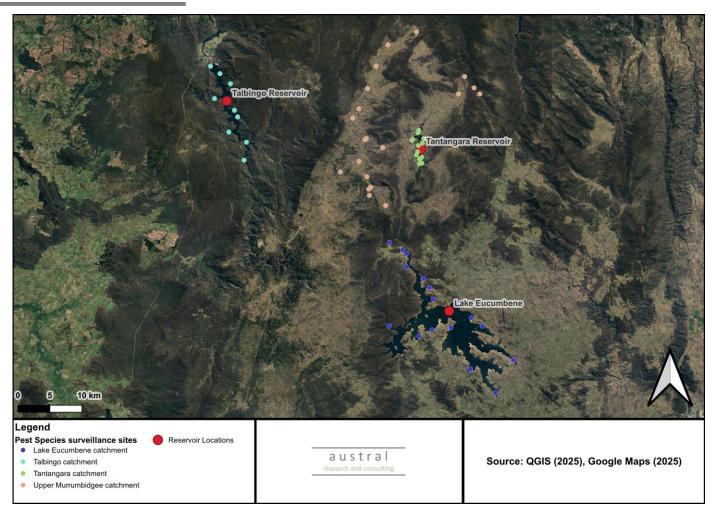


Figure 1: Site locations of Pest Fish Surveillance Program.

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## 2. Methods

The Pest fish surveillance program consisted of physical sampling including backpack electrofishing and boat electrofishing across selected sites. These activities were completed from the 9<sup>th</sup> to the 26<sup>th</sup> of February 2025. Environmental DNA (eDNA) samples were also collected at each pest fish monitoring site. The methods and results of this sampling has been reported separately (Griffiths, Reid, & Licul, 2025).

Fish collection for the Snowy 2.0 Disease Surveillance Program in Talbingo reservoir occurred from the 3<sup>rd</sup> to the 17<sup>th</sup> of February 2025. As boat electrofishing proved ineffective, a variety of methods including gill netting, line fishing and bait traps were also employed. This program of sampling was more intensive than that within other catchments which explains the higher catch rates.

All captured fished were assessed for presence of parasites including Lernaea.

Section 2.1 details the methods used during the surveillance program.

#### 2.1. Survey Methodology

#### 2.1.1. Backpack electrofishing

Backpack electrofishing was performed at suitable sites using a Smith-Root LR-24 electrofisher. The sampling procedure involved electrofishing all wadable areas of the waterways targeting available fish habitat such as rocks, snags and riffles. The amount of survey effort employed at each site was determined by the size of the waterway and availability of wadable water. The survey effort was recorded at each site.

#### 2.1.2. Boat Electrofishing

Boat electrofishing was performed across all navigable areas at selected sites, targeting suitable fish habitat. Austral used a 4.3 m electrofishing vessel "MSV11735" for the surveys. The vessel was equipped with a Grassl electrofisher unit, two boom arms with 16-dropper anode arrays and hull cathode. During each shot immobilised fish were netted from the water and transferred to a holding tank to recover until the end of the 90 second duration shot. At the end of each shot all fish were identified, counted and their body lengths were measured. Fish observed as affected by the electric field and positively identified, but not captured, were also counted. The fishing effort at each site was recorded.

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#### 2.1.3. Gill Netting

Gill nets are a passive trap that are 10 to 30m long with varying mesh sizes (20 to 100mm). Floats and weights are used to hold the net in position. The nets are monitored continuously and are physically inspected a minimum of every 30 minutes.

#### 2.1.4. Line Fishing

Line fishing with lures was used in areas unsuitable for boat electrofishing. 10lb braided line was used with a mixture of vibe lures, soft plastics and trawling lures. Vibe lures proved the most successful.

#### 2.1.5. Bait Traps

Bait traps were set within appropriate habitat. Bait traps consisted of a 2mm mesh and are approximately 250mm x 250mm x 450mm. This size rating complies with restrictions set by NSW Fisheries (i.e. that a single bait trap must not be larger than 450mm x 350mm x 250mm with an entrance not larger than 60mm and mesh between 10mm and 40mm). Bait traps have a small aperture and are a passive trap.

#### 2.2. Limitations

These surveys gather data in the form of a 'snap shot in time'. Results may vary depending on the time of year surveys are undertaken.

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## 2.3. Surveillance Effort

The methods used and electrofishing total effort for each site are detailed in Table 1 and Table 2.

Table 1: Method and effort completed at each Pest Fish surveillance site.

Site	Catchment	Backpack Electrofishing	Boat Electrofishing	EF On-time (secs)
BC1	Upper Murrumbidgee	✓		600
GR1	Upper Murrumbidgee	$\checkmark$		1200
GR2	Upper Murrumbidgee	$\checkmark$		1200
GR3	Upper Murrumbidgee	$\checkmark$		900
GU1	Upper Murrumbidgee	$\checkmark$		750
TA1	Upper Murrumbidgee	$\checkmark$		1200
TA2	Upper Murrumbidgee	$\checkmark$		1200
TA3	Upper Murrumbidgee	$\checkmark$		1200
TA4	Upper Murrumbidgee	$\checkmark$		900
TA5 <sup>1</sup>	Upper Murrumbidgee	$\checkmark$		8562
TA6 <sup>2</sup>	Upper Murrumbidgee	$\checkmark$		
SF1 <sup>2</sup>	Upper Murrumbidgee	$\checkmark$		
TAT1	Upper Murrumbidgee	$\checkmark$		600
UMU1	Upper Murrumbidgee	$\checkmark$		1200
UMU2	Upper Murrumbidgee	$\checkmark$		1200
UMU3	Upper Murrumbidgee	$\checkmark$		1200
UMU4	Upper Murrumbidgee	$\checkmark$		1200
UMU5	Upper Murrumbidgee	$\checkmark$		1200
UMU6	Upper Murrumbidgee	$\checkmark$		1200

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Site	Catchment	Backpack Electrofishing	Boat Electrofishing	EF On-time (secs)
UMU7	Upper Murrumbidgee	<b>√</b>		1200
GG1C	Lake Eucumbene	$\checkmark$		600
LE1	Lake Eucumbene		✓	1080
LE10	Lake Eucumbene		$\checkmark$	1080
LE11	Lake Eucumbene		$\checkmark$	1080
LE12	Lake Eucumbene		$\checkmark$	1080
LE13	Lake Eucumbene		✓	1080
LE14	Lake Eucumbene		✓	1080
LE15	Lake Eucumbene		✓	1080
LE16	Lake Eucumbene		✓	1080
LE2	Lake Eucumbene		✓	1080
LE3	Lake Eucumbene		✓	1080
LE4	Lake Eucumbene		✓	1080
LE5	Lake Eucumbene		✓	1080
LE6	Lake Eucumbene		✓	1080
LE7	Lake Eucumbene		$\checkmark$	1080
LE8	Lake Eucumbene		$\checkmark$	1080
LE9	Lake Eucumbene		$\checkmark$	1080
TR1	Tantangara		✓	1080
TR10	Tantangara		✓	1080
TR11	Tantangara		✓	1080
TR12	Tantangara		$\checkmark$	1080
TR13	Tantangara		$\checkmark$	1080
TR2	Tantangara		$\checkmark$	1080
TR3	Tantangara		$\checkmark$	1080

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Site	Catchment	Backpack Electrofishing	Boat Electrofishing	EF On-time (secs)
TR4	Tantangara		✓	1080
TR5	Tantangara		$\checkmark$	1080
TR6	Tantangara		<b>✓</b>	1080
TRA1	Tantangara		<b>✓</b>	1080
TRA2	Tantangara		<b>✓</b>	1080
TRA3	Tantangara		<b>✓</b>	1080

Note 1 – High EF effort due to Stocky galaxias salvage for the Tantangara Creek barrier project occurring concurrently (Austral Research and Consulting, 2025).

Note 2 – Sites sampled by M. Lintermans and H. Allan (Lintermans & Allan, 2025).

Table 2: Method and effort completed at each disease surveillance area in Talbingo Reservoir.

Site	Boat Electrofishing	EF On-time (secs)	Bait Traps	Gill Netting (min)	Line Fishing (min)	Seine Netting
TAL1	✓	2340	10	120	1350	
TAL2				360	1080	
TAL3	$\checkmark$	1560		480	2400	
TAL4	$\checkmark$	1500		120	540	
TAL5					960	
TAL6	$\checkmark$	120			261	
TAL7					360	
TAL8		Not	Surveyed <sup>1</sup>			
TAL9 (TL1C)				420	600	
TAL10 (SUE1)				150	750	✓

Note 1 – Not surveyed due to weather and access at time of survey.

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## 3. Results

The species and number of individuals captured during the surveys in each catchment are detailed below (Table 3, Table 4, Table 5, Table 6).

Native species captured during the surveillance program included Flathead gudgeon (*Philypnodon grandiceps*), Trout cod (*Maccullochella macquariensis*), Climbing galaxias, Common Yabby (*Cherax destructor destructor*), Spiny crayfish (*Euastacus* sp.), Mountain galaxias (*Galaxias olidus*), Two-spined blackfish (*Gadopsis bispinosus*) and Stocky galaxias. Climbing galaxias was only captured within the Lake Eucumbene catchment where it is considered endemic.

Spiny crayfish were only identified to genus due to the similar external morphological characteristics and requirement to destructively sample to accurately differentiate species via gastric mill morphology (T. Raadik pers. com; Morgan, 1997).

Three Platypus (*Ornithorhynchus anatinus*) were also observed during the program, one in Lake Eucumbene and two within Tantangara Reservoir.

No captured fish was observed with parasites or *Lernaea* during the Pest Fish surveillance program or the Disease surveillance program.

In Talbingo Reservoir a total of 493 introduced individuals were captured with 386 Redfin, 105 Eastern gambusia, one Rainbow trout (*Oncorhynchus mykiss*) and one Goldfish (*Carassius auratus*) recorded (Table 3). Additionally, a further 40 Redfin at TAL3 and 20 Eastern gambusia at TAL4 were observed.

A total of 133 introduced individuals were captured in Lake Eucumbene including three Goldfish, five Oriental weatherloach (*Misgurnus anguillicaudatus*), three Rainbow trout and 122 Brown trout (*Salmo trutta*) recorded (Table 4).

A total of 10 introduced individuals were captured in the Tantangara Reservoir including seven Rainbow trout and three Brown trout. Additionally, one unidentified trout was observed (Table 5). Due to low water levels at the time of survey, sites TR7, TR8 and TR9 were replaced with sites TRA1, TRA2 and TRA3 (new locations).

A total of 178 introduced individuals were captured in the Upper Murrumbidgee catchment with 155 Brown trout and 23 Rainbow trout recorded. One additional unidentified trout was observed (Table 6).

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Table 3: Fish Species and numbers of individuals captured during surveys in Talbingo Reservoir. Values in brackets are observed numbers.

Taxon Common Name	Taxon Scientific Name	TAL1	TAL2	TAL3	TAL4	TAL5	TAL6	TAL7	TAL9 (TL1C)	TAL10 (SUE1)	Total
Native species											
Common yabby	Cherax destructor destructor	1									1
Flathead gudgeon	Philypnodon grandiceps	11		6			150			200 (1000+)	367
Trout cod	Maccullochella macquariensis	3									3
Two-spined blackfish	Gadopsis bispinosus			1							1
Introduced species											
Eastern gambusia	Gambusia holbrooki			5	(20)		100				105
Goldfish	Carassius auratus								1		1
Redfin	Perca fluviatilis	34	6	89 (40)	75	30	45	42	10	55	386
Rainbow trout	Oncorhynchus mykiss									1	1
Total		49	6	101	75	30	295	42	11	256	865

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Table 4: Fish Species and numbers of individuals captured during surveys in the Lake Eucumbene catchment.

Taxon Common Name	Taxon Scientific Name	GG1C*	LE1	LE2	LE3	LE4	LE5	LE6	LE7	LE8	LE9	LE10	LE11	LE12	LE13	LE14	LE15	LE16	Total
Native species																			
Climbing galaxias	Galaxias brevipinnis	4																	4
Common yabby	Cherax destructor destructor																100	50	150
Spiny crayfish	Euastacus sp.	1																	1
Introduced species																			
Brown trout	Salmo trutta	81	19	2	3		5				1	1		3	1		2	4	122
Goldfish	Carassius auratus		3																3
Oriental weatherloach	Misgurnus anguillicaudatus		3					1									1		5
Rainbow trout	Oncorhynchus mykiss								1				2						3
Total		86	25	2	3	0	5	1	1	0	1	1	2	3	1	0	103	54	288

<sup>\*</sup> Site GG1C is located on Gang Gang Creek, a tributary of Lake Eucumbene. This is a site where Climbing Galaxias are known to be present so serves as a positive control site to confirm that techniques applied are able to detect this species. All other sites in this table are located within Lake Eucumbene.

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Table 5: Species and numbers of individuals captured during surveys in Tantangara Reservoir.

Taxon Common Name	Taxon Scientific Name	TR1	TR2	TR3	TR4	TR5	TR6	TR10	TR11	TR12	TR13	TRA1	TRA2	TRA3	Total
Native species															
Common yabby	Cherax destructor destructor											5			5
Introduced species															
Brown trout	Salmo trutta			1				1	1						3
Rainbow trout	Oncorhynchus mykiss				2				2	2				1	7
Trout	Trout sp.			1											1
Total		0	0	2	2	0	0	1	3	2	0	5	0	1	16

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Table 6: Species and numbers of individuals captured during surveys in the Upper Murrumbidgee catchment.

Taxon Common Name	Taxon Scientific Name	BC1	GR1	GR2	GR3	GU1	TA1	TA2	TA3	TA4	TA5	TAT1	UMU1	UMU2	UMU3	UMU4	UMU5	OMU6	UMU7	Total
Native species																				
Mountain galaxias	Galaxias olidus						3							1						4
Spiny crayfish	Euastacus sp.	14	1			5	5	2	6				1	3		3	4	4	3	51
Stocky galaxias	Galaxias tantangara										87									87
Introduced species																				
Brown trout	Salmo trutta	1				6	2	3	2	27		2	6	3	1	8	11	82	1	155
Rainbow trout	Oncorhynchus mykiss		4	9	6							2				1	1			23
Trout	Trout sp.														1					1
Total		15	5	9	6	11	10	5	8	27	87	4	7	7	2	12	16	86	4	321

#### research and consulting

#### 4. Summary

Across all surveillance sites a total of 1490 individuals from 14 different fish species (674 individuals from 8 native species and 816 individuals from 6 introduced species) were captured.

