Pest fish surveillance for Snowy 2.0: mid to upper Murrumbidgee, and Lake Eucumbene catchments

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Acknowledgment

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Front cover photo: (clockwise from top) Murrumbidgee River at junction with Tantangara Creek; Macquarie Perch; alpine plain in snow; Stocky Galaxias (Images: Tarmo A. Raadik).

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1 Introduction

Snowy Hydro Limited received approval in 2020 to construct a new large-scale pumped hydro-electric storage and generation scheme (Snowy 2.0), to increase hydro-electric capacity within the existing Snowy Mountains Hydro-electric Scheme. This will involve the connection of the existing Talbingo and Tantangara reservoirs via a series of underground pipes and an underground power generation station. Water will be transferred in both directions between the reservoirs, which are in separate river catchments.

The Arthur Rylah Institute for Environmental Research has been engaged by Snowy Hydro Ltd. to provide specialist advice that can inform the selection of options and preparation of various aquatic Management Plans required as part of the NSW and Commonwealth approvals for the Snowy 2.0 project (the Project).

Construction and operational activities associated with Snowy 2.0 Main Works have the potential to impact aquatic ecology in some waterbodies in the project area, in particular the existing Talbingo and Tantangara reservoirs and inflowing and outflowing streams to Tantangara Reservoir (Cardno et al. 2019). The main potential for impact would be from construction activities within the reservoirs and from the new two-way hydrologic connection between the reservoirs, connecting the separate Tumut catchment and Upper Murrumbidgee Catchment. Specifically, this new hydrologic pathway poses biosecurity risks, namely the spread of pest fish, phytoplankton and aquatic pathogens. This issue is covered in detail by Cardno (2019) and is based on investigations related to pest fish species and disease (Baumgartner *et al.* 2016; Griffith et al. 2017; Allan and Lintermans 2019; Lintermans 2019; Hick *et al.* 2019; Ning et al. 2019; Raadik 2019; Robinson *et al.* 2019; Weeks *et al.* 2019; Wright and Horsfield 2019; Doyle *et al.* 2022.)

The NSW Biosecurity Act 2015, which came into effect on 1 July 2017 (Biosecurity Regulation), provides a consolidated regulatory framework to effectively respond to and manage biosecurity risks. The broad objectives for biosecurity in NSW are to manage biosecurity risks from animal and plant pests and diseases, weeds and contaminants through a flexible and responsive statutory framework for the benefit of the NSW economy, environment and community. Consequently, as a consent condition for Snowy 2.0 works, a Biosecurity Risk Management Plan (BRMP) will be prepared which will set out objectives and potential activities for effective surveillance of potential pest fish incursion, including consideration of the Epizootic Haematopoietic Necrosis (EHN) Virus, during pre- and post-connection of Talbingo and Tantangara reservoirs. The broad aim of the BRMP is to contribute to improving the security and resilience of the threatened native species within the area of the Snowy 2.0 scheme, by potentially assisting to reduce the risk and impact of the project from pest fish species and disease.

The key purpose of this document is to provide advice on pest fish surveillance and management measures that may be implemented to protect the Macquarie Perch (*Macquaria australasica*) and Stocky Galaxias (*Galaxias tantangara*) in the Mid to Upper Murrumbidgee catchment and the salmonid fishery in Lake Eucumbene as part of the BRMP for Snowy 2.0. This work has links to other activities associated with Snowy 2.0 including aquatic disease surveillance (i.e. EHNV), conservation measures associated with the Threatened Fish Management Plan (TFMP) and salmonid restocking associated with the Snowy 2.0 Recreational Fishing Management Plan (RFMP). Consideration on how each of these activities may interact with any proposed pest fish surveillance program is required.

Whilst the key biosecurity risk of potential transfer of pest fish and pathogens is from the Talbingo catchment into the Tantangara Reservoir in the upper Murrumbidgee catchment, introductions via other pathways into Tantangara Reservoir could occur at any stage during the life of the scheme, though the transfer of fish is less likely (Cardno *et al.* 2019). Tantangara Reservoir also has hydrologic connectivity with inflowing tributaries, particularly the upper Murrumbidgee River, the Mid Murrumbidgee catchment via environmental flow releases or spills from Tantangara Dam, and via the existing Tantangara-Eucumbene Tunnel, into Lake Eucumbene (NSW DPIE 2020a). A downstream hydrologic connection from Tantangara Reservoir to Talbingo Reservoir already exists via the connection to Lake Eucumbene, from where water is subsequently transferred to reservoirs on the Tumut River via a sequence of power stations.

To prevent the spread of pest fish downstream of the Tantangara Reservoir and to protect a population of the Macquarie Perch in the mid-Murrumbidgee catchment, Snowy Hydro Ltd. has proposed installation of fish screens on the Tantangara Dam outlet and the Murrumbidgee-Eucumbene Tunnel, and a new fish barrier on Tantangara Creek to protect the largest of the two remaining populations of the critically endangered Stocky Galaxias (NSW DPIE 2020b; Lintermans *et al.* 2021). Further, restocking of Tantangara Reservoir and Lake Eucumbene with salmonid fish to minimise the impact of the development on recreational fishing by pest fish (Redfin Perch, *Perca fluviatilis*) is also proposed, should impacts occur (NSW

DPIE 2020b). Whilst the screening/barrier measures seek to reduce adverse biosecurity impacts of the project, there is uncertainty about how effective the fish screens and fish barrier will be over the operational life of the project (potentially 100 years) (NSW DPIE 2020a).

Pest fish surveillance is an essential, initial component in detecting and managing the spread of unwanted/harmful species of fish and involves the detection of a species at a location, which then triggers management actions. Early detection of pest fish is important to maximise the potential ability for eradication or management actions, such as containment and control, to commence before the species can establish and increase in abundance; this will influence the efficacy of the actions. Further, surveillance at multiple locations, and continuing surveillance once a detection is made, are important monitoring activities which inform pest species spread or decline.

2 Surveillance objectives, constraints, and considerations

The objective of the pest fish surveillance activities detailed in this document is to:

- Provide early detection of incursions or range expansion of pest fish, following the commencement of the Snowy 2.0 Project (direct or indirect, pre- and post-connection). Early detection is crucial to facilitate rapid management responses to protect the following target aquatic assets:
  - Macquarie Perch and Stocky Galaxias in the Mid to Upper Murrumbidgee catchment.
  - the salmonid fishery in Lake Eucumbene.