All catch data for both native and introduced species was in accordance with the previously reported known distributions (Snowy Hydro, 2024). The catch data for the target pest fish species is also aligned with the results from eDNA samples collected in the same locations (Griffiths, Reid, & Licul, 2025).

Redfin and Eastern gambusia were confirmed present in Talbingo Reservoir, and they were not detected in any other location targeted in this survey. Climbing galaxias was not detected in Talbingo reservoir, although this is not unexpected as they have only ever previously been detected in the Yarrangobilly River catchment, not the reservoir itself (Snowy Hydro, 2024).

Introduced salmonid species, including Brown and Rainbow trout, were ubiquitous within all catchments but, as expected, were not detected within the Stocky Galaxias habitat above the waterfall on Tantangara Creek.

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# Appendix B

# Snowy 2.0 Environmental DNA Surveillance Report





Monitoring for the presence of pest fish species within the Snowy Scheme using environmental DNA, 2025.

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This report is to be referenced as:

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Date	Version	Description	Author	Reviewed By
17/07/2025	1.0	Draft report	JG	SHL
12/08/2025	2.0	Final report	JG	

#### **Abbreviations**

Abbreviations	Description
eDNA	environmental DNA
qPCR	quantitative polymerase chain reaction
EIS	Environmental Impact Statement



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#### **Executive Summary**

A comprehensive monitoring program using environmental DNA to detect *Perca fluviatilis*, *Gambusia holbrooki* and *Galaxias brevipinnis* has been implemented since 2019 as part of environmental investigations for the Snowy 2.0 project. EnviroDNA, with assistance from Austral Research and Consulting, were engaged to repeat these surveys across six subcatchment areas in February 2025 with the sampling program following the requirements of the Snowy 2.0 Biosecurity Risk Management Plan, Pest Fish Surveillance Program.

A total of 246 water samples from 92 sites, plus additional field negative controls (6), were collected throughout the area with 220 samples from 79 sites analysed for the presence of *P. fluviatilis DNA*, 192 samples from 72 sites analysed for *G. holbrooki* DNA and 198 samples from 80 sites analysed for *G. brevipinnis* DNA. Two independent assays for *P. fluviatilis* targeting different gene regions were used for all samples to provide additional confidence in results for this species. A small subset of 10 sites were also analysed for several trout species.

Perca fluviatilis was detected in Talbingo Reservoir (positive control site) as well one site in the downstream section of the Middle Murrumbidgee where this species is known to occur. Widespread detections of Gambusia holbrooki were recorded throughout waterways of the Middle Murrumbidgee. Galaxias brevipinnis was detected at one site in the Upper Eucumbene catchment where it is considered endemic. All positive detections were within areas the species' are known to occur.

A possible detection of *P. fluviatilis* was recorded in a single sample within Tantangara Reservoir but was not supported by the second assay. Follow-up sampling within the Reservoir also did not detect the species.

Brown and rainbow trout were detected at sites Tantangara and Eucumbene Reservoirs and adjacent waterways where they are expected to occur. Samples upstream of the waterfall along Tantangara Creek where critical *Galaxias tantangara* habitat exists were negative for trout species.



#### **Background**

The Snowy Hydro Scheme incorporates a number of reservoirs and river systems in the Kosciuszko National Park, New South Wales. New hydrologic connections between separate catchment areas are being established to increase the capacity of Snowy Hydro's hydro-electric Scheme (Snowy 2.0) with associated construction and operational activities. This increases the risk of facilitating dispersal of aquatic organisms into catchment areas where they don't currently occur. Alien species (native or introduced) have the potential for significant detrimental impacts (i.e. predation, competition, pathogens) on resident native and/or recreational fish species if they were to become established (Koehn 2004; Crowl et al. 1992; Forsyth et al. 2013; Russell et al. 2012).

To understand the current distribution of pest fish within catchments connected to the Snowy Scheme, on behalf of Snowy Hydro, EnviroDNA has implemented a comprehensive surveillance program using environmental DNA to assess the potential presence of several invasive fish species in different catchment areas. Environmental DNA (eDNA) is a noninvasive sampling technique that detects traces of genetic material from a target species secreted into its surrounding environment (i.e. water). Quantitative comparisons with traditional sampling methods have demonstrated that eDNA methods can be superior in terms of sensitivity and cost efficiency, particularly for scarce, elusive or cryptic species (Biggs et al. 2015; Smart et al. 2015; Thomsen et al. 2012; Valentini et al. 2016; Lugg et al. 2018; McColl-Gausden et al. 2020), enabling effective detection of species at low densities and at all life stages. High detection probabilities are critical to reduce the incidence of false negatives in monitoring surveys (assuming a species is absent when it is actually present), which is particularly important when assessing early incursions of invasive species (Mehta et al. 2007). However, the replication required to achieve high detection rates with traditional techniques is often unfeasible. The high sensitivity of eDNA methods is particularly powerful for early detection of incursions of alien species (Dejean et al. 2012; Furlan and Gleeson 2016; Robson et al. 2016).

The current pest fish eDNA surveys complement and build on previous monitoring undertaken annually since 2019 (e.g. Weeks et al. 2019; Griffiths et al. 2024). During this project, eDNA was used to primarily assess the presence of redfin (*Perca fluviatilis*), eastern mosquitofish (*Gambusia holbrooki*), and climbing galaxias (*Galaxias brevipinnis*) in Lake Eucumbene and Tantangara Reservoir, as well as waterways of the middle and upper Murrumbidgee catchment areas upstream and downstream of Tantangara Reservoir. *Gambusia holbrooki* and *P. fluviatilis* are known to be present in Talbingo Reservoir and sections of the Murrumbidgee catchment below Tantangara Reservoir. *G. brevipinnis* is considered endemic to the Lake Eucumbene catchment and has previously been detected in the Yarrangobilly River, a tributary of Talbingo Reservoir (Griffiths et al. 2017; Weeks et al. 2019; Griffiths et al. 2020, 2021). These species are expected to be absent elsewhere in the surveyed area outside of these known distributions. Recent surveys also included samples to explore the presence of several trout species (*Salmo trutta*, *Oncorhynchus mykiss*, *S. fontinalis*).



#### **Methods**

#### Water sampling

A comprehensive pest fish surveillance program has been developed based on existing knowledge of distributions and expected activities associated with Snowy 2.0 (Raadik and Lintermans 2022). The purpose of the program is to ensure early detection of new incursions or range expansions of key pest fish species to protect existing aquatic values. Sites surveyed during the 2025 monitoring were in accordance with the program and are largely consistent with those surveyed previously (Griffiths *et al.* 2022, 2023) with additional sites in the Middle Murrumbidgee catchment area added (Griffiths *et al.* 2024). Sites were sampled at Talbingo Reservoir as positive controls for *P. fluviatilis*, in Gang Gang Creek as a positive control for *G. brevipinnis* and in the Upper Murrumbidgee for trout species.

Water sampling attempted to target suitable habitat for the relevant species where possible. For example, sampling sites within reservoirs were focused around edge habitat, tributary inlets and where potentially suitable habitat was identified for *P. fluviatilis* and *G. holbrooki* (e.g. vegetation, rocks, snags). In river and stream locations, sampling focused on *G. brevipinnis* habitat preferences where possible (e.g. clear fast-flowing shady streams with rocks, boulders and logs).

Sampling was undertaken by EnviroDNA and Austral Research and Consulting with staff from Snowy Hydro during February 2025. Sampling in Lake Eucumbene and Tantangara Reservoir was primarily by boat while other sites were accessed by vehicle and foot. In total, 92 sites were sampled across the six subcatchment areas – Talbingo Reservoir (n = 3), Upper Eucumbene (n = 1), Lake Eucumbene (n = 16), Tantangara Reservoir (n = 15), Upper Murrumbidgee (n = 19) and Middle Murrumbidgee (n = 38).

At each site, water samples were collected and filtered by passing 0.51-8.00 L of water (average 3.30 L) through a 5  $\mu M$  self-preserving Smith Root filter using an eDNA sampling system (Smith-Root, USA; Thomas et~al. 2018). If any initial sample volume was <1 L due to the filter clogging from in-stream sediments or algae, a second sample was taken at the site to improve detectability. Two replicate samples were achieved in the smaller lotic systems where DNA is expected to be distributed more homogenously due to the flow of water. Four replicate samples were obtained in the larger lentic systems to improve detection where DNA may be more patchily dispersed and overall water volumes are greater. Each filter was divided in half prior to analysis to create two replicates (apart from sites where a second sample was taken and each sample was processed independently). A total of 246 samples were analysed across all sites.

Filtration was undertaken on site to reduce DNA degradation during transport of whole water samples (Yamanaka et al. 2016). Care was taken to minimise contamination between sites by using clean equipment and gloves at each site and avoiding the transfer of water, soil, or organic matter. The Smith-Root eDNA sampler was designed to minimise contamination risk in the field by using a negative pressure filtration pump where only the filter housing comes in contact with the water at any site with the remainder of the system "upstream" of the filter (Thomas *et al.* 2018). In-field negative controls were taken (n = 6) to check for any sampling or equipment contamination. Negative controls were obtained by filtering approximately 500 mL of DNA-free water, following sampling at sites where the target species are expected to

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occur from previous surveys (Appendix 2). Following filtration, all samples were placed in individual snap-lock bags, stored out of sunlight and at ambient temperature before being transported to the laboratory for processing.



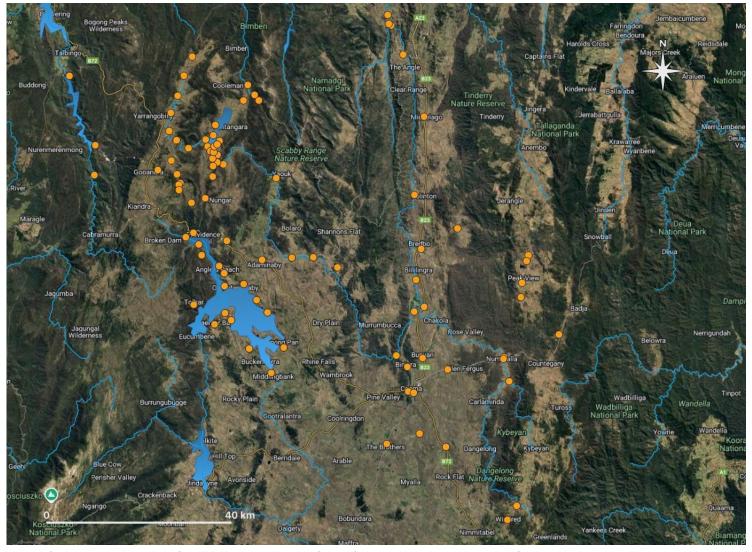


Figure 1. Location of 2025 sampling sites for eDNA surveillance of pest fish species throughout the Snowy Hydro surveillance catchments (see Appendix 1 for further details).

#### Target species assays

DNA was extracted from the filters using a commercially available DNA extraction kit (Qiagen Power Soil Pro Kit) which minimises compounds that can inhibit PCR reactions. As part of the quality control process, a portion of the sample elution is retained after the cellular material has been lysed and DNA removed from the filter but prior to DNA extraction and qPCR steps. This allows independent testing of the original samples to determine whether initial results are consistent with repeat testing if required (see below). Species-specific markers and assays for the target species had already been developed and validated (Griffiths *et al.* 2017; Weeks *et al.* 2019; Furlan and Gleeson 2016). Additional species assays targeted brown trout (*Salmo trutta*)(Capo *et al.* 2020), rainbow trout (*Oncorhynchus mykiss*)(Piggott *et al.* 2021) and brook trout (*Salvelinus fontinalis*)(Wilcox *et al.* 2013).

Using a PrimeTime® probe assay (Integrated DNA Technologies), real-time quantitative polymerase chain reactions (qPCR) were carried out on samples to amplify the target DNA. Three laboratory technical replicates (qPCR's) were undertaken for all samples providing 6 to 12 qPCR assays for each site. Negative controls were included at both the DNA extraction and qPCR stages to help identify possible laboratory contamination. If any contamination is found throughout the DNA extraction or qPCR process, qPCR and/or DNA extractions (using the retained portion of the original elution) are repeated as part of the quality control process.

For this project we consider three possible outcomes for testing based on single species target assays using gPCR across samples at the site level; 'negative' for the target species DNA (no qPCR technical replicates positive out of all samples tested), 'positive' for the target species DNA (at least 3 of 12 or at least 2 of 6 qPCR positives per site) or an 'equivocal' result (less than 3 of 12 or less than 2 of 6 qPCR positives per site). Having an 'equivocal' category can be considered somewhat conservative (see Hyatt et al. 2007) but can minimise the chance of false positives. While trace amounts of DNA may indicate the target species is actually present in low abundance, it may also arise from sample contamination through the sampling or laboratory screening process which is minimised though careful field and laboratory protocols as described above. Facilitated movement of DNA between waterbodies can also occur in situ via water birds, recreational anglers, water transfers, predator scats, or natural dispersal of DNA from further upstream locations in lotic systems (considered sitelevel false positives). When 'equivocal' detections occur, further sampling is recommended to confirm the presence or absence of the target species if greater confidence is required. All samples from sites that returned an equivocal result from any replicate sample were reanalysed for confirmation. If the equivocal result could not be corroborated with the second analysis (i.e. all assays were negative), the sample was considered negative.

In recognition of the importance of this project for Snowy Hydro and consequences of both false negatives and false positives, EnviroDNA undertook additional research in 2023 and developed a new *P. fluviatilis* assay targeting a different mitochondrial gene region (ND5). Comparison with the original *P. fluviatilis* assay (Furlan and Gleeson 2016) that targets the 12S mitochondrial gene demonstrates similar performance with Limit of Detection (Thalinger *et al.* 2021) suggesting equivalent sensitivity between the two assays (Figure 2). To provide additional confidence in results, all samples analysed for *P. fluviatilis* used both assays in parallel. This approach was implemented during the Covid-19 pandemic surveillance and can be considered the 'gold standard' for biosecurity or health applications. Samples were only considered positive for *P. fluviatilis* if detections are recorded in both assays. Any

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samples with equivocal results were still re-analysed with both assays. If a sample returns a negative result on the re-run, it is considered negative.