Any activities associated with pest fish surveillance for Snowy 2.0 must also consider related activities including disease (EHNV) surveillance, threatened fish conservation measures (impacts on Macquarie Perch and Stocky Galaxias) and salmonid stocking in related catchments.

2.1 Constraints and considerations

The ability to detect target taxa as early as possible following an incursion is a major constraint of a surveillance program aiming to achieve early detection . Many biological factors can influence the success of early detection, such as:

- Biological/ecological characteristics of target species (e.g. habitat preferences, behaviour, life history stages/size, juvenile or sexually mature, etc.).
- Incursion pathways (obvious, known, unknown).
- Incursion location (remote or accessible, suitable habitat or unsuitable).
- Number of incursions (single or multiple, infrequent or frequent).
- Number of individuals (few, many).
- Survival of individuals (short, moderate, long).
- Time since incursion (short (days or week) to long (weeks to months).
- Spatial density of surveillance sites (sparse, dense, restricted, widespread).
- Frequency of surveillance (infrequent or frequent, e.g. semi-annual, bi-annual or tri-annual).
- Timing of surveillance (appropriate time of year for target life-history stages or behavioural characteristics).
- Detection methods employed (single, multiple and complimentary, appropriate for species, life-history stages, abundance, habitats, etc.).
- Environmental conditions (influencing incursion success, survivorship, efficiency of detection methods).

A key factor in the success of early detection is detection probability, which is related to the above constraints. Maximising detection probability is therefore a key goal and requires consideration of all constraints in the program design, including the ability for re-evaluation and refinement/flexibility.

Other non-biological constraints are also important to consider, such as site access/safety issues at biologically suitable surveillance times.

2.2 Design considerations

Key broad considerations for the design of a pest fish surveillance program are target species, pathways for incursion, probability and potential frequency for incursion, and when pathways for incursion may operate. These factors will influence the specific surveillance requirements, which will relate to various combinations of factors and may potentially differ between species, locations and timeframes. Other specific factors listed in Section 2.1 will also need to be considered, requiring the surveillance plan to be flexible and adaptive.

2.2.1 Target pest species

The target pest species for surveillance associated with Snowy 2.0 (with additional details provided in Table 1) are:

- The exotic Redfin Perch, a carrier of EHN Virus; both are notifiable under Schedule 1 of the NSW Biosecurity Regulation 2017.
- The native Climbing Galaxias (*Galaxias brevipinnis*), translocated outside of its natural range and present in the Murray and Tumut (Murrumbidgee) River catchments.
- The exotic Eastern Gambusia (*Gambusia holbrooki*).

These three species have potential to impact populations of the threatened Macquarie Perch and Stocky Galaxias, as well as the Lake Eucumbene trout fishery (Cardno 2019).

Redfin Perch

A viable population of Redfin Perch exists in Talbingo Reservoir but the species is currently considered absent from Tantangara Reservoir, Lake Eucumbene and the mid-Murrumbidgee River below Tantangara Reservoir, upstream of the Australian Capital Territory (Cardno 2019). There is a likelihood of transfer of the species within the Snowy pumped hydro system if entrained and it would have potential to survive in Tantangara Reservoir (Cardno 2019; Ning et al. 2019, Doyle et al. 2022). Fish screens proposed as part of the project are intended to prevent subsequent transfer of Redfin Perch to the mid-Murrumbidgee River and Lake Eucumbene, should they establish in Tantangara Reservoir. Should these controls fail, the species may impact Macquarie Perch located in the Mid Murrumbidgee and salmonids in Lake Eucumbene through predation and competition for resources (Cardno, 2019). Redfin Perch is unlikely to colonise the habitat of Stocky Galaxias as it is a slow-flow specialist that is unlikely to invade very far upstream of Tantangara Reservoir. Should Redfin Perch establish in Tantangara Reservoir and subsequently become infected with EHNV, there is a small risk that this disease may affect the nearby populations of Macquarie Perch and/or Stocky Galaxias, however in the absence of Redfin Perch within the habitats of these species, the infection pressure is considered low (Hick et. al. 2019).

Climbing Galaxias

Climbing Galaxias has not been observed in Talbingo Reservoir, however adult individuals were observed for the first time in this catchment in the Yarrangobilly River, a tributary of Talbingo Reservoir during aquatic surveys for the Snowy 2.0 project (Cardno 2019). They have also previously been observed in a tributary of Blowering Reservoir, further downstream (Raadik 2003). Climbing Galaxias are considered a migratory species, where newly hatched larvae are washed downstream into marine environments and juveniles migrate upstream, back into freshwater habitats, The species, however, is known to fulfill this life cycle when land-locked in freshwater (Augspurger et al. 2017) where newly hatched larvae wash downstream into a lake or reservoir, and juveniles disperse upstream (Matveev et al. 2002; Matveev 2003; Hicks et al. 2017, 2021). They are considered to have some likelihood of transfer within the Snowy pumped hydro system if entrained and have potential to survive in Tantangara Reservoir (Cardno 2019; Ning *et al.* 2019). A fish barrier proposed as part of the project is intended to prevent subsequent transfer of Climbing Galaxias to upper Tantangara Creek, should they establish within the Upper Murrumbidgee catchment. Should these controls fail, this species may predate on and compete with Stocky Galaxias within this catchment (Cardno 2019).

Eastern Gambusia

Eastern Gambusia is present in Talbingo Reservoir and absent from Tantangara Reservoir and the very upper reaches of the mid-Murrumbidgee River and has some likelihood of transference via the Snowy pumped hydro system (Ning *et al.* 2019; Doyle *et al.* 2020). Eastern Gambusia is a slow-flow specialist that is unlikely to invade upstream of Tantangara Reservoir and impact Stocky Galaxias. Eastern Gambusia is already known to be present in the Murrumbidgee River downstream of Tantangara Reservoir, having been detected as far upstream as Adaminaby using eDNA analysis (Weeks *et al.* 2019; Griffith *et al.* 2020; Griffith *et al.* 2022), which is also the known upstream extent of Macquarie Perch. However, if it establishes in Tantangara Reservoir, and if the screening controls fail, the species may be displaced downstream into the Mid-Murrumbidgee where it may impact Macquarie Perch if present above Adaminaby.

Brown Trout (*Salmo trutta*) and Rainbow Trout (*Oncorhynchus mykiss*) are the major threat to Stocky Galaxias (Lintermans and Allan 2019; Lintermans *et al.* 2020; TSSC 2021), and both are already present in Tantangara Reservoir and tributary streams. If Redfin Perch establish and cause a decline in the recreational fishery, trout populations within Tantangara Reservoir are proposed to be maintained by stocking (NSW DPIE 2020b; see also the Recreational Fishing Management Plan). Consequently, whilst surveillance monitoring for trout is not required, any salmonid stockings in Tantangara Reservoir should avoid potential impact to Stocky Galaxias, such as increasing the density and size of trout in tributary streams below galaxiid populations above pre-stocking levels.

Table 1. Target pest fish species considered, including their impact on Macquarie Perch and Stocky Galaxias, and trout in Lake Eucumbene, including presence in the Mid to Upper Murrumbidgee catchment, Talbingo Reservoir and Eucumbene Reservoir

Pest species	Current distribution	Aquatic assets potentially impacted and location	Potential impact	Comment
Redfin Perch (*Perca fluviatilis*)	Present in Talbingo Reservoir Considered absent from Tantangara and Lake Eucumbene Up to lower end of Mid Murrumbidgee catchment	Macquarie Perch in the Mid Murrumbidgee catchment	Predation, Competition, Disease (EHNV). (Cardno 2019; Wedderburn and Barnes al. 2016)	Northern Hemisphere exotic species. Slow range expansion upstream of ACT border in the Murrumbidgee River. Also present in Tumut/Yarrangobilly catchments (Lintermans 2002, 2019)
		Stocky Galaxias in Upper Murrumbidgee catchment	Disease (EHNV)	EHNV potentially also impacts Stocky Galaxias
		Trout spp. in Eucumbene Reservoir	Disease (EHNV)	
Climbing Galaxias (*Galaxias brevipinnis*)	Present in Talbingo Reservoir and Eucumbene Reservoir.	Stocky Galaxias in Upper Murrumbidgee Catchment	Competition; Predation. (McDowall and Allibone 1994; Allibone and McDowall 1997; Allibone 1999; Hardie et al. 2006)	Native species in coastal catchments, a pest when introduced outside of its natural range. Present in Eucumbene (natural) and Tumut (outside range) catchments (Waters *et al.* 2002; Raadik 2003, 2019)
Eastern Gambusia (*Gambusia holbrooki*)	Present in lower part of Mid Murrumbidgee catchment below Adaminaby Present in Talbingo Reservoir	Macquarie Perch in upper Mid Murrumbidgee catchment	Competition; aggressive interactions. (Ivantsoff & Aarn 1999; Pyke 2008; Hinchcliffe et al. 2017)	Exotic species from mainland North America. Also present in Tumut/Yarrangobilly catchments

An additional exotic species has been flagged as of potential concern, though following a review of threats, low likelihood of between catchment transference, likelihood of survival and establishment, and potential for ecological impact (Cardno 2019), is not included in this program:

- Goldfish (*Carassius auratus*) – present in Talbingo Reservoir, Eucumbene Reservoir and Mid Murrumbidgee catchment, has some likelihood of transference from Talbingo Reservoir to Tantangara Reservoir, and survival. Not considered to pose a threat to Stocky Galaxias or Macquarie Perch. Also unlikely to move upstream into Murrumbidgee River headwaters.

Whilst not assessed by Cardno (2019), the following exotic species is also not considered a major threat and is similarly not included in this plan:

- Oriental Weatherloach (*Misgurnus anguillicaudatus*) – Present in Eucumbene Reservoir tributaries, the Murrumbidgee River up to Bredbo, but considered to be absent in Talbingo and Tantangara Reservoirs and therefore there is no current risk of cross catchment transference via the Snowy pumped hydro system. This species has a steadily expanding range, largely via angler use as baitfish (Lintermans 1993, 2004) and consequently may establish in the popular recreational fisheries in Talbingo and/or Blowering reservoirs over the life of Snowy 2.0. The species is also not considered to impact Stocky Galaxias or Macquarie Perch, although the species is suspected of being an egg predator that has impacted local Mountain Galaxias (*Galaxias olidus*) populations in the Canberra region (Lintermans *et al.* 1990a). The species is a poor upstream disperser (Koster *et al.* 2002).

The surveillance area for the target pest fish species varies, based on their present distribution and habitat preference (Table 1):

- Redfin Perch – a still water (e.g. lake, reservoir) and river/creek specialist. Currently considered absent in Tantangara and Eucumbene reservoirs and rivers and creeks in the Mid and Upper Murrumbidgee catchment.
- Climbing Galaxias – rivers and creeks specialist. Currently considered absent in the Murrumbidgee River and its tributaries upstream and downstream of Tantangara Reservoir.
- Eastern Gambusia – a shallow, still to slow water specialist. Currently considered absent in Tantangara and Eucumbene reservoirs. Not considered important in the upper Murrumbidgee River and tributaries due to its poor upstream colonisation ability and in the mid Murrumbidgee River as it is already present.

The following section provides some key characteristics of each target pest species, important for consideration in the surveillance plan (also see Cardno 2019):

Redfin Perch

- Not generally found in fast water habitats
- Prefer slow flowing habitats with abundant macrophytes structure (rock, timber).
- Adults solitary, juveniles and sub-adults form large schools.
- Newly hatched larvae pelagic.

Climbing Galaxias

- Adults and juveniles mainly in streams (all habitat types).
- In streams found on the substrate and amongst cover, or swimming midwater in pools.
- Newly hatched larvae pelagic in lakes for short period following hatching.

Eastern Gambusia

- Not generally found in fast water habitats.
- Prefer slow flowing habitats, particularly near the bank of emergent vegetation but can venture further out from the bank at night.
- Found in open water, usually throughout the water column in areas of streams or margins of lakes/reservoirs < 1 m deep (Pen and Potter 1991; Pyke 2005, 2008).