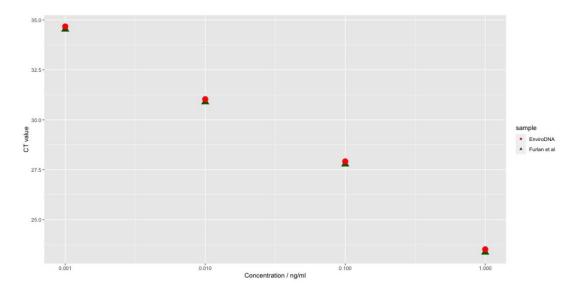


Figure 2. qPCR cycles values (Ct) for detection of redfin DNA at various concentrations using the original 12S probe (Furlan and Gleeson 2016) and the ND5 probe (EnviroDNA).

#### Quality assurance

No contamination was detected in either lab (extraction and PCR) or field controls. Positive controls using serial dilutions of extracted tissue DNA for each species returned positive detections and high R<sup>2</sup> values indicating species probes performed as expected.



#### **Findings**

#### Perca fluviatilis

A total of 220 samples from 79 sites were analysed for P. fluviatilis eDNA using two assays. The target DNA was positively detected at 3 sites within Talbingo Reservoir and 1 site in the downstream end of the Middle Murrumbidgee (Figure 3, Appendix 1). These are sites where the species is known to occur (Weeks et~al.~2019; Griffiths et~al.~2020,~2021,~2022) and there was good agreement between the two assays. One site within the Tantangara Reservoir returned an equivocal result from the 16S assay only with the same result achieved on a reanalysis of the sample. To provide further confidence around this result, the site and surrounding areas were re-sampled in June 2025 (n=14 samples). All samples returned negative results for both P. fluviatilis assays (Figure 4, Appendix 2). There was no evidence of P. fluviatilis presence Lake Eucumbene (n=16), the Upper Murrumbidgee catchment (n=16), or Middle Murrumbidgee catchment upstream of Angle Crossing (n=34).

#### Gambusia holbrooki

A total of 192 samples from 72 sites were analysed for *G. holbrooki* eDNA. Positive detections of *G. holbrooki* were recorded at 16 sites throughout the Middle Murrumbidgee catchment (Figure 5, Appendix 1) where the species has been consistently detected previously (Weeks *et al.* 2019; Griffiths *et al.* 2020, 2021, 2022). No evidence of *G. holbrooki* presence was detected in either Lake Eucumbene (n = 16), Tantangara Reservoir (n = 6), or Talbingo Reservoir (n = 3) subcatchments.

#### Galaxias brevipinnis

A total of 198 samples from 80 sites were analysed for G. brevipinnis eDNA. One positive detection was recorded in Gang Gang Creek (GG01) in the Upper Eucumbene subcatchment where the species is endemic and known to occur (Figure 6, Appendix 1). No evidence of G. brevipinnis presence was detected in any of the Middle Murrumbidgee (n = 38), Tantangara Reservoir (n = 13), Talbingo Reservoir (n = 3), or Upper Murrumbidgee (n = 19) subcatchments.

#### Trout species

Brown trout eDNA was positively detected at 7 of the 10 sites surveyed (Figure 6, Appendix 1). Rainbow trout eDNA was positively detected at 5 of the 10 sites surveyed with one further site returning an equivocal result (Figure 7, Appendix 1). No brook trout eDNA was detected at any of the sites (Figure 8, Appendix 1). The distributions of brown and rainbow trout appear very similar with detections in Lake Eucumbene, middle Murrumbidgee River, and lower Tantangara Creek. No trout species were detected at the 2 upstream sites in Tantangara Creek above the Tantangara Creek waterfall and within the habitat of *Galaxias tantangara*.



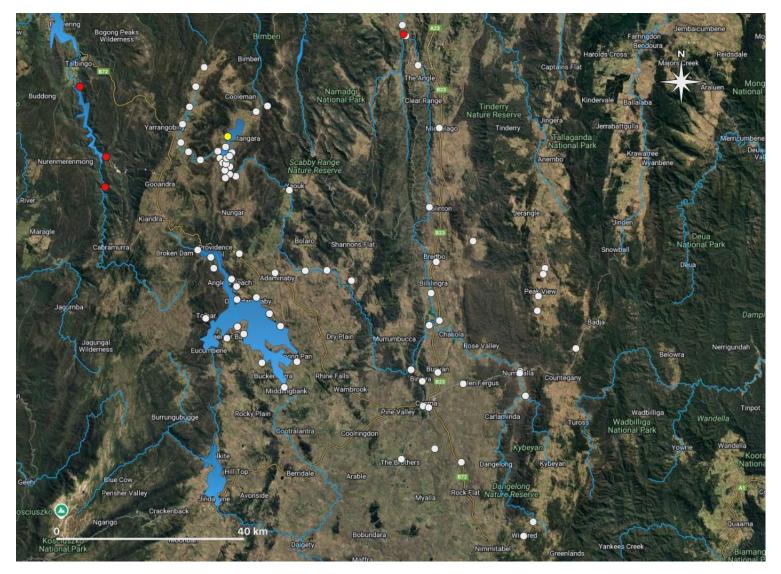


Figure 3. Results of 2025 eDNA surveys for *P. fluviatilis* (red = positive, yellow = equivocal (16S assay only), grey = negative).



Figure 4. Results of follow-up eDNA surveys for *P. fluviatilis* in Tantangara Reservoir (grey = negative).

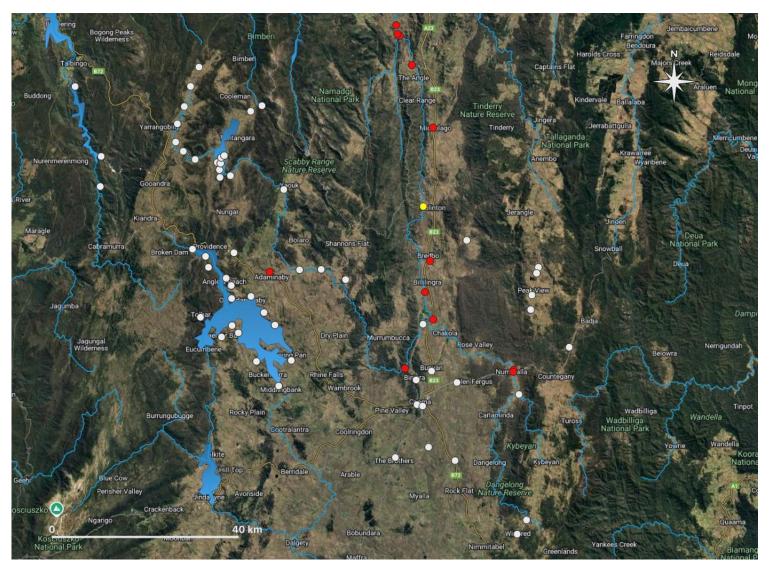


Figure 5. Results of 2025 eDNA surveys for *G. holbrooki* (red = positive, yellow = equivocal, grey = negative).

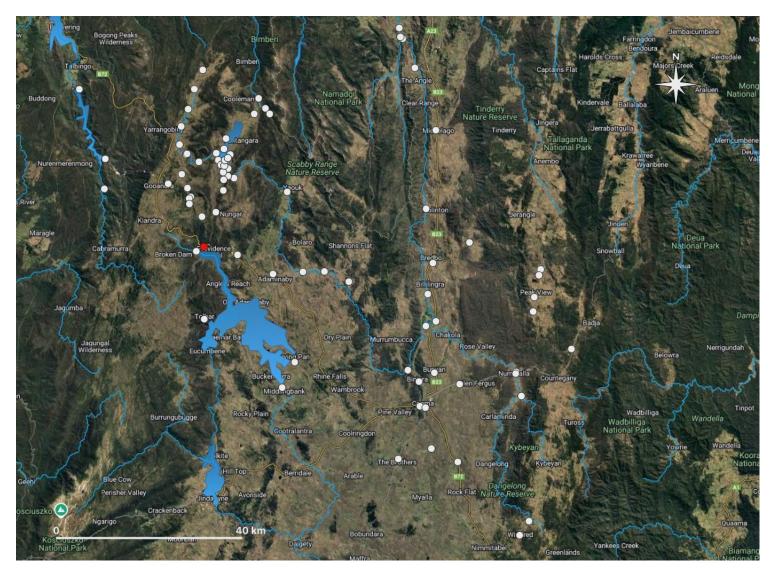


Figure 6. Results of 2025 eDNA surveys for *G. brevipinnis* (red = positive, yellow = equivocal, grey = negative).

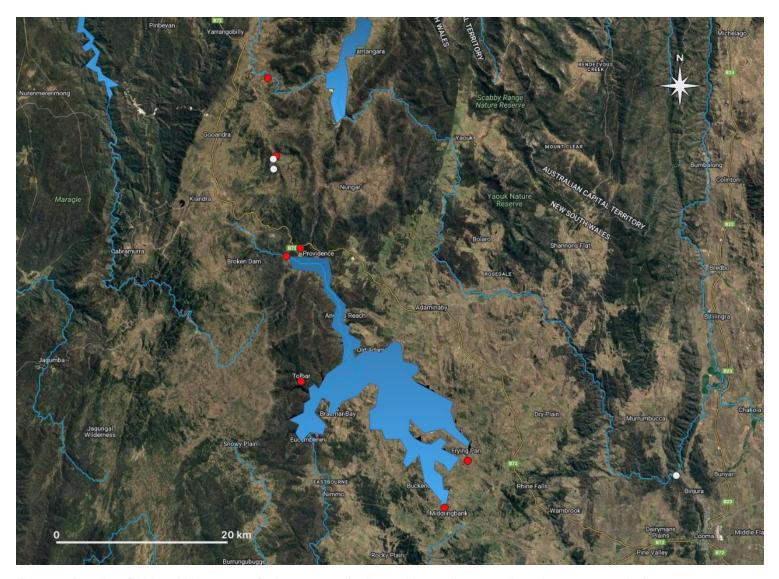


Figure 7. Results of 2025 eDNA surveys for brown trout (red = positive, yellow = equivocal, grey = negative).

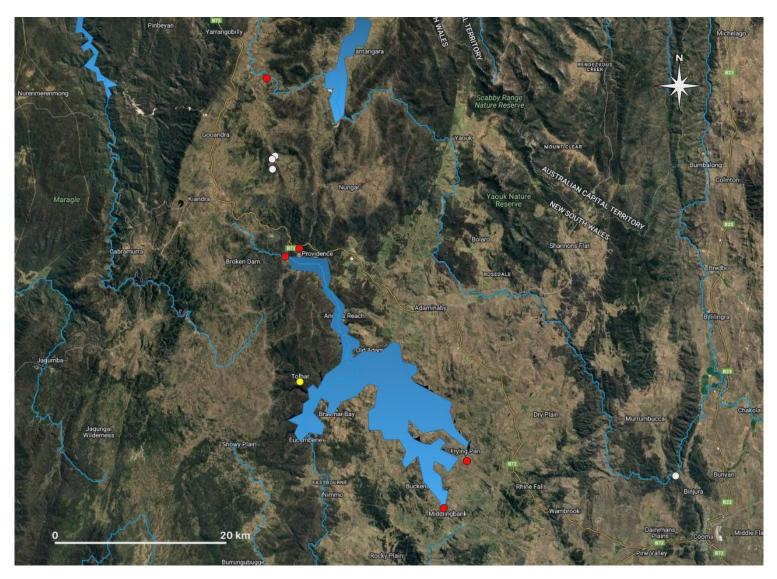


Figure 8. Results of 2025 eDNA surveys for rainbow trout (red = positive, yellow = equivocal, grey = negative).

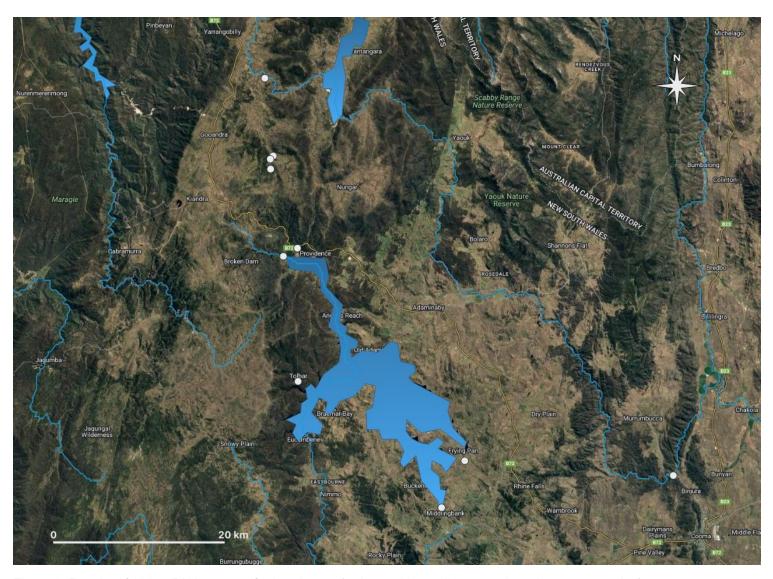


Figure 9. Results of 2025 eDNA surveys for brook trout (red = positive, yellow = equivocal, grey = negative).

#### **Conclusions**

Results from the 2025 eDNA pest fish surveillance program recorded detections of target species where they are expected to occur and no evidence for their presence in subcatchments where they are expected to be absent. *Perca fluviatilis* was detected in Talbingo Reservoir and the most downstream surveyed reach of the Murrumbidgee River. An equivocal detection in one sample from Tantangara Reservoir was not supported by the second assay and could not be repeated with further sampling. Widespread detections of *Gambusia holbrooki* were recorded throughout waterways of the Middle Murrumbidgee. *Galaxias brevipinnis* was only detected at one site in the Upper Eucumbene catchment where the species is expected to occur. The current surveys found no evidence for incursions of the three target species outside of their expected distributions.

Results from the surveys for trout species at a limited subset of sites found no evidence of encroachment into critical *Galaxias tantangara* habitat.