2.2.2 Pathways/locations of potential introductions and primary surveillance areas

There are three potential pathways for the introduction of pest fish to the primary surveillance areas (Table 2). The main pathway (Cardno 2019) is the connection of Tantangara Reservoir to Talbingo Reservoir by the new tunnel and pumped hydroelectric station, which has the potential to allow transference of pest fish to Tantangara Reservoir (Area 1; Cardno 2019; Doyle *et al.* 2022). A consequence of the incursion of these species is the potential for them to survive and colonise more widely to connected waterways, which may vary for the different pest fish species (see below). Consequently, whilst Tantangara Reservoir near the outlet/inlet for the Talbingo-Tantangara Tunnel will be a primary surveillance site due to the area being an early location of potential incursion, the various interconnected or nearby waterbodies, listed above, are key focal points for surveillance.

Human assisted transfer of pest fish (i.e. Lintermans 2004; Fernández *et al.* 2019) is also considered a possibility and poses a current and future risk (Table 2). Whilst independent of the Snowy 2.0 Scheme operation, there may be an increased possibility of this method of transfer occurring through media coverage of the Snowy 2.0 scheme, and enhanced angler visitation. The spread of pest fish is commonly related to angler visitation (Davis and Darling 2017) and bait-bucket introductions are a common vector (Lintermans

2004). Therefore, the potential locations of deliberate incursions via this pathway are broad. Surveillance sites should be established with consideration for this potential pathway across a wide area and cover the period both before and after the connection between Talbingo and Tantangara Reservoir is established.

Natural colonisation of pest fish from outside to inside of the catchments is a third pathway of proposed introduction, though its probability of occurrence is extremely low. For Climbing Galaxias, the natural population in Eucumbene Reservoir has not moved into Tantangara Reservoir via the existing tunnel or from the Upper Eucumbene and upper Murray catchments to the Upper Murrumbidgee via dispersal over narrow catchment divides during wet conditions (Raadik 2019; Lintermans 2019). Further, whilst this species has been recorded recently at one location in the lower Yarrangobilly River, it may not have spread far up this system, and therefore may not be near any headwater reaches between the Yarrangobilly and upper Murrumbidgee catchments.

Redfin Perch have spread very slowly and sporadically upstream in the mid Murrumbidgee River (Lintermans 2002; 2019), over a long time, indicating that upstream dispersal ability is poor for this species. Similarly, significant dispersal of Redfin Perch upstream of Talbingo Reservoir has also not occurred (Cardno 2019).

Consequently, the broader area of surveillance proposed for these three species to cover potential post-connection incursion and spread, and pre-connection spread, will be sufficient to also monitor for the extremely low probability of natural colonisation.

Specifically, the important catchment areas at risk of incursion of each target species are:

- Climbing Galaxias – upper Murrumbidgee River system upstream of Tantangara Reservoir (Area 2a; Cardno 2019), including Tantangara Creek and Sally’s Flat Creek in the Goodradigbee River system (currently second known population of Stocky Galaxias; Lintermans *et al.* 2021) either by crossing the narrow catchment divide, or via colonising the Goodradigbee River system via the existing Goodradigbee River aqueduct (Area 2b; Cardno 2019).
- Redfin Perch – Lake Eucumbene by transfer from Tantangara Reservoir via the Murrumbidgee-Eucumbene Tunnel (Area 5; Cardno 2019), or downstream into the mid Murrumbidgee River system via the Tantangara Reservoir dam wall/spillway during environmental release of spill (Area 4; Cardno 2019).
- Eastern Gambusia – into the upper Murrumbidgee catchment via the Talbingo-Tantangara Tunnel and then displacement downstream in the upper reaches of the mid-Murrumbidgee River system potentially affecting Macquarie Perch in the upper reaches of the Mid Murrumbidgee catchment upstream of Adaminaby (Bobeyan Road) where they are not currently known to be present. Also potential transfer to Lake Eucumbene from Tantangara Reservoir via the Murrumbidgee-Eucumbene Tunnel (Area 5; Cardno 2019), though potential impact on trout stocks considered minimal due to the small size of the species and preference for marginal, shallow, still water habitat.

Table 2. Considered pathways for the introduction of pest fish to the Mid and Upper Murrumbidgee River catchment, including species, connection phase, probability of incursion and primary surveillance areas

Pathway and connection phase	Specifics	Pest species	Probability	Primary surveillance areas
1. Through **new water connectivity pathways** created by the Snowy 2.0 Scheme	Primarily via the tunnel connecting Tantangara Reservoir and Talbingo Reservoir	Redfin Perch	Low but possible	Tantangara Reservoir; mid to upper Murrumbidgee catchment; Lake Eucumbene
	If primary incursion occurs, secondary incursion may occur: – via existing water tunnel between Tantangara Reservoir and Lake Eucumbene. Note: Prior to the connection between Talbingo and Tantangara being established, the tunnel and dam outlet are to be fitted with fish screens – via overland cross-catchment movement or existing diversion tunnel between Tantangara Reservoir and Goodradigbee River system	Climbing Galaxias		Tantangara Reservoir; upper Murrumbidgee catchment (including Tantangara Creek if controls fail); upper Goodradigbee River system
		Eastern Gambusia		Tantangara Reservoir; upper sections of mid Murrumbidgee catchment
2. Human-assisted dispersal – the deliberate, or uninformed, illegal release of fish into new locations (e.g. Lintermans 2004; Fernandez *et al.* 2019)	Release of pest fish into any waterway in the mid to upper Murrumbidgee catchment or Lake Eucumbene	Redfin Perch	Very low, but possible	Mid to upper Murrumbidgee catchment, including Tantangara Reservoir and all tributaries
		Climbing Galaxias		Lake Eucumbene (for Redfin Perch)
		Eastern Gambusia		Tantangara Reservoir and Murrumbidgee River downstream of reservoir
3. Natural colonisation – range expansion from existing pest fish population centres	Redfin Perch colonising upstream in Murrumbidgee River	Redfin Perch	Extremely low (extremely limited upstream potential)	Murrumbidgee River main stem and tributaries upstream of current extent of range
	Climbing Galaxias moving across catchment divide between Tumut or Eucumbene catchments to Murrumbidgee catchment	Climbing Galaxias	Extremely low but can potentially move across catchment divides	Upper Murrumbidgee River tributaries adjacent to the Tumut River or Eucumbene River catchment boundaries

2.2.3 Monitoring methods

To meet the objective of the early detection of an incursion of the target pest species (see Section 2.2.1, above) monitoring methods must be effective in detecting:

1. The target species.
2. The different life-history stages of the species which may be present in the early stages of an incursion (i.e. larval, juvenile, adult).
3. Very low abundance of all life-history stages.
4. The species within relevant habitats (i.e. deep, shallow, lotic (flowing water) and lentic (still water – impoundments).