#### **Acknowledgments**

EnviroDNA thank Elizabeth Pope (Snowy Hydro) for project support and comments on this report and Mic Clayton (Snowy Hydro) for help with site access and water sampling field work. We also thank Austral Research and Consulting staff for collecting samples at sites throughout the Talbingo, Tantangara, and Upper Murrumbidgee regions.



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#### Appendix 1. Site details and results of eDNA analysis for target species.

Site	Catchment	Waterway	Latitude	Longitude	Collection date	Volume filtered (ml)	Perca fluviatilis 12S	Perca fluviatilis ND5	Gambusia holbrooki	Galaxias brevipinnis
TL1C	Talbingo Reservoir	Tumut River	-35.7623	148.3688	13/2/2025	12000	positive (12/12)	positive (12/12)	negative (0/12)	negative (0/12)
SUE1	Talbingo Reservoir	Talbingo Reservoir	-35.8204	148.3665	13/2/2025	12000	positive (11/12)	positive (11/12)	negative (0/12)	negative (0/12)
TAL1	Talbingo Reservoir	Talbingo Reservoir	-35.6274	148.3068	13/2/2025	1400	positive (6/12)	positive (7/12)	negative (0/12)	negative (0/12)
GG1C	Upper Eucumbene	Gang Gang Ck	-35.93246	148.60421	16/4/2024	5880				positive (6/6)
LE1	Lake Eucumbene	Lake Eucumbene	-35.941	148.586	17/4/2024	5160	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
LE2	Lake Eucumbene	Lake Eucumbene	-35.955	148.617	17/4/2024	3160	negative (0/12)	negative (0/12)	negative (0/12)	
LE3	Lake Eucumbene	Lake Eucumbene	-35.976	148.624	17/4/2024	6900	negative (0/12)	negative (0/12)	negative (0/12)	
LE4	Lake Eucumbene	Lake Eucumbene	-35.997	148.666	17/4/2024	9990	negative (0/12)	negative (0/12)	negative (0/12)	
LE5	Lake Eucumbene	Lake Eucumbene	-36.011	148.678	17/4/2024	10050	negative (0/12)	negative (0/12)	negative (0/12)	
LE6	Lake Eucumbene	Lake Eucumbene	-36.036	148.679	17/4/2024	10020	negative (0/12)	negative (0/12)	negative (0/12)	
LE7	Lake Eucumbene	Lake Eucumbene	-36.032	148.725	17/4/2024	6450	negative (0/12)	negative (0/12)	negative (0/12)	
LE8	Lake Eucumbene	Lake Eucumbene	-36.063	148.756	17/4/2024	9970	negative (0/12)	negative (0/12)	negative (0/12)	
LE9	Lake Eucumbene	Lake Eucumbene	-36.087	148.782	17/4/2024	9880	negative (0/12)	negative (0/12)	negative (0/12)	
LE10	Lake Eucumbene	Lake Eucumbene	-36.155	148.821	17/4/2024	10020	negative (0/12)	negative (0/12)	negative (0/12)	negative (0/12)
LE11	Lake Eucumbene	Lake Eucumbene	-36.204	148.791	17/4/2024	9930	negative (0/12)	negative (0/12)	negative (0/12)	negative (0/12)
LE12	Lake Eucumbene	Lake Eucumbene	-36.157	148.738	17/4/2024	10080	negative (0/12)	negative (0/12)	negative (0/12)	
LE13	Lake Eucumbene	Lake Eucumbene	-36.102	148.695	17/4/2024	10110	negative (0/12)	negative (0/12)	negative (0/12)	







Site	Catchment	Waterway	Latitude	Longitude	Collection date	Volume filtered (ml)	Perca fluviatilis 12S	Perca fluviatilis ND5	Gambusia holbrooki	Galaxias brevipinnis
LE14	Lake Eucumbene	Lake Eucumbene	-36.088	148.68	17/4/2024	8330	negative (0/12)	negative (0/12)	negative (0/12)	
LE15	Lake Eucumbene	Lake Eucumbene	-36.11	148.655	17/4/2024	10010	negative (0/12)	negative (0/12)	negative (0/12)	
LE16	Lake Eucumbene	Lake Eucumbene	-36.072	148.605	17/4/2024	7530	negative (0/12)	negative (0/12)	negative (0/12)	
BC1	Upper Murrumbidgee		-35.8741	148.5996	12/2/2025	2000				negative (0/6)
GR1	Upper Murrumbidgee		-35.645	148.7348	17/2/2025	2000				negative (0/6)
GR2	Upper Murrumbidgee		-35.6644	148.7511	17/2/2025	2000	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
GR3	Upper Murrumbidgee		-35.6752	148.7613	17/2/2025	2000				negative (0/6)
GU1	Upper Murrumbidgee		-35.6752	148.7244	17/2/2025	2000	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
TA1	Upper Murrumbidgee		-35.7541	148.562	10/2/2025	2000				negative (0/6)
TA2	Upper Murrumbidgee		-35.7921	148.5513	10/2/2025	2000				negative (0/6)
TA3	Upper Murrumbidgee		-35.8188	148.565	11/2/2025	2000				negative (0/6)
TA4	Upper Murrumbidgee	Tantangara Creek	-35.8357	148.5728	12/2/2025	2000				negative (0/6)
TA5	Upper Murrumbidgee	Tantangara Creek	-35.839	148.569	20/2/2025	2000				negative (0/6)
TA6	Upper Murrumbidgee	Tantangara Creek	-35.8495	148.5695	20/2/2025	2000				negative (0/6)
Tat1	Upper Murrumbidgee		-35.8105	148.5188	10/2/2025	2000				negative (0/6)
UMU1	Upper Murrumbidgee		-35.7681	148.5923	10/2/2025	2000	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
UMU2	Upper Murrumbidgee		-35.7525	148.564	10/2/2025	2000	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
UMU3	Upper Murrumbidgee		-35.7346	148.546	11/2/2025	2000	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
UMU4	Upper Murrumbidgee		-35.6994	148.55	9/2/2025	2000	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)







Site	Catchment	Waterway	Latitude	Longitude	Collection date	Volume filtered (ml)	Perca fluviatilis 12S	Perca fluviatilis ND5	Gambusia holbrooki	Galaxias brevipinnis
UMU5	Upper Murrumbidgee		-35.666	148.5665	9/2/2025	2000	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
UMU6	Upper Murrumbidgee		-35.6271	148.5813	9/2/2025	2000	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
UMU7	Upper Murrumbidgee		-35.5899	148.6014	9/2/2025	2000	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
16	Upper Murrumbidgee	Nungar Creek	-35.865439	148.63270	14/2/2025	2000				negative (0/6)
23	Upper Murrumbidgee	Kelly's Plain Creek	-35.823311	148.65051	14/2/2025	2000				negative (0/6)
TRA1	Tantangara Reservoir	Tantangara Reservoir	-35.7755	148.6528	21/2/2025	4000	negative (0/12)	negative (0/12)	negative (0/12)	negative (0/12)
TRA2	Tantangara Reservoir	Tantangara Reservoir	-35.7647	148.6543	21/2/2025	3600	negative (0/12)	negative (0/12)	negative (0/12)	negative (0/12)
TRA3	Tantangara Reservoir	Tantangara Reservoir	-35.7607	148.6614	21/2/2025	4000	negative (0/12)	negative (0/12)	negative (0/12)	negative (0/12)
TR1	Tantangara Reservoir	Tantangara Reservoir	-35.788	148.65	21/2/2025	4000	negative (0/12)	negative (0/12)	negative (0/12)	negative (0/12)
TR2	Tantangara Reservoir	Tantangara Reservoir	-35.774	148.645	21/2/2025	4000	negative (0/12)	negative (0/12)	negative (0/12)	negative (0/12)
TR3	Tantangara Reservoir	Tantangara Reservoir	-35.7648	148.6385	19/2/2025	12000	negative (0/12)	negative (0/12)		negative (0/12)
TR4	Tantangara Reservoir	Tantangara Reservoir	-35.7513	148.6337	19/2/2025	12000	negative (0/12)	negative (0/12)		negative (0/12)
TR5	Tantangara Reservoir	Tantangara Reservoir	-35.743	148.652	19/2/2025	4000	negative (0/12)	negative (0/12)		negative (0/12)
TR6	Tantangara Reservoir	Tantangara Reservoir	-35.723	148.657	19/2/2025	3400	equivocal (1/12)	negative (0/12)		negative (0/12)
TR10	Tantangara Reservoir	Tantangara Reservoir	-35.754	148.667	19/2/2025	3200	negative (0/12)	negative (0/12)		negative (0/12)
TR11	Tantangara Reservoir	Tantangara Reservoir	-35.781	148.662	20/2/2025	4000	negative (0/12)	negative (0/12)		negative (0/12)
TR12	Tantangara Reservoir	Tantangara Reservoir	-35.795577	148.66145	20/2/2025	4000	negative (0/12)	negative (0/12)		negative (0/12)
TR13	Tantangara Reservoir	Tantangara Reservoir	-35.80275	148.65132	21/2/2025	4000	negative (0/12)	negative (0/12)	negative (0/12)	negative (0/12)
ALUM1	Middle Murrumbidgee	Alum Creek	-35.999664	148.95011	23/2/2025	2550	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)







Site	Catchment	Waterway	Latitude	Longitude	Collection date	Volume filtered (ml)	Perca fluviatilis 12S	Perca fluviatilis ND5	Gambusia holbrooki	Galaxias brevipinnis
Badja1	Middle Murrumbidgee	Big Badja River	-36.173513	149.34946	23/2/2025	5720	negative (0/6)	negative (0/6)	positive (3/6)	negative (0/6)
Badja2	Middle Murrumbidgee	Big Badja River	-36.12959	149.48159	23/2/2025	5690	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
Bred01	Middle Murrumbidgee	Bredbo River	-35.963709	149.15091 1	24/2/2025	3120	negative (0/6)	negative (0/6)	positive (6/6)	negative (0/6)
Bred03	Middle Murrumbidgee	Bredbo River	-35.976	149.4083	23/2/2025	5470	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
CAM01	Middle Murrumbidgee	Camerons Creek	-35.94797	148.68453	23/2/2025	800	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
CBC1	Middle Murrumbidgee	Cooma Back Creek	-36.239848	149.11985 4	24/2/2025	2460	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
CBC2	Middle Murrumbidgee	Cooma Back Creek	-36.34106	149.0683	24/2/2025	5330	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
Cele01	Middle Murrumbidgee	Celeys Creek	-35.987278	149.40407	23/2/2025	5340	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
COOMA 1	Middle Murrumbidgee	Cooma Creek	-36.192411	149.11767 9	25/2/2025	2260	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
Cooma2	Middle Murrumbidgee	Cooma Creek	-36.24247	149.1332	24/2/2025	3600	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
Cooma3	Middle Murrumbidgee	Cooma Creek	-36.321326	149.14729	24/2/2025	1620	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
Cooma4	Middle Murrumbidgee	Cooma Creek	-36.17537	149.15443	25/2/2025	3310	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
Cowra2	Middle Murrumbidgee	Cowra Creek	-36.05743	149.39019	23/2/2025	4340	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
GOOR01	Middle Murrumbidgee	Goorudee River	-35.98488	148.7691	24/2/2025	2660	negative (0/6)	negative (0/6)	positive (6/6)	negative (0/6)
Gudg01	Middle Murrumbidgee	Gudgenby River	-35.525942	149.07333	24/2/2025	5000	positive (2/6)	equivocal (1/6)	positive (6/6)	negative (0/6)
Kybeyan 1	Middle Murrumbidgee	Kybeyan River	-36.36386	149.42213	24/2/2025	2540	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
Kybeyan 2	Middle Murrumbidgee	Kybeyan River	-36.2202	149.36223	24/2/2025	4050	negative (0/6)	negative (0/6)	positive (6/6)	negative (0/6)
Mich01	Middle Murrumbidgee	Michelago Creek	-35.706686	149.15791	24/2/2025	3140	negative (0/6)	negative (0/6)	positive (6/6)	negative (0/6)
MR01	Middle Murrumbidgee	Murrumbidgee Rv	-35.7995	148.6762	14/2/2025	2000	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)







Site	Catchment	Waterway	Latitude	Longitude	Collection date	Volume filtered (ml)	Perca fluviatilis 12S	Perca fluviatilis ND5	Gambusia holbrooki	Galaxias brevipinnis
MR03	Middle Murrumbidgee	Murrumbidgee Rv	-35.82624	148.80301	25/2/2025	4890	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
MR06	Middle Murrumbidgee	Murrumbidgee Rv	-35.980784	148.84063	23/2/2025	5020	negative (0/6)	negative (0/6)	positive (6/6)	negative (0/6)
MR06a	Middle Murrumbidgee	Murrumbidgee Rv	-35.979951	148.89195	23/2/2025	3330	negative (0/6)	negative (0/6)	positive (6/6)	negative (0/6)
MR08	Middle Murrumbidgee	Murrumbidgee Rv	-36.170377	149.09115 1	25/2/2025	5720	negative (0/6)	negative (0/6)	positive (2/6)	negative (0/6)
MR09a	Middle Murrumbidgee	Murrumbidgee Rv	-36.02368	149.13857	24/2/2025	910	negative (0/6)	negative (0/6)	positive (4/6)	negative (0/6)
MR09b	Middle Murrumbidgee	Murrumbidgee Rv	-36.08527	149.13405	25/2/2025	3740	negative (0/6)	negative (0/6)	positive (6/6)	negative (0/6)
MR10	Middle Murrumbidgee	Murrumbidgee Rv	-36.076175	149.15817 7	25/2/2025	3370	negative (0/6)	negative (0/6)	positive (3/6)	negative (0/6)
MR11	Middle Murrumbidgee	Murrumbidgee Rv	-35.883118	149.12699	24/2/2025	2520	negative (0/6)	negative (0/6)	equivocal (1/6)	negative (0/6)
MR12.1	Middle Murrumbidgee	Murrumbidgee Rv	-35.58589	149.10778	24/2/2025	1540	negative (0/6)	negative (0/6)	positive (6/6)	negative (0/6)
MR15	Middle Murrumbidgee	Murrumbidgee Rv	-35.52915	149.0789	24/2/2025	2200	negative (0/6)	negative (0/6)	positive (4/6)	negative (0/6)
MR16	Middle Murrumbidgee	Murrumbidgee Rv	-35.50839	149.0706	24/2/2025	2570	negative (0/6)	negative (0/6)	positive (6/6)	negative (0/6)
NUM2	Middle Murrumbidgee	Numeralla River	-36.176884	149.34853	23/2/2025	5380	negative (0/6)	negative (0/6)	positive (6/6)	negative (0/6)
NumCk0 1	Middle Murrumbidgee	Numeralla Creek	-36.4607	149.3803	23/2/2025	5510	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
RFC1	Middle Murrumbidgee	Rock Flat Creek	-36.19728	149.21468	24/2/2025	5330	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
RFC2	Middle Murrumbidgee	Rock Flat Creek	-36.34698	149.21026	24/2/2025	3060	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
SaL02	Middle Murrumbidgee	Strike-a-light River	-35.92401	149.23806	24/2/2025	2490	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
Scott01	Middle Murrumbidgee	Scotts Creek	-36.487742	149.35795	24/2/2025	1470	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)
Yell01	Middle Murrumbidgee	Yellow Creek	-36.029638	149.39286	24/2/2025	2930	negative (0/6)	negative (0/6)	negative (0/6)	negative (0/6)