Different monitoring methods and amount of effort to be employed, may be required to enable effective detection. These will differ with the various incursion scenarios, ranging from difficult (small number of larvae in deep still habitat) to relatively easy (e.g. many large adult fish in a small flowing stream). Obviously the distance between the site of the incursion to a monitoring site will also influence early detection probability (see Section 3.1.1, below).

It is proposed to base surveillance on a combination of indirect sensing (using analysis of water samples for the presence of DNA from target species (eDNA)), and direct sensing (using conventional, active and passive, fish sampling techniques) (Table 3). The methods vary in their detection effectiveness depending on habitat, water quality conditions and biological characteristics of fish (i.e. size, behaviour, etc.).

Regardless of surveillance techniques, the ability to detect target fauna is related to their abundance and biomass, and distribution, and these may increase over time. Biomass can increase due to the growth of individuals or an increase in abundance due to reproduction, and distribution can increase due to the spread of individuals, which can also be promoted by increasing abundance. However, the sampling techniques suggested above, used in varying combinations with varying effort, adjusted for habitat types across the proposed surveillance locations (see Section 3.1.1, below) and scheme connection phase, are considered to provide a robust method for surveillance, and importantly in the early stage when fish abundance or size may be very low and the incursion site very restricted: the ‘needle in the haystack’ scenario.

Reports of target species capture by recreational anglers or via observation or image capture by the public, are extremely valuable data sources, particularly in the early phase of incursion when numbers may be low. Other methods of detection (e.g. baited remote underwater video (BRUV) stations, larval trawling,, etc.) should also be considered and evaluated. In particular these may be useful in reservoirs where early detection probability is lower due to the large volume of aquatic habitat, increased depth, and water clarity which may influence avoidance of passive capture gear types. These methods can be time consuming and expensive but may add value to more rigorous techniques for early detection of establishment and range expansion, for target fish species.

eDNA

Environmental DNA (eDNA) is a relatively new and evolving technique and relies on the ability to detect short strands of DNA, unique to each target species, from a water sample (Wood *et al.* 2013; Wilcox *et al.* 2016; Taberlet *et al.* 2018). It has been shown to be more sensitive than physical sampling of fish in some circumstances (Smart *et al.* 2015, McColl-Gausden *et al.* 2021), however, many environmental factors can influence the presence, persistence and amount of DNA in the water column, and the ability to isolate and detect DNA in the sample, leading to potential false negatives (non-detection when present) or false positives (detection when absent) (Furlan and Gleeson 2016a; Hinlo *et al.* 2017a; Taberlet *et al.* 2018; Stewart 2019, Tingley *et al.* 2021). Consequently, eDNA monitoring, whilst increasingly valuable as an indirect detection method, is currently primarily considered an effective, complimentary, tool when used in conjunction with physical sampling for target species, particularly in new environments which may present novel conditions.

Table 3. Comparison of indirect and direct sampling methods for capturing different life history stages of the target pest fish from various water body types and depths

Waterbody type (WB): S – small stream, L – large stream, R – reservoir; Water depth (WD): Sh – shallow (< 2 m), De – deep (> 2 m); Life-history stage (LH): La – larval, Ju – juvenile, Ad – adult; ? – possibly low effectiveness.

Gear Type		Redfin Perch	Climbing Galaxias	Eastern Gambusia[1]	Comments
eDNA (indirect)	WB WD LH	S, L, R? Sh, D? n/a	S, L, R? Sh, D? n/a	S, L, R Sh, D? n/a	– Effectiveness influenced by amount of DNA which can be influenced by water volume and amount of mixing, fish abundance, DNA inhibitors
Electrofishing Backpack (direct, active)		S Sh La?, Ju, Ad	S Sh La?, Ju, Ad	S Sh Ju?, Ad	– Can be used along edge of reservoir but limited – Effectiveness can be reduced in low conductivity waters
Electrofishing Boat (direct, active)		L,R Sh, D Ju?, Ad	L,R? Sh Ad	L Sh Ad	– Increasing depth (>3 m) and clear water can reduce effectiveness in reservoirs – Effectiveness can be reduced in low conductivity waters
Larval Light Traps (direct, passive)		S, L, R Sh La	S, L, R Sh La	S, L, R Sh Ju?	– Best set in in streams in quiet water along edge; can be set suspended in reservoirs or within structural habitat – Only targets larvae but may collect early juveniles
Plankton Tow (direct, active)		S, L, R Sh, D La, Ju	S, L, R Sh, D La, Ju	S, L, R Sh, D La, Ju	– Best used for larvae in shallower water – Targets slower swimming life history stages
Fyke Nets (direct, passive)		S, L, R Sh, De? Ju, Ad	S, L, R Sh, De? Ju, Ad	S, L, R Sh, De? Ju, Ad	– Larval fyke nets for larvae; Fine-mesh fyke nets for juveniles – Can only be set along edge (excludes at depth in reservoirs)
Gill Nets (direct, passive)		L, R Sh, De? Ad	-	-	– Effective for fish with spines – Can be suspended at the surface or at given depth in reservoirs – Clear water, and large water volumes compared to habitat, can reduce effectiveness in reservoirs – Requires fish to move
Baited Remote Underwater Video (BRUV) (direct, passive)		R Sh, De La?, Ju, Ad	R? Sh, De La?, Ju, Ad	R? Sh, De La?, Ju, Ad	– Common technique in marine environments – May be effective in reservoirs with good water clarity – research is emerging
Line fishing (direct, active)		L, R Sh, De Ju, Ad	N/A	N/A	– High effort to catch ratio – Unlikely to be effective if density is low

[1] Lacking larval stage, live young released.

[2] Larvae reside in stream bed in riffles.

False positive detections (detections when not present) can also occur, however, these are often due to sample contamination (Evans *et al.* 2017; Sepulveda *et al.* 2020; Tingley *et al.* 2021). In the context of this surveillance program, a primary situation which will lead to false positives in Tantangara Reservoir and locations further downstream where target pest species are not found, is during the post-connection phase.