Site	Catchment	Waterway	Latitude	Longitud e	Collectio n date	Volume filtered (L)	Brown trout	Rainbow trout	Brook trout
GG1C	Upper Eucumbene	Gang Gang Ck	-35.93246	148.60421	12/2/2025	2000	positive (6/6)	positive (6/6)	negative (0/6)
LE1	Lake Eucumbene	Lake Eucumbene	-35.941	148.586	24/2/2025	5160	positive (6/6)	positive (6/6)	negative (0/6)
LE10	Lake Eucumbene	Lake Eucumbene	-36.155	148.821	25/2/2025	1020	positive (9/12)	positive (5/12)	negative (0/12)
LE11	Lake Eucumbene	Lake Eucumbene	-36.204	148.791	25/2/2025	9930	positive (8/12)	positive (5/12)	negative (0/12)
LE16	Lake Eucumbene	Lake Eucumbene	-36.072	148.605	17/4/2024	7530	equivocal (1/12)	equivocal (1/12)	negative (0/12)
TA1	Upper Murrumbidgee		-35.7541	148.562	10/2/2025	2000	positive (6/6)	positive (6/6)	negative (0/6)
TA4	Upper Murrumbidgee	Tantangara Creek	-35.8357	148.5728	12/2/2025	2000	positive (6/6)	negative (0/6)	negative (0/6)
TA5	Upper Murrumbidgee	Tantangara Creek	-35.839	148.569	20/2/2025	2000	negative (0/6)	negative (0/6)	negative (0/6)
TA6	Upper Murrumbidgee	Tantangara Creek	-35.8495	148.5695	20/2/2025	2000	negative (0/6)	negative (0/6)	negative (0/6)
MR08	Middle Murrumbidgee	Murrumbidgee Rv	-36.170377	149.09115 1	25/2/2025	5720	negative (0/6)	negative (0/6)	negative (0/6)



# Appendix 2. Site details and results of eDNA analysis for follow-up *P. fluviatilis* surveys in Tantangara Reservoir.

Site	Catchment	Waterway	Latitude	Longitude	Collection date	Volume filtered (ml)	Perca fluviatilis 12S	Perca fluviatilis ND5
TR-1	Tantangara Reservoir	Tantangara Reservoir	-35.788	148.65	6/3/2025	2000	negative (0/3)	negative (0/3)
TR-10	Tantangara Reservoir	Tantangara Reservoir	-35.754	148.667	6/3/2025	600	negative (0/3)	negative (0/3)
TR-10A	Tantangara Reservoir	Tantangara Reservoir	-35.754	148.667	6/3/2025	1200	negative (0/3)	negative (0/3)
TR-11	Tantangara Reservoir	Tantangara Reservoir	-35.781	148.662	6/3/2025	2000	negative (0/3)	negative (0/3)
TR-12	Tantangara Reservoir	Tantangara Reservoir	-35.79558	148.66145	6/3/2025	2000	negative (0/3)	negative (0/3)
TR-13	Tantangara Reservoir	Tantangara Reservoir	-35.80275	148.6513	6/3/2025	1200	negative (0/3)	negative (0/3)
TR-2	Tantangara Reservoir	Tantangara Reservoir	-35.774	148.645	6/3/2025	2000	negative (0/3)	negative (0/3)
TR-3	Tantangara Reservoir	Tantangara Reservoir	-35.76477	148.63851	6/3/2025	2000	negative (0/3)	negative (0/3)
TR-4	Tantangara Reservoir	Tantangara Reservoir	-35.75127	148.63374	6/3/2025	2000	negative (0/3)	negative (0/3)
TR-5	Tantangara Reservoir	Tantangara Reservoir	-35.743	148.652	6/3/2025	2000	negative (0/3)	negative (0/3)
TR-6	Tantangara Reservoir	Tantangara Reservoir	-35.723	148.657	6/3/2025	2000	negative (0/3)	negative (0/3)
TR-A1	Tantangara Reservoir	Tantangara Reservoir	-35.77552	148.65276	6/3/2025	1800	negative (0/3)	negative (0/3)
TR-A2	Tantangara Reservoir	Tantangara Reservoir	-35.76475	148.65425	6/3/2025	1700	negative (0/3)	negative (0/3)
TR-A3	Tantangara Reservoir	Tantangara Reservoir	-35.7607	148.66136 6	6/3/2025	2000	negative (0/3)	negative (0/3)



#### Appendix 3. Negative controls collected to assess for field sampling contamination.

Site	Catchment	Waterway	Latitude	Longitud e	Collectio n date	Volume filtered (L)	Perca fluviatilis 12S	Perca fluviatilis ND5	Gambusia holbrooki	Galaxias brevipinnis
EC- MR06	Middle Murrumbidgee	Murrumbidgee Rv	-35.9808	148.8406	23/2/2025	590			negative (0/6)	
EC- MR06a	Middle Murrumbidgee	Murrumbidgee Rv	-35.98	148.892	23/2/2025	570			negative (0/6)	
EC- MR09b	Middle Murrumbidgee	Murrumbidgee Rv	-36.08527	149.1340 5	25/2/2025	580			negative (0/6)	
EC- MR15	Middle Murrumbidgee	Murrumbidgee Rv	-35.52915	149.0789	24/2/2025	540	negative (0/6)	negative (0/6)		
EC- MR16	Middle Murrumbidgee	Murrumbidgee Rv	-35.50839	149.0706	24/2/2025	510	negative (0/6)	negative (0/6)		

Site	Catchment	Waterway	Latitude	Longitud e	Collection date	Volume filtered (L)	Brown trout	Rainbow trout	Brook trout
EC-LE7	Lake Eucumbene	Lake Eucumbene	-36.032	148.725	25/2/2024	650	negative (0/6)	negative (0/6)	negative (0/6)



# Appendix C

# 2025 EHNV Sample Collection Protocol





#### Appendix C – 2025 EHNV Sample Collection Protocol

This protocol outlines the sample collection process that was followed by the contractor in February 2025 to achieve compliance with the Disease Surveillance component of the Snowy 2.0 Biosecurity Risk Management Plan.

#### **Sample Collection**

Personal and public safety are the key priority at all times. Sampling will only occur when it is safe to do so and will be in accordance with all safety documentation and legislation.

The target fish species is Redfin. Sampling will be biased towards young of year (YOY) and juvenile fish. Best efforts will be made to lethally sample the numbers of fish presented in Table 4 for EHNV analysis using qPCR testing and serology.

Fish collection will be undertaken using electrofishing, netting or other methods of capturing fish that aim to collect fish that may be elusive and/or are not necessarily feeding. The collection of unwell or injured fish or fish showing unusual external signs (including signs of disease) will be prioritised.

Table 4: Surveillance design for detecting EHNV in Talbingo reservoir.

Location	Water Temperature	Time of Year	No. of samples and species targeted for lethal testing by qPCR and serology
Talbingo Reservoir	>19°C	Dec- Feb	Redfin YOY (n ≥ 156) (qPCR testing only)
Talbingo Reservoir	>19°C	Dec- Feb	Redfin juveniles or adults (n ≥ 156)

Every fish collected will be visually examined for the presence of Lernaea. Non-lethal sampling of desirable species will involve brief, gentle handling of freshly captured fish including close visual observation of the entire body surface (including fins and under opercula) prior to their release at the site of capture.

In order to confirm a positive detection in an area where the parasite is not already confirmed to occur (which includes Talbingo), where appropriate, voucher specimens will be dissected from the infected fish, and fixed in 70% ethanol before being sent to an appropriate person/facility for official identification. If the affected fish species is uncommon or rare, photographs may be taken in lieu of a sample.

#### **Geographic Partitioning and effort**

The reservoir will be partitioned into at least 10 units with fish in each unit to be collected and bagged separately (Figure 5). Fish from each unit will be analysed to account for disease clustering.

The level of sampling effort applied to each area will be at the discretion of the ecologist undertaking the works. As an indication, each area will be sampled for up to one full day or until at least 16-30 fish of each age class are collected.

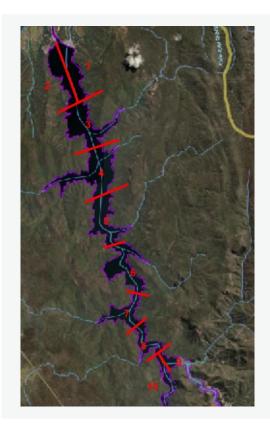
If more than the required number of Redfin are collected, additional fish will be analysed for qPCR using pooled sampling. If insufficient Redfin of each age class can be collected, other age classes will be substituted.

#### Record keeping and reporting

Records of all Redfin are to be collected and provided in a table indicating their sample number, age class, analysis type, the geographic area they were collected from and whether Lernaea was present. Records of other fish species observed are also to be made.

The level of effort (hours in area, methods used, equipment settings, on time etc. are all to be recorded and provided to Snowy Hydro.

Each day, an email will be sent to Snowy Hydro detailing the activities and number of fish caught that day and the plan for the following day.



Tal01	Talbingo Dam Area
Tal02	Talbingo Spillway Area
Tal03	Landers Creek Arm
Tal04	Honeysuckle Creek Arm
Tal05	Lick Hole Creek Arm
Tal06	Glendower Creek Arm
Tal07	Long Creek Arm
Tal08	Plain Creek Arm
Tal09	Yarrangobilly River Arm
Tal010	Tumut River Arm

Figure 5: Indicative geographic partitioning of Talbingo Reservoir

#### **EHNV Sample Preparation**

#### **qPCR**

Fish will be euthanised and placed in household snaplock bags, or equivalent, with identifier labels both inside and outside of the sample bag and frozen.

Every fish will be given a unique identifier including the geographical area it was collected from.

Small Juvenile fish may be bagged together when collected from the same location

Frozen fish will be sent to the EMAI every Monday in an esky.

The COC will advise if individual fish are to be analysed individually, analysed in a pooled sample or retained for potential future analysis.

As a general rule, for qPCR sampling Fish 1-16 from each age class and each area will be analysed with any additional fish to be retained in storage by the EMAI until the completion of sampling. At this time, any additional fish will be analysed at random individually to make up a total of 156 individual samples with the remainder pooled into groups of 5 and analysed.

Similarly, for the blood sampling the first 16 samples of adult fish from each geographic area will be analysed, while the remainder will be held until the completion of sampling to determine if analysis is required to meet the target number of samples.

#### Serology

Blood samples will be attempted to be collected from all adult fish captured prior to euthaniasia (these same fish will also be retained for qPCR testing)

1-2mL of blood will be attempted to be collected in each sample using 0.75" needles and stored in paediatric blood tubes. The samples do not need to be centrifuged. If more than 0.2mL can be collected, samples with will still be sent for analysis.

Samples will be refrigerated, not frozen, and sent to the EMAI in an esky with an ice brick (or frozen fish) (insulation e.g. bubble wrap between blood tubes and ice to prevent freezing)

Samples will be shipped on a Monday and Wednesday or Thursday using an overnight courier if available with a view to arriving at the EMAI chilled and within 5 days of collection.

## Appendix D

# EHNV and Lernaea Results



Batch	Date Collected	Date Shipped	Collection Method	Site No	Site Name	Fish Type	Length	Weight	qPCR Sample No.	EHNV Real-time PCR Result	ELISA Sample No.	EHNV Antibody ELISA Result	Lernaea?
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	214	160	M25-01605/0013	Negative			No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	264	313	M25-01605/0014	Positive			No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	251	251	M25-01605/0015	Negative			No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	243	224	M25-01605/0016	Negative	M25-01605/0001	Negative	No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	190	94	M25-01605/0017	Positive			No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	199	123	M25-01605/0018	Negative	M25-01605/0002	Negative	No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	223	179	M25-01605/0019	Negative	M25-01605/0003	Unsuitable	No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	186	99	M25-01605/0020	Negative	M25-01605/0004	Negative	No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	185	90	M25-01605/0021	Negative	M25-01605/0005	Negative	No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	162	57	M25-01605/0022	Negative	M25-01605/0006	Negative	No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	196	119	M25-01605/0023	Negative	M25-01605/0007	Negative	No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	177	84	M25-01605/0024	Negative			No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	239	207	M25-01605/0025	Negative	M25-01605/0008	Negative	No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	165	69	M25-01605/0026	Negative	M25-01605/0009	Negative	No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	216	153	M25-01605/0027	Negative	M25-01605/0010	Negative	No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	172	82	M25-01605/0028	Negative	M25-01605/0011	Negative	No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	154	48	M25-01605/0029	Negative	M25-01605/0012	Negative	No Lernaea
1	2/4/2025	2/5/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	135	35	M25-01605/0030	Negative			No Lernaea
2	2/5/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	220	165	M25-01806/0027	Negative			No Lernaea

Batch	Date Collected	Date Shipped	Collection Method	Site No	Site Name	Fish Type	Length	Weight	qPCR Sample No.	EHNV Real-time PCR Result	ELISA Sample No.	EHNV Antibody ELISA Result	Lernaea?
2	2/5/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	181	84	M25-01806/0028	Negative			No Lernaea
2	2/5/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	190	110	M25-01806/0029	Negative			No Lernaea
2	2/5/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	198	115	M25-01806/0030	Negative			No Lernaea
2	2/5/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	184	83	M25-01806/0031	Negative			No Lernaea
2	2/8/2025	2/10/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	151	51	M25-01806/0013	Negative	M25-01806/0001	Negative	No Lernaea
2	2/8/2025	2/10/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	160	60	M25-01806/0014	Negative	M25-01806/0002	Negative	No Lernaea
2	2/8/2025	2/10/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	164	61	M25-01806/0015	Negative			No Lernaea
2	2/8/2025	2/10/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	183	104	M25-01806/0016	Negative	M25-01806/0003	Negative	No Lernaea
2	2/8/2025	2/10/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	163	59	M25-01806/0017	Negative	M25-01806/0004	Negative	No Lernaea
2	2/8/2025	2/10/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	184	84	M25-01806/0018	Negative	M25-01806/0005	Negative	No Lernaea
2	2/8/2025	2/10/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	135	36	M25-01806/0019	Negative	M25-01806/0006	Negative	No Lernaea
2	2/8/2025	2/10/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	164	58	M25-01806/0020	Negative	M25-01806/0007	Negative	No Lernaea
2	2/8/2025	2/10/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	184	88	M25-01806/0021	Negative	M25-01806/0008	Negative	No Lernaea
2	2/8/2025	2/10/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	184	99	M25-01806/0022	Negative	M25-01806/0009	Negative	No Lernaea
2	2/8/2025	2/10/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	174	75	M25-01806/0023	Negative	M25-01806/0010	Negative	No Lernaea
2	2/8/2025	2/10/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	182	92	M25-01806/0024	Negative	M25-01806/0011	Negative	No Lernaea
2	2/8/2025	2/10/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	170	73	M25-01806/0025	Negative			No Lernaea
2	2/8/2025	2/10/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	166	68	M25-01806/0026	Negative	M25-01806/0012	Negative	No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	256	203	M25-01806/0032	Negative	M25-01806/0044	Negative	No Lernaea

Batch	Date Collected	Date Shipped	Collection Method	Site No	Site Name	Fish Type	Length	Weight	qPCR Sample No.	EHNV Real-time PCR Result	ELISA Sample No.	EHNV Antibody ELISA Result	Lernaea?
2	2/9/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	197	114	M25-01806/0033	Negative	M25-01806/0045	Negative	No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	199	111	M25-01806/0034	Negative	M25-01806/0046	Negative	No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	218	133	M25-01806/0035	Negative	M25-01806/0047	Negative	No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	225	154	M25-01806/0036	Negative	M25-01806/0048	Negative	No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	250	247	M25-01806/0037	Negative	M25-01806/0049	Negative	No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	193	92	M25-01806/0038	Negative			No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	219	138	M25-01806/0039	Negative	M25-01806/0050	Negative	No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	195	100	M25-01806/0040	Negative	M25-01806/0051	Negative	No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	179	71	M25-01806/0041	Negative	M25-01806/0052	Negative	No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	203	119	M25-01806/0042	Negative	M25-01806/0053	Negative	No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	3	Landers Creek Arm	Adult	217	155	M25-01806/0043	Negative	M25-01806/0054	Negative	No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0055	Negative			No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	1	Talbingo Dam Area	Adult	158	60	M25-01806/0056	Negative			No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	1	Talbingo Dam Area	Adult	183	65	M25-01806/0057	Negative	M25-01806/0061	Negative	No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	1	Talbingo Dam Area	Adult	187	102	M25-01806/0058	Negative	M25-01806/0062	Negative	No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	1	Talbingo Dam Area	Adult	111	21	M25-01806/0059	Negative			No Lernaea
2	2/9/2025	2/10/2025	Line Fishing	1	Talbingo Dam Area	Adult	165	67	M25-01806/0060	Negative	M25-01806/0063	Negative	No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0064	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0065	Negative			No Lernaea

Batch	Date Collected	Date Shipped	Collection Method	Site No	Site Name	Fish Type	Length	Weight	qPCR Sample No.	EHNV Real-time PCR Result	ELISA Sample No.	EHNV Antibody ELISA Result	Lernaea?
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0066	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0067	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0068	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0069	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0070	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0071	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0072	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0073	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0074	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0075	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0076	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0077	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0078	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0079	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0080	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0081	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0082	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0083	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0084	Negative			No Lernaea

Batch	Date Collected	Date Shipped	Collection Method	Site No	Site Name	Fish Type	Length	Weight	qPCR Sample No.	EHNV Real-time PCR Result	ELISA Sample No.	EHNV Antibody ELISA Result	Lernaea?
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0085	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0086	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0087	Negative			No Lernaea
2	2/9/2025	2/10/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-01806/0088	Negative			No Lernaea
3	2/10/2025	2/13/2025	Line Fishing	1	Talbingo Dam Area	Adult	200	130	M25-02089/0001	Negative	M25-02089/0011	Negative	No Lernaea
3	2/10/2025	2/13/2025	Line Fishing	1	Talbingo Dam Area	Adult	165	69	M25-02089/0002	Negative			No Lernaea
3	2/10/2025	2/13/2025	Line Fishing	1	Talbingo Dam Area	Adult	172	77	M25-02089/0003	Negative			No Lernaea
3	2/10/2025	2/13/2025	Line Fishing	1	Talbingo Dam Area	Adult	174	84	M25-02089/0004	Negative	M25-02089/0012	Negative	No Lernaea
3	2/10/2025	2/13/2025	Line Fishing	1	Talbingo Dam Area	Adult	205	97	M25-02089/0005	Negative	M25-02089/0013	Negative	No Lernaea
3	2/10/2025	2/13/2025	Line Fishing	1	Talbingo Dam Area	Adult	182	93	M25-02089/0006	Negative	M25-02089/0014	Negative	No Lernaea
3	2/10/2025	2/13/2025	Line Fishing	1	Talbingo Dam Area	Adult	189	99	M25-02089/0007	Negative	M25-02089/0015	Negative	No Lernaea
3	2/10/2025	2/13/2025	Line Fishing	1	Talbingo Dam Area	Adult	175	71	M25-02089/0008	Negative	M25-02089/0016	Negative	No Lernaea
3	2/10/2025	2/13/2025	Line Fishing	1	Talbingo Dam Area	Adult	174	69	M25-02089/0009	Negative	M25-02089/0017	Negative	No Lernaea
3	2/10/2025	2/13/2025	Line Fishing	1	Talbingo Dam Area	Adult	176	79	M25-02089/0010	Negative	M25-02089/0018	Negative	No Lernaea
3	2/10/2025	2/13/2025	Gillnetting	1	Talbingo Dam Area	YOY			M25-02089/0019	Negative			No Lernaea
3	2/10/2025	2/13/2025	Gillnetting	1	Talbingo Dam Area	YOY			M25-02089/0020	Negative			No Lernaea
3	2/11/2025	2/13/2025	Line Fishing	2	Talbingo Spillway Area	Adult	179	88	M25-02089/0021	Negative	M25-02089/0115	Negative	No Lernaea
3	2/11/2025	2/13/2025	Gillnetting	2	Talbingo Spillway Area	Adult	200	122	M25-02089/0022	Negative			No Lernaea
3	2/11/2025	2/13/2025	Gillnetting	2	Talbingo Spillway Area	Adult	267	319	M25-02089/0023	Negative			No Lernaea

Batch	Date Collected	Date Shipped	Collection Method	Site No	Site Name	Fish Type	Length	Weight	qPCR Sample No.	EHNV Real-time PCR Result	ELISA Sample No.	EHNV Antibody ELISA Result	Lernaea?
3	2/11/2025	2/13/2025	Gillnetting	2	Talbingo Spillway Area	YOY			M25-02089/0024	Negative			No Lernaea
3	2/11/2025	2/13/2025	Gillnetting	2	Talbingo Spillway Area	YOY			M25-02089/0025	Negative			No Lernaea
3	2/11/2025	2/13/2025	Gillnetting	2	Talbingo Spillway Area	YOY			M25-02089/0026	Negative			No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	253	226	M25-02089/0027	Negative	M25-02089/0047	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	207	117	M25-02089/0028	Negative	M25-02089/0048	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	225	159	M25-02089/0029	Negative	M25-02089/0049	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	249	215	M25-02089/0030	Negative	M25-02089/0050	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	235	169	M25-02089/0031	Negative	M25-02089/0051	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	175	70	M25-02089/0032	Negative	M25-02089/0052	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	180	73	M25-02089/0033	Negative	M25-02089/0053	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	199	118	M25-02089/0034	Negative	M25-02089/0054	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	184	85	M25-02089/0035	Negative	M25-02089/0055	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	185	93	M25-02089/0036	Negative	M25-02089/0056	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	206	122	M25-02089/0037	Negative	M25-02089/0057	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	183	82	M25-02089/0038	Negative	M25-02089/0058	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	205	119	M25-02089/0039	Negative	M25-02089/0059	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	202	121	M25-02089/0040	Negative	M25-02089/0060	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	194	90	M25-02089/0041	Negative	M25-02089/0061	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	203	119	M25-02089/0042	Negative	M25-02089/0062	Negative	No Lernaea

Batch	Date Collected	Date Shipped	Collection Method	Site No	Site Name	Fish Type	Length	Weight	qPCR Sample No.	EHNV Real-time PCR Result	ELISA Sample No.	EHNV Antibody ELISA Result	Lernaea?
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	204	108	M25-02089/0043	Negative	M25-02089/0063	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	186	90	M25-02089/0044	Negative	M25-02089/0064	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	182	77	M25-02089/0045	Negative	M25-02089/0065	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	7	Long Creek Arm	Adult	197	109	M25-02089/0046	Negative	M25-02089/0066	Negative	No Lernaea
3	2/12/2025	2/13/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02089/0067	Negative			No Lernaea
3	2/12/2025	2/13/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02089/0068	Negative			No Lernaea
3	2/12/2025	2/13/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02089/0069	Negative			No Lernaea
3	2/12/2025	2/13/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02089/0070	Negative			No Lernaea
3	2/12/2025	2/13/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02089/0071	Negative			No Lernaea
3	2/12/2025	2/13/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02089/0072	Negative			No Lernaea
3	2/12/2025	2/13/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02089/0073	Negative			No Lernaea
3	2/12/2025	2/13/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02089/0074	Negative			No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	187	93	M25-02089/0075	Negative	M25-02089/0095	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	200	111	M25-02089/0076	Negative	M25-02089/0096	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	228	198	M25-02089/0077	Negative	M25-02089/0097	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	194	105	M25-02089/0078	Negative	M25-02089/0098	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	204	123	M25-02089/0079	Negative	M25-02089/0099	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	230	167	M25-02089/0080	Negative	M25-02089/0100	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	204	115	M25-02089/0081	Negative	M25-02089/0101	Negative	No Lernaea

Batch	Date Collected	Date Shipped	Collection Method	Site No	Site Name	Fish Type	Length	Weight	qPCR Sample No.	EHNV Real-time PCR Result	ELISA Sample No.	EHNV Antibody ELISA Result	Lernaea?
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	194	105	M25-02089/0082	Negative	M25-02089/0102	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	213	137	M25-02089/0083	Negative	M25-02089/0103	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	182	86	M25-02089/0084	Negative	M25-02089/0104	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	190	94	M25-02089/0085	Negative	M25-02089/0105	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	231	187	M25-02089/0086	Negative	M25-02089/0106	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	182	89	M25-02089/0087	Negative	M25-02089/0107	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	224	145	M25-02089/0088	Negative	M25-02089/0108	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	161	61	M25-02089/0089	Negative	M25-02089/0109	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	189	97	M25-02089/0090	Negative	M25-02089/0110	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	189	99	M25-02089/0091	Negative	M25-02089/0111	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	231	174	M25-02089/0092	Negative	M25-02089/0112	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	251	200	M25-02089/0093	Negative	M25-02089/0113	Negative	No Lernaea
3	2/12/2025	2/13/2025	Line Fishing	6	Glendower Creek Arm	Adult	189	91	M25-02089/0094	Negative	M25-02089/0114	Negative	No Lernaea
4	2/10/2025	2/17/2025	Line Fishing	1	Talbingo Dam Area	Adult	165	61	M25-02339/0003	Negative			No Lernaea
4	2/10/2025	2/17/2025	Line Fishing	1	Talbingo Dam Area	Adult	170	68	M25-02339/0004	Negative			No Lernaea
4	2/10/2025	2/17/2025	Line Fishing	1	Talbingo Dam Area	Adult	178	86	M25-02339/0005	Negative			No Lernaea
4	2/10/2025	2/17/2025	Line Fishing	1	Talbingo Dam Area	Adult	150	39	M25-02339/0006	Negative			No Lernaea
4	2/10/2025	2/17/2025	Line Fishing	1	Talbingo Dam Area	Adult	168	68	M25-02339/0007	Negative			No Lernaea
4	2/10/2025	2/17/2025	Line Fishing	1	Talbingo Dam Area	Adult	158	54	M25-02339/0008	Negative			No Lernaea