When water is pumped from Talbingo Reservoir to Tantangara Reservoir, it is highly likely to contain DNA from the pest species present in Talbingo Reservoir or nearby, (i.e. Redfin Perch, Climbing Galaxias and Eastern Gambusia), and eDNA sampling from Tantangara Reservoir will likely test positive for the DNA of one or more of these species even if larvae, juvenile or adults have not moved through the scheme into the reservoir. This type of false positive detection can be avoided by shifting the collection of water samples for analysis to locations where water may contain the DNA of target species only if they are physically present, e.g. inflowing tributary streams to reservoirs or larger rivers, above the reservoir full supply level (FSL) or recent flood levels.

False negative detections can be reduced by developing effective genetic probes which have high sensitivity to the DNA of target species, particularly at very low amounts, as well as specificity, to avoid positive detection of evolutionarily closely related species (i.e. similar DNA) (Furlan *et al.* 2016; Wilcox *et al.* 2016; Bylemans *et al.* 2019; Hinlo *et al.* 2018). Once a probe is developed, it should be field tested alongside physical sampling to provide a level of confidence in its detection ability and modified if required to be improve (e.g. Hinlo et al. 2017b). Only once a probe has been optimised can a high level of confidence in results be reached from the sole use of this technique. Whilst probes have been developed for the study area for Redfin Perch, Climbing Galaxias and Eastern Gambusia, only the Redfin Perch probe has been thoroughly tested. Consequently, the probes for the other species should be further refined for specificity using a broad range of fish tissue from a wide range across or nearby to the surveillance area, as well as sensitivity testing, to ensure precision in detection of these taxa and in a range of differing water bodies (i.e. still, flowing, cold, deep, etc.).

DNA detection surveillance, using multispecies metabarcoding, has been employed in the study area for the target pest species, (Griffith et al. 2017, Cardno 2019, Robinson et al. 2019) and surveillance has recently shifted to single-species detection (Weeks et al. 2019; Griffith et al. 2020, 2022). A species-specific probe for Redfin Perch has been developed and refined (Furlan and Gleeson 2016b; Hinlo et al. 2017b; Rojhan et al. 2021), however, species-specific probes for Climbing Galaxias and Eastern Gambusia have only been recently developed and may benefit from additional optimisation to improve detection and specificity (e.g. by using additional tissue from individuals collected from the monitoring catchments). For these species, information on detection probability is essential and can be informed by trials to determine species DNA production rates and downstream persistence (Wilcox *et al.* 2016; Hinlo 2018; Hinlo *et al.* 2018). Detection sensitivity/probability will be affected by a range of parameters such as target species ecology (burrowing, pelagic/benthic, spawning season), adult size, fish density, stream sampling position, turbidity, flow etc).

A complication of using eDNA sampling is that following scheme connection, DNA from Redfin Perch, which is present in Talbingo Reservoir, and Climbing Galaxias, which are highly likely to also be present, will be transferred in the water pumped to Tantangara Reservoir, and transferred downstream of Tantangara to the mid-Murrumbidgee during flow releases, or to Eucumbene Reservoir via the Tantangara-Eucumbene Tunnel. An option following connection is to shift the eDNA monitoring locations into adjacent tributary streams (above FSL for reservoirs, or flood level in streams), thereby monitoring only for DNA from fish which have established and moved into tributaries but continue physical sampling in reservoirs.

Consideration should also be given to the potential to detect an ‘abundance signature’ for an established pest fish species in the surveillance are by analysing DNA amount via copy number of target species eDNA (i.e. DNA from established populations will be in higher abundance than DNA in water derived from Talbingo Reservoir). However, this technique will be insensitive during an early incursion phase as establishing populations outside of Talbingo Reservoir will also produce a low abundance of DNA. Further, Climbing Galaxias is already naturally present in Lake Eucumbene, and therefore only Redfin Perch eDNA is of value to analyse in this system.

To compensate for potential issues with eDNA surveillance, every positive detection of target species DNA in an area where the species has previously been considered absent, should be confirmed by physical sampling techniques (see below).

Physical sampling techniques

The physical methods suggested are commonly used in the detection and/or management of pest fish species, particularly Redfin Perch (e.g. Closs *et al.* 2003; Norris and Low 2005; West et al. 2007; Knight 2010; Ayres and Clunie 2010; Faulks et al. 2011; Ingram 2016; Raadik et al. 2015; Raadik 2017) or Climbing Galaxias larvae (Matveev et al. 2002; Matveev 2003). Therefore the following discussion will only deal with specifics related to their use in the surveillance area and with the target pest species (also see Table 3).

Physical sampling using backpack electrofishing equipment can be effective for surveillance of fish incursion, however, detection probability can be very low if only a small number (e.g. < 5) of fish are present, such as in the early phase of invasion. Therefore, as it is important to detect small numbers of invading fish, incorporating environmental DNA (eDNA) surveillance will improve detection probability (e.g. Bylemans *et al.* 2016; Hinlo *et al.* 2018). As mentioned above, the eDNA method must have high selectivity for each target species, but also very high sensitivity to a low abundance of DNA in the stream (Furlan *et al.* 2016; Hinlo *et al.* 2017a).

• Backpack electrofishing

Suitable habitats: Effective in sampling fish species in small to moderate sized streams or small wetlands of wadeable depth (e.g. less than 1.5 m deep, > 10 m wide).

Species: All target species, though some inefficiency with Eastern Gambusia.

Life-history stages: Juvenile and adult , though ineffective for small juvenile Eastern Gambusia.

Disadvantage: In low conductivity waters, such as alpine environments, can be power-limited, though this can be compensated for by use of higher-powered units (> 700 watts) and modifying sampling technique (Raadik and Lintermans 2022b). Power-limited in larger bodies of water.

Advantages: Active technique so rapid (e.g. > 1 hr for sample), non-selective for species and generally for juveniles and adults, except larvae but can be modified to specifically target these. Efficient and easy, non-destructive. More efficient than fine mesh Bait Traps (passive technique) in small to medium flowing streams.

Comments: The most efficient method in shallow, smaller water bodies, an active technique, can be used over large spatial areas and habitat or flow types, has relatively low species bias and immediate results. Particularly effective for galaxiids and trout (Ayres *et al.* 2012, Raadik and Nicol 2013, Raadik *et al.* 2015, Raadik 2018, Allan *et al.* 2021). Operator is followed by a second person using a fine mesh dipnet to retrieve fish missed by the operator. Therefore, smaller fish, such as larvae, may also be captured, or fish difficult to electrofish (stun) effectively, such as Eastern Gambusia, can be collected. Both operators can also visually detect the presence of Eastern Gambusia if water clarity is good.

• Boat electrofishing

Suitable habitats: Effective in sampling fish species in moderate to large streams, large wetlands and lakes/reservoir (effective depth to 3–4 m).

Species: Redfin Perch, trout species, Climbing Galaxias, ineffective for Eastern Gambusia in larger waterbodies.

Life-history stages: Mainly adults, marginal for smaller juveniles.

Disadvantage: In low conductivity waters, such as alpine environments, can be power-limited, though this can be compensated for by use of higher-powered units and modifying sampling technique. Limited by sampling depth. Requires sufficient depth to navigate. Usually setup, and more efficient for, larger sized fish.

Advantages: Active technique so rapid (e.g. > 1–1.5 hours for sample), non-selective for species and generally for adults. Efficient and easy, non-destructive.

Comments: Different sized boats can be deployed into various waterbodies either by vehicle or helicopter. Operator and crew can also visually detect the presence of Eastern Gambusia and other species if the water is clear. Has been shown to be effective for Redfin Perch in lakes and reservoirs (Faulks *et al.* 2011; Cardno 2019).

• Larval light traps

Suitable habitats: Effective in sampling fish larvae (when available) in very slow flowing to still areas of creek, rivers, wetlands, lakes and reservoirs, with light stick actively attracting larvae to trap.

Species: Redfin Perch, Climbing Galaxias.

Life-history stages: Larvae, possibly some smaller juveniles.

Disadvantage: Passive technique so must be set for a long period of time (e.g. 12 hrs). Can only be used during the breeding period of target species. Can be affected by increasing water flow.

Advantages: Can be set just below the surface or suspended at greater depth to sample where larvae may be. Specifically trap fish larvae so less non-target fauna captured (e.g. pelagic macroinvertebrates). Larvae are not damaged by abrasion against fine mesh material by flow.

Comments: Light traps, suspended in the limnetic zone of a reservoir, have been used successfully to trap Climbing Galaxias larvae (e.g. Matveev *et al.* 2002; Matveev 2003), and in rivers and billabongs for Redfin

Perch larvae (Humphries *et al.* 2002; King *et al.* 2007). Potentially more effective if deployed in conjunction with plankton tows.

• Plankton tows

Suitable habitats: Effective in sampling fish larvae (when available) and juveniles in the water column, from below the surface or at greater depth, in flowing to still areas of creek, rivers, wetlands, lakes and reservoirs.

Species: Redfin Perch, possibly Climbing Galaxias.

Life-history stages: Larvae, possibly some smaller juveniles.

Disadvantage: Unable to be used amongst timber debris and requires a motorised punt to haul the net. Larvae may be damaged by abrasion.

Advantages: Rapid active technique which does not rely on flow or fauna swimming into device. Can be set to trawl just below the surface, or at greater depth, to sample where larvae may be.

Comments: A standard technique to capture fish larvae, often in conjunction with light traps (e.g. Cheshire and Ye 2008; Cheshire 2010; Humphries *et al.* 2002; Humphries and King 2004). Potentially more effective if undertaken with deployment of larval light traps.

• Gill nets

Suitable habitats: Effective in sampling larger size classes of fish (depending on mesh size) in slowly flowing or still reaches of rivers, lakes and reservoirs.

Species: Redfin Perch, trout species.

Life-history stages: Usually adults, but possibly some larger sized juvenile fish.

Disadvantage: Passive technique so must be set for a period of time (few to many hours) and efficiency affected by water flow and by visual detection by target species in clear water. Relies on movement of fish with spines in fins or on the body (e.g. fish running into the mesh and becoming trapped enmeshed by the spines). Selective for fish species, type and size, usually destructive (resulting in fish death).

Advantages: Targets fish movement. Can be used in open water and at depth (e.g. surface set or suspended at depth)

Comments: Effective in detecting Redfin Perch at moderate to large population sizes in impoundments (Cardno 2019) but limited when fish are at low density. Useful in reservoirs, particularly if set near habitat near the shore, if boat electrofishing is marginal.

• Fyke nets (larval and medium mesh nets)

Suitable habitats: Depending on mesh and fyke net length, effective in sampling larval, juvenile and adult size classes of fish in to medium flowing or still reaches of creeks, rivers, lakes and reservoirs.

Species: Redfin Perch, Climbing Galaxias, trout species.

Life-history stages: Depending on mesh size, larvae, juveniles and adults.

Disadvantage: Passive technique so must be set for a period of time (few to many hrs) and efficiency affected by water flow and by visual detection by target species in clear water. Relies on the movement of fish.

Advantages: Non-destructive and targets fish movement, but flowing water can direct other species of fish into the net if the net is set facing upstream. Captures fish without entangling them. Usually set in open water but near habitat and can also operate at greater depth than backpack electrofishing.

Comments: Effective in detecting Redfin Perch at moderate to large population sizes in impoundments (Cardno 2019) but limited when fish are at low density. Useful in reservoirs, particularly if set near habitat near the shore, if boat electrofishing is marginal.

Where direct physical sampling, and potentially indirect sampling using eDNA, is difficult, e.g. large, deep, clear water reservoirs, where fish may be easily disturbed by approaching equipment or may avoid sampling equipment, consideration should be given to alternative methods of surveillance, e.g. use of baited remote underwater video (BRUVs), deployment of bait traps, larval tows, etc. However, these techniques need to be assessed for value related to capture efficiency, effort, and cost.

2.2.4 Pre and post connection considerations

The main pathway for potential pest fish incursion related to the Snowy 2.0 Scheme is via the new Tantangara-Talbingo Tunnel (Cardno 2019). Consequently, post connection pest fish surveillance must commence once any water transfers occur between the two reservoirs; this includes the testing and commissioning phase of the scheme, which will be earlier than the commencement of normal operation.

The important connection phase for pest fish surveillance related to the new Talbingo-Tantangara Tunnel is therefore post-connection (Table 4), though establishment of base-line data on the presence/absence of all target species should be undertaken pre-connection.