Batch	Date Collected	Date Shipped	Collection Method	Site No	Site Name	Fish Type	Length	Weight	qPCR Sample No.	EHNV Real-time PCR Result	ELISA Sample No.	EHNV Antibody ELISA Result	Lernaea?
4	2/12/2025	2/17/2025	Line Fishing	7	Long Creek Arm	Adult	168	60	M25-02339/0001	Negative			No Lernaea
4	2/12/2025	2/17/2025	Line Fishing	7	Long Creek Arm	Adult	168	69	M25-02339/0002	Negative			No Lernaea
4	2/12/2025	2/17/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	189	101	M25-02339/0009	Negative			No Lernaea
4	2/12/2025	2/17/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	165	59	M25-02339/0010	Negative			No Lernaea
4	2/12/2025	2/17/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	227	177	M25-02339/0011	Negative			No Lernaea
4	2/12/2025	2/17/2025	Line Fishing	5	Lick Hole Creek Arm	Adult	176	72	M25-02339/0012	Negative			No Lernaea
4	2/12/2025	2/17/2025	Line Fishing	6	Glendower Creek Arm	Adult	154	45	M25-02339/0013	Negative			No Lernaea
4	2/12/2025	2/17/2025	Line Fishing	6	Glendower Creek Arm	Adult	182	88	M25-02339/0014	Negative			No Lernaea
4	2/12/2025	2/17/2025	Line Fishing	6	Glendower Creek Arm	Adult	183	79	M25-02339/0015	Negative			No Lernaea
4	2/12/2025	2/17/2025	Line Fishing	6	Glendower Creek Arm	Adult	211	143	M25-02339/0016	Negative			No Lernaea
4	2/12/2025	2/17/2025	Line Fishing	6	Glendower Creek Arm	Adult	192	91	M25-02339/0017	Negative			No Lernaea
4	2/13/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	206	115	M25-02339/0044	Negative	M25-02339/0073	Negative	No Lernaea
4	2/13/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	174	67	M25-02339/0045	Negative	M25-02339/0074	Negative	No Lernaea
4	2/13/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	161	59	M25-02339/0046	Negative	M25-02339/0075	Negative	No Lernaea
4	2/13/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	226	109	M25-02339/0047	Negative	M25-02339/0076	Negative	No Lernaea
4	2/13/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	168	66	M25-02339/0048	Negative	M25-02339/0077	Negative	No Lernaea
4	2/13/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	210	123	M25-02339/0049	Negative	M25-02339/0078	Negative	No Lernaea
4	2/13/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	210	116	M25-02339/0050	Negative	M25-02339/0079	Negative	No Lernaea
4	2/13/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	171	72	M25-02339/0051	Negative	M25-02339/0080	Negative	No Lernaea

Batch	Date Collected	Date Shipped	Collection Method	Site No	Site Name	Fish Type	Length	Weight	qPCR Sample No.	EHNV Real-time PCR Result	ELISA Sample No.	EHNV Antibody ELISA Result	Lernaea?
4	2/13/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	242	193	M25-02339/0052	Negative	M25-02339/0081	Negative	No Lernaea
4	2/13/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	253	217	M25-02339/0053	Negative			No Lernaea
4	2/13/2025	2/17/2025	Gillnetting	10	Tumut River Arm	YOY			M25-02339/0054	Negative			No Lernaea
4	2/13/2025	2/17/2025	Gillnetting	10	Tumut River Arm	YOY			M25-02339/0055	Negative			No Lernaea
4	2/13/2025	2/17/2025	Gillnetting	10	Tumut River Arm	YOY			M25-02339/0056	Negative			No Lernaea
4	2/13/2025	2/17/2025	Gillnetting	10	Tumut River Arm	YOY			M25-02339/0057	Negative			No Lernaea
4	2/14/2025	2/17/2025	Line Fishing	9	Yarrangobilly River Arm	Adult	186	83	M25-02339/0018	Negative			No Lernaea
4	2/14/2025	2/17/2025	Line Fishing	9	Yarrangobilly River Arm	Adult	213	127	M25-02339/0019	Negative	M25-02339/0024	Negative	No Lernaea
4	2/14/2025	2/17/2025	Line Fishing	9	Yarrangobilly River Arm	Adult	203	107	M25-02339/0020	Negative	M25-02339/0025	Negative	No Lernaea
4	2/14/2025	2/17/2025	Line Fishing	9	Yarrangobilly River Arm	Adult	180	87	M25-02339/0021	Negative	M25-02339/0026	Negative	No Lernaea
4	2/14/2025	2/17/2025	Line Fishing	9	Yarrangobilly River Arm	Adult	235	169	M25-02339/0022	Negative	M25-02339/0027	Negative	No Lernaea
4	2/14/2025	2/17/2025	Line Fishing	9	Yarrangobilly River Arm	Adult	148	41	M25-02339/0023	Negative			No Lernaea
4	2/15/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	198	103	M25-02339/0028	Negative	M25-02339/0058	Negative	No Lernaea
4	2/15/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	205	110	M25-02339/0029	Negative	M25-02339/0059	Negative	No Lernaea
4	2/15/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	174	63	M25-02339/0030	Negative	M25-02339/0060	Negative	No Lernaea
4	2/15/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	269	290	M25-02339/0031	Negative	M25-02339/0061	Negative	No Lernaea
4	2/15/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	208	119	M25-02339/0032	Negative	M25-02339/0062	Negative	No Lernaea
4	2/15/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	182	89	M25-02339/0033	Negative	M25-02339/0063	Negative	No Lernaea
4	2/15/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	196	86	M25-02339/0034	Negative	M25-02339/0064	Negative	No Lernaea

Batch	Date Collected	Date Shipped	Collection Method	Site No	Site Name	Fish Type	Length	Weight	qPCR Sample No.	EHNV Real-time PCR Result	ELISA Sample No.	EHNV Antibody ELISA Result	Lernaea?
4	2/15/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	194	91	M25-02339/0035	Negative	M25-02339/0065	Negative	No Lernaea
4	2/15/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	238	174	M25-02339/0036	Negative	M25-02339/0066	Negative	No Lernaea
4	2/15/2025	2/17/2025	Line Fishing	10	Tumut River Arm	Adult	237	186	M25-02339/0037	Negative	M25-02339/0067	Negative	No Lernaea
4	2/15/2025		Line Fishing	10	Tumut River Arm	Adult	220	135	M25-02339/0038	Negative	M25-02339/0068	Negative	No Lernaea
4			Line Fishing	10	Tumut River Arm	Adult	279	315	M25-02339/0039	Negative	M25-02339/0069	Negative	No Lernaea
4	2/15/2025		Line Fishing	10	Tumut River Arm	Adult	170	58	M25-02339/0040	Negative			No Lernaea
4	2/15/2025		Line Fishing	10	Tumut River Arm	Adult	179	72	M25-02339/0041	Negative	M25-02339/0070	Negative	No Lernaea
4	2/15/2025		Line Fishing	10	Tumut River Arm	Adult	194	109	M25-02339/0042	Negative	M25-02339/0071	Negative	No Lernaea
4			Line Fishing	10	Tumut River	Adult	194	133	M25-02339/0043	Negative	M25-02339/0072	Negative	No Lernaea
5			Line Fishing	3	Landers Creek Arm	Adult	193	97	M25-02333/0001	Negative			No Lernaea
5			Line Fishing	3	Landers Creek Arm	Adult	216	166	M25-02333/0002	Negative			No Lernaea
5			Line Fishing	3	Landers Creek	Adult	185	94	M25-02333/0003	Negative			No Lernaea
5			Line Fishing	3	Landers Creek Arm	Adult	164	69	M25-02333/0004	Negative			No Lernaea
5			Line Fishing	3	Landers Creek	Adult	185	92	M25-02333/0005	Negative			No Lernaea
5			Line Fishing	3	Landers Creek	Adult	166	77	M25-02333/0006	Negative			No Lernaea
5			Line Fishing	3	Landers Creek	Adult	154	55	M25-02333/0007	Negative			No Lernaea
5			Line Fishing	3	Landers Creek Arm	Adult	194	112	M25-02333/0008	Negative			No Lernaea
5			Line Fishing	3	Landers Creek	Adult	179	90	M25-02333/0009	Negative			No Lernaea
5			Line Fishing	3	Landers Creek Arm	Adult	173	77	M25-02333/0010	Negative			No Lernaea

Batch	Date Collected	Date Shipped	Collection Method	Site No	Site Name	Fish Type	Length	Weight	qPCR Sample No.	EHNV Real-time PCR Result	ELISA Sample No.	EHNV Antibody ELISA Result	Lernaea?
5	2/15/2025	2/18/2025	Line Fishing	3	Landers Creek Arm	Adult	196	123	M25-02333/0011	Negative			No Lernaea
5	2/15/2025	2/18/2025	Line Fishing	3	Landers Creek Arm	Adult	168	75	M25-02333/0012	Negative			No Lernaea
5	2/15/2025	2/18/2025	Line Fishing	3	Landers Creek Arm	Adult	199	117	M25-02333/0013	Negative			No Lernaea
5	2/15/2025	2/18/2025	Line Fishing	3	Landers Creek Arm	Adult	164	72	M25-02333/0014	Negative			No Lernaea
5	2/15/2025	2/18/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02333/0015	Negative			No Lernaea
5	2/15/2025	2/18/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02333/0016	Negative			No Lernaea
5	2/15/2025	2/18/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02333/0017	Negative			No Lernaea
5	2/15/2025	2/18/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02333/0018	Negative			No Lernaea
5	2/17/2025	2/18/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02333/0019	Negative			No Lernaea
5	2/17/2025	2/18/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02333/0020	Negative			No Lernaea
5	2/17/2025	2/18/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02333/0021	Negative			No Lernaea
5	2/17/2025	2/18/2025	Boat electrofishing	3	Landers Creek Arm	YOY			M25-02333/0022	Negative			No Lernaea
5	2/17/2025	2/18/2025	Line Fishing	3	Landers Creek Arm	Adult	170	63	M25-02333/0023	Negative	M25-02333/0026	Negative	No Lernaea
5	2/17/2025	2/18/2025	Line Fishing	3	Landers Creek Arm	Adult	168	35	M25-02333/0024	Negative	M25-02333/0027	Negative	No Lernaea
5	2/17/2025	2/18/2025	Line Fishing	3	Landers Creek Arm	Adult	220	156	M25-02333/0025	Negative	M25-02333/0028	Negative	No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	176	73	M25-02333/0029	Negative	M25-02333/0060	Negative	No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	173	80	M25-02333/0030	Negative			No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	169	78	M25-02333/0031	Negative	M25-02333/0061	Negative	No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	203	131	M25-02333/0032	Negative			No Lernaea

Batch	Date Collected	Date Shipped	Collection Method	Site No	Site Name	Fish Type	Length	Weight	qPCR Sample No.	EHNV Real-time PCR Result	ELISA Sample No.	EHNV Antibody ELISA Result	Lernaea?
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	228	174	M25-02333/0033	Negative			No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	183	90	M25-02333/0034	Negative			No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	192	114	M25-02333/0035	Negative	M25-02333/0062	Negative	No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	191	115	M25-02333/0036	Negative			No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	181	91	M25-02333/0037	Negative	M25-02333/0063	Negative	No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	195	107	M25-02333/0038	Negative			No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	193	111	M25-02333/0039	Negative	M25-02333/0064	Negative	No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	211	152	M25-02333/0040	Negative	M25-02333/0065	Negative	No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	262	254	M25-02333/0041	Negative	M25-02333/0066	Negative	No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	221	174	M25-02333/0042	Negative			No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	194	115	M25-02333/0043	Negative	M25-02333/0067	Negative	No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	154	53	M25-02333/0044	Negative			No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	170	65	M25-02333/0045	Negative			No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	196	127	M25-02333/0046	Negative			No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	173	72	M25-02333/0047	Negative			No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	170	71	M25-02333/0048	Negative	M25-02333/0068	Negative	No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	159	57	M25-02333/0049	Negative			No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	181	91	M25-02333/0050	Negative	M25-02333/0069	Negative	No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	174	84	M25-02333/0051	Negative			No Lernaea

Batch	Date Collected	Date Shipped	Collection Method	Site No	Site Name	Fish Type	Length	Weight	qPCR Sample No.	EHNV Real-time PCR Result	ELISA Sample No.	EHNV Antibody ELISA Result	Lernaea?
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	180	90	M25-02333/0052	Negative	M25-02333/0070	Negative	No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	187	107	M25-02333/0053	Negative			No Lernaea
5	2/17/2025	2/18/2025	Gillnetting	4	Honeysuckle Creek Arm	Adult	208	151	M25-02333/0054	Negative	M25-02333/0071	Negative	No Lernaea
5	2/17/2025	2/18/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	206	111	M25-02333/0055	Negative			No Lernaea
5	2/17/2025	2/18/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	167	58	M25-02333/0056	Negative			No Lernaea
5	2/17/2025	2/18/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	196	109	M25-02333/0057	Negative	M25-02333/0072	Negative	No Lernaea
5	2/17/2025	2/18/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	166	77	M25-02333/0058	Negative			No Lernaea
5	2/17/2025	2/18/2025	Line Fishing	4	Honeysuckle Creek Arm	Adult	181	87	M25-02333/0059	Negative	M25-02333/0073	Negative	No Lernaea

## Appendix E

# CSIRO Positive Detection Verification Report



#### **CSIRO**

#### Australian Centre for Disease Preparedness (ACDP)

5 Portarlington Road, Geelong VIC 3220

Postal Address: Private Bag 24 Geelong, VIC 3220 Australia

acdp.csiro.au ABN 41 687 119 230

#### Diagnostic Testing Report

Department of Regional NSW (PO 4500000314)

Attn: Nicholas Yuen

Primary Industries State Diagnostic Vet Lab

Private Bag 4008

NARELLAN NSW 2567 AUSTRALIA

Tel: +61 3 5227 5000

Final Report

**SAN:** 25-00878

Collection Date: 05-FEB-2025
Received at AAHL: 05-Mar-2025
Report Date: 07-Mar-2025

Report Version: 25-00878-F-V1

No. Pages: 2

Ref: M25-01605\_CV25-0783

Sender: Nicholas Yuen

Department of Regional NSW (PO 4500000314)

Owner:

Elizabeth Pope

NA

NA NSW NA AUSTRALIA

**Examination Requested:** 

EHNV confirmation

Submission Information:

fish 2x tissue homogenate (supernatant)

**Molecular Diagnostics** 

Test: 18S Ribosomal RNA Control - Standard TaqMan PCR

No.	Specimen ID	Species	Specimen Type	Test Result
1	M25-01605/0014	Redfin Perch	Tissue	POSITIVE
			homogenate	
2	M25-01605/0017	Redfin Perch	Tissue	POSITIVE
			homogenate	

Date Test(s) Completed:

06-Mar-2025

Comment:

NATA accreditation does not cover the performance of this service. Positive results demonstrate that nucleic acid was extracted successfully and there were no PCR inhibitors present thus validating negative results by the pathogen-specific

test(s).

Test: Ranavirus - PCR (Hyatt et al. 2000)

No.	Specimen ID	Species	Specimen Type	Test Result
1	M25-01605/0014	Redfin Perch	Tissue homogenate	POSITIVE
2	M25-01605/0017	Redfin Perch	Tissue homogenate	POSITIVE

Date Test(s) Completed:

07-Mar-2025

Comment:

NATA accreditation does not cover the performance of this service.

The amplicon from sample 1 was sequenced. The nucleotide sequence shared 100% identity with EHNV reference

sequences.

Test: Ranavirus - TaqMan PCR (WOAH, Pallister et al. 2007)

No.	Specimen ID	Species	Specimen Type	Test Result
1	M25-01605/0014	Redfin Perch	Tissue homogenate	POSITIVE
2	M25-01605/0017	Redfin Perch	Tissue homogenate	POSITIVE

This report was originally issued as a digitally signed PDF file, allowing verification that the document has not been altered after it was issued. To verify the authenticity of a hard copy of the report, please contact the laboratory at acdp-reports@csiro.au

Please note: Online verification is not available for this report.

#### **CSIRO**

#### Australian Centre for Disease Preparedness (ACDP)



Date Test(s) Completed:

Nick Moody - Authorised Signatory

06-Mar-2025

Comment:

NATA accreditation does not cover the performance of this service.

#### Summary

Two tissue homogenate samples were processed and tested with a Ranavirus-specific real-time PCR assay. All samples tested POSITIVE with mean Ct values from 21.96 to 26.02. ADDITIONAL TESTING: All samples also tested POSITIVE with a Ranavirus-specific conventional PCR. The amplicon from 1 sample was sequenced. The nucleotide sequence shared 100% nucleotide identity with EHNV reference sequences. All positive and negative controls performed as expected.

Yours Faithfully

25-00878-F-V1

## Appendix F

# Revised BRMP Disease Surveillance Program



#### Appendix F – Revised BRMP Disease Surveillance Program

Revision 2. August 2025

This disease surveillance program is based on advice provided by Diggles (2022b), Dr Paul Hick from the Elizabeth Macarthur Agricultural Institute (EMAI) and Dr Jeffrey Go from NSW DPIRD, Aquatic Biosecurity.

Surveillance will include both active and passive components.

Following a positive detection of EHNV in Talbingo Reservoir in February 2025 the disease surveillance program has been reviewed. As EHNV has been confirmed present in the Talbingo Reservoir Redfin population, the focus for the program has shifted to determining the potential presence of EHNV in Tantangara Reservoir which is currently considered Redfin and EHNV free.

#### **Active Surveillance**

Targeted active sampling for EHNV will be undertaken in Tantangara Reservoir targeting the summer months between December and February once water temperatures exceed 19°C. Annual surveillance surveys will occur for two consecutive years, to meet the international requirements for declaration of freedom from disease (OIE, 2015).

As Redfin is considered absent from Tantangara Reservoir, the target fish species is Rainbow trout although any non-native species collected during sampling will be sent for laboratory testing for EHNV. Each sampling season, best efforts will be made to lethally sample the numbers of fish presented in Table 5 for EHNV analysis using qPCR testing and if available, serology. Fish collection will be undertaken using electrofishing, netting, lures, baited hooks or other suitable approved methods of capturing fish.

Table 5: Surveillance design for detecting EHNV in Tantangara reservoir.

Location	Water Temperature	Time of Year	No. of samples and species targeted for lethal testing by qPCR and serology
Tantangara Reservoir	>19°C	Dec- Feb	Rainbow Trout (n ≥ 156)

The reservoir will be partitioned into 5-10 units, depending on water levels, with fish in each unit to be collected and bagged separately. Fish from each unit will be analysed to account for disease clustering. The collection of unwell or injured fish or fish showing unusual external signs (including signs of disease) will be prioritised. Sampling effort will occur for up to 10 days. If more than the required number of fish are collected, additional fish will be analysed for qPCR using pooled sampling. In the event that insufficient numbers of fish can be collected, Snowy Hydro will consult with NSW DPIRD on the appropriate course of action.

Chilled samples of whole fish will be maintained at 2-4°C and sent to the laboratory within three days. Alternatively, the samples will be frozen at -20°C prior to transport to the laboratory as soon as practicable. The preferred laboratory to conduct the testing is the OIE Reference Laboratory for infection with EHNV, namely, EMAI. Any non-normal results from qPCR analysis will be tested by conventional PCR and sequence analysis to increase sensitivity and specificity.

If analysis capability is available at the EMAI, blood samples will also be collected from the caudal vein of up to 156 adult fish and placed in labelled tubes and placed on ice or in a portable fridge. The same fish can be tested using qPCR and serology.

Notification of suspected emergency disease and positive test results for EHNV will be undertaken according to legislative requirements.

During pest fish and EHNV surveillance activities, opportunistic visual examination of captured fish for the presence of Lernaea will occur. This will be done by visually examining all relevant fish species, including juvenile and adult Redfin, native species such as Trout Cod, Golden Perch, Mountain Galaxias, as well as exotic Rainbow and Brown Trout and Goldfish that are collected during sampling for EHNV or pest fish. Non-lethal sampling of desirable species will aim to minimise

handling time and stress, and involve brief, gentle handling of freshly captured fish including close visual observation of the entire body surface (including fins and under opercula) prior to their release at the site of capture.

Specific diagnostic tests are not required for Lernaea, however in order to confirm a positive detection in an area where the parasite is not already confirmed to occur, where appropriate, voucher specimens will be dissected from the infected fish and fixed in 70% ethanol before being sent to an appropriate person/facility for official identification.

This surveillance program will be reviewed following a positive detection of EHNV within the surveillance catchments or following two consecutive years of negative samples within the target area.

#### **Passive Surveillance**

Passive surveillance for EHNV will occur across all surveillance catchments for the duration of the BRMP.

Reports of fish kill<sup>3</sup> events or fish disease in any of the surveillance catchments will trigger a notification to NSW DPIRD in accordance with legislative requirements and the deployment of a sample collection team as soon as reasonably practicable who will seek to collect dead or moribund fish from the relevant location and submit them for EHNV testing. Any affected fish species will be collected and will include a minimum of five and up to 30 moribund or freshly dead fish per location where possible. Visual examination of any affected fish species for Lernaea will also occur.

<sup>&</sup>lt;sup>3</sup>See: https://www.dpi.nsw.gov.au/fishing/habitat/threats/fish-kills

## Appendix G

# Tantangara Barrier Pre-clearance Report



#### research and consulting

#### Stocky Galaxias Pre-disturbance Salvage Tantangara Creek Barrier Construction



- Final
- October 2025

Austral Research and Consulting ABN 73 007 840 779 23 Buntings Rd Kirkstall Vic 3283 Australia Web: www.austral.net.au

#### research and consulting

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#### research and consulting

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#### 1. Introduction

Austral Research and Consulting (Austral) were contracted by Snowy Hydro to undertake the salvage of Stocky galaxias (*Galaxias tantangara*) within the dewatering zone of the Tantangara Creek Barrier Project, the construction of which, is a requirement of the Snowy 2.0 Project Consent.

The Project required the diversion of a section of Tantangara Creek upstream of Tantangara Creek waterfall (Figure 1), to facilitate the construction of a concrete barrier to prevent the potential upstream migration of Climbing galaxias (*Galaxias brevipinnis*) into critical habitat for Stocky galaxias, which are listed nationally as critically endangered.

The objective of the salvage was to collect any Stocky galaxias located within the planned dewatering zone and translocate them to an upstream area of Tantangara Creek (Figure 1).



Figure 1: Tantangara barrier/weir site, fish clearance/salvage area and translocation site.

This report details the methods used to undertake the salvage and translocation efforts; and details of the results of the surveys including basic biometric information of translocated individuals.

All surveys and activities were conducted under NSW Animal Research Authority – RVF21/3375 (Animal Ethics) (Austral), NSW DPIRD Scientific Collection Permit No. FP 23/108 (Dr Mark Lintermans) and NSW Department of Planning and Environment Scientific Licence No. SL101755 (Dr Mark Lintermans).

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#### 2. Methods

The Stocky galaxias salvage was completed using a range of techniques including backpack electrofishing, netting (bait nets with light sticks), and dip netting in consultation with Dr Mark Lintermans. Mark Lintermans and Hugh Allen were present on site and assisted with fish collection and translocation on the 5<sup>th</sup> and 6<sup>th</sup> of February 2025.

Provided below are details of the two stages of the salvage operation and the methods used over the period of the salvage operation.

#### 2.1. Pre-Diversion Clearance

The area of fish clearance was located on Tantangara Creek, downstream of the area known as 'Chinese cut' and upstream of the existing waterfall as shown in Figure 1. Three consecutive barriers (haybales and gravel filled sandbags) were established immediately upstream of the clearance area to prevent any new fish from entering the area after clearance works were complete.

Pre-diversion fish clearance works initially occurred on the 5<sup>th</sup> and 13<sup>th</sup> of February 2025.

Following high rainfall on the 14<sup>th</sup> of February, there was evidence that the barriers upstream of the site had been temporarily overtopped. As such, diversion works were delayed, and a further day of survey and pre-clearance occurred on the 15<sup>th</sup> of February.

Any Stocky galaxias captured were translocated to the release site located immediately upstream of the Tantangara Creek fenced area (Figure 1). All fish had lengths recorded and those of an appropriate length (greater than 40mm) were fin clipped from the dorsal tip of the caudal fin for tissue samples and marked recapture. Additionally, any Riek's crayfish (*Euastacus rieki*) captured were released approximately 30m upstream of the established barriers.

#### 2.2. Post-diversion Clearance

Post-diversion clearance work was undertaken on the 16<sup>th</sup> of February 2025. The dewatering process began with a 30% bypass that reduced flow down the creek and was maintained for an hour to allow fish within riffle habitat to move into the deeper pools. The bypass was then increased to 100% and completed the dewatering of the creek clearance area. Backpack electrofishing was then used to target the remnant pools for fish once flow had ceased.

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#### 2.3. Fish Survey Methods

A summary of the fish survey techniques used to capture and identify numbers of Stocky galaxias is provided below.

#### 2.3.1. Spotlighting

Torches were used at night to identify numbers of fish remaining in pools and riffles.

#### 2.3.2. Bait traps

Bait traps consisted of 2mm mesh and approximate dimensions of 250mm x 250mm x 450mm. A light stick was included in each trap as an attractant device. The traps were set around pools with rock and snag habitat overnight and retrieved the next morning.

#### 2.3.3. Backpack electrofishing

Backpack electrofishing was performed in the clearance area using a Smith-Root LR-24 electrofisher. The sampling procedure involved electrofishing all wadable areas of the creek targeting available habitat such as rock, snags and riffles. The survey effort was recorded at each site. Settings used were 990v, 25% duty cycle and 90Hz frequency.

#### 2.4. Clearance Effort

A morning and afternoon effort of backpack electrofishing was completed on each of the three days of pre-diversion clearance work. Bait traps with light sticks and spotlighting were only used once overnight (Table 1).

Table 1: Effort for each session of pre-clearance work.

Date	Morning EF on-time (sec)	Afternoon EF on-time (sec)	Bait trap with light stick (No. traps)	Spotlighting
05/02/2025	3,076	2,336	-	No
06/02/2025			15	Yes
13/02/2025	750	700	-	No
15/02/2025	650	650	-	No
16/02/2025		400	-	No

Note: EF = electrofishing.

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#### 2.5. Translocation

All individuals were translocated in aerated buckets to the translocation site (Figure 1).

#### 2.6. Fin Clipping and Data Collection

All captured fish were measured and adult fish were fin clipped on the top of the tail. The clips will be retained for genetic analysis and this will enable these fish to be identified during further surveys later this year (marked recapture).

#### 2.7. Biosecurity

Aquatic biosecurity was identified as a particular concern for the site and species based on the conservation status of the species. New equipment (buckets, waders, etc) was used where possible, and all equipment was treated with Virkon-S as directed by the Department of Primary Industries and Regional Development (DPIRD) NSW. Disinfection was completed using a contact time of 1 to 5 minutes of Virkon-S at a concentration of 1% (in accordance with manufacturer's instructions).

#### 2.8. Clearance Limitations

Every effort was made to ensure as many Stocky galaxias and Riek's crayfish were captured and translocated from the clearance area. However, capturing all individuals was unlikely due to the species' cryptic nature and habitat preferences.

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#### 3. Results

Total numbers of translocated fish and crayfish for the pre-diversion clearance work are presented in Table 2. A total of 87 Stocky galaxias and 16 Riek's crayfish were translocated across the three days of pre-diversion clearance work.

The majority of fish were captured via backpack electrofishing, with bait traps yielding no fish and spotlighting assisting in the capture of just six fish on one occasion.

Sixty-nine of the Stocky galaxias were fin clipped for genetic analysis and to assist with the ongoing population study at the translocation site where it was necessary to be able to identify translocated individuals via marked recapture.

Table 2: Total numbers of translocated Stocky galaxias and Riek's crayfish.

Date	Stocky galaxias		Riek's crayfish	
	Morning	Afternoon	Morning	Afternoon
5/02/2025	55 (47)	16 (12)	4	7
13/02/2025	5 (3)	6 (3)	1	2
15/02/2025	1 (1)	2 (1)	0	2
16/02/2025		3 (2)		

Note: Value in brackets is number of individuals fin clipped.

#### 3.1. Population Structure

Two distinct cohorts of Stocky galaxias were captured and translocated (Figure 2). The first cohort of 25 – 50mm were considered to be juvenile and may have included young of year and 1+ year individuals. And the second cohort of 60mm and larger were considered to be mature, reproductive individuals.

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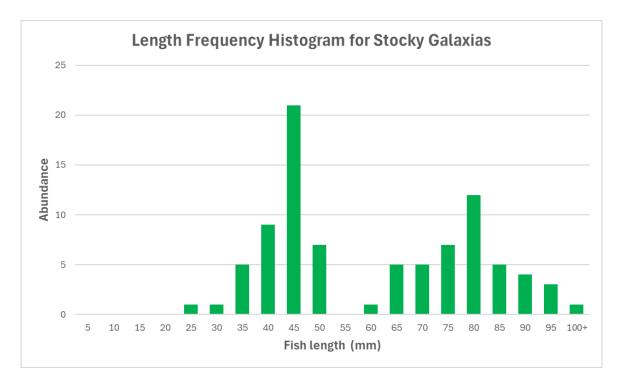


Figure 2: Length frequency histogram of Stocky galaxias captured and translocated in Tantangara Creek.

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#### 4. Ongoing Monitoring

It is recommended that the integrity of the temporary fish barriers and the water diversion continue to be monitored daily.

In the event of the barriers or diversion being overtopped or breached, further survey and salvage operations for Stocky galaxias will need to be undertaken.