The pathways of human-assisted dispersal and natural colonisation of pest fish do not rely on the scheme connection, may operate at any time, and include any of the target pest fish species (Table 2). In addition, although studies for Snowy 2.0 established the current distribution of the target pest species with a reasonable degree of certainty, there is value in replicating sampling to improve levels of confidence regarding the presence/absence of the target species across the areas of interest. Therefore, surveillance should operate during the pre- and post-connection phases.

Table 4. Pest fish surveillance requirement related to potential incursion pathways and scheme connection phase. PRE – pre-scheme connection, POST – post-scheme connection

Potential incursion pathway	Pathway frequency	Probability	Important connection phase	Specific surveillance considerations
Talbingo-Tantangara tunnel	Infrequent to frequent during commissioning, frequent during pumping phase in normal operation	Possible	Post	Incursion pathway will operate once scheme connection is established
Human – assisted	Rare	Very low but possible	Pre, Post	No published evidence exists of the deliberate translocation of Redfin Perch, Climbing Galaxias or Eastern Gambusia to Upper Murrumbidgee catchment in the past 100 years. They are however located elsewhere and the potential for human assisted translocation exists, particularly for Redfin Perch which is a popular species for recreational fishing. Recent evidence of deliberate translocations of redfin in other catchments (e.g. Lake Lyell) suggests this remains a contemporary problem Eastern Gambusia are commonly moved to control mosquitoes in domestic ponds. Contamination of recreational fish stocking is possible, with Redfin Perch contamination of trout stockings documented in the ACT (Lintermans *et al.* 1990b)
Natural colonisation	Extremely rare	Extremely low	Pre, Post	There remains an extremely low possibility that Climbing Galaxias may naturally colonise the upper Murrumbidgee River catchment from the Eucumbene or Talbingo catchments. Consequently, surveillance is required

3 Pest fish surveillance

This program for pest fish surveillance is focussed on identifying potential pest fish incursions once a two-way hydrologic connection is established between Talbingo and Tantangara reservoirs. It also considers alternative pathways for pest fish incursion (see above).

The program aims to maximise the early detection of the target pest species in streams and impounded habitats by addressing the constraints and considerations listed above, and specific details of the plan are provided below. Whilst pre- and post-connection stages of the Snowy 2.0 scheme are important to consider, the aquatic connectivity conditions and pest fish incursion risk between the stages differ significantly. Consequently the pre-connection component of this program is designed to carry through to post-connection, but with modification as required for post-connection conditions/constraints.

Regular review of the surveillance program (i.e. locations, methods, effort, frequency) is recommended to ensure that it will remain effective through the pre- and post-connection phases. At the minimum, a review should be triggered in any phase of the scheme by the report or detection, spread or establishment of a target pest species in a novel location in the surveillance catchments. This will necessitate a re-evaluation of the potential risk of incursion to connected, adjacent surveillance catchments and fish assets being protected, which will influence which sites are monitored, at what frequency and for which target pest fish species. Consequently, a pest fish surveillance program can only be developed for an initial period, until a pest fish incursion is detected, and the amount of modification required to the program will depend on factors such as the:

- Number and localities of pest fish incursion.
- Species of pest fish.
- Degree and rate of spread of the pest fish incursion.
- Success of any management actions.

This may lead to minor or significant modifications to the program, either increasing or decreasing effort, surveillance sites or catchments.

The risk of target pest fish incursion is relatively low in the pre-connection phase, and surveillance will also be informed by catchment surveys and regular monitoring of Macquarie Perch and Stocky Galaxias (Lintermans *et al.* 2022a,b; Raadik and Lintermans 2022a,b). Therefore, unless the length of this phase exceeds three years, a review of the surveillance program may not be necessary in the absence of a pest fish incursion or other conditions which may influence a major change to stream connectivity between the surveillance area pathways (e.g. major flood reaching or exceeding FSL of Tantangara or Eucumbene reservoirs, etc.).

However, as the risk of pest fish incursion in the post-connection phase is high, biennial reviews of the surveillance program are recommended in the absence of pest fish incursions, large floods or scheme operation changes in the first four years. The surveillance program review period may increase following this to every five years assuming the plan is informed and modified by the events listed above and accumulating surveillance data.

This section identifies areas of catchments important for surveillance, actions to undertake surveillance, and actions to undertake if pest fish are detected. Factors important to consider for the selection of surveillance catchments are the distribution of the asset being protected (i.e. Macquarie Perch and Stocky Galaxias in the Mid to Upper Murrumbidgee catchment and the salmonid fishery in Lake Eucumbene), target pest fish and their known distribution, potential pathways for incursion and constraints imposed by the operating conditions of the reservoirs.

3.1 Surveillance details

Pest fish surveillance is proposed to be undertaken at five broad localities, each with different surveillance requirements pre- and post-connection phases – target pest species, primary methods and sampling equipment, and sampling details (Table 5). The primary surveillance method proposed is eDNA screening in combination with varying levels of physical sampling using a range of equipment (see Section 2.2.3, above), selected to suite the target pest species, life-history stages and characteristics of each specific monitoring site.

Periodic eDNA surveillance has occurred since 2017 (Griffith *et al.* 2017, Cardno 2019, Robinson *et al.* 2019, Weeks *et al.* 2019; Griffith *et al.* 2020, 2022) and these sites from should be maintained with appropriate modification post-connection. The data generated from previous surveillance events, including any learnings from sample collection to analysis, are important to incorporate into this program. Due to the developing nature of eDNA detection, and the potential for false positive or negative detections, some physical sampling is also proposed as complimentary methods to verify eDNA results, thereby contributing to eDNA method development and increasing confidence in eDNA detection.

eDNA needs to ensure that the detection probabilities for the target pest fish species are maximised (e.g. highly specific for target species, able to detect very low concentrations of DNA, methods optimised for maximum extraction of DNA from collected water samples, etc.). As such, the use of single species DNA probes is preferable to a metabarcoding method, which has been used in recent periodic eDNA sampling in the surveillance catchments (Weeks *et al.* 2019; Griffith *et al.* 2020, 2022). Consideration should also be given to optimising the DNA probe for Climbing Galaxias and Eastern Gambusia for additional specificity (using additional target species tissue from within or nearby to the surveillance catchment) and sensitivity and undertaking some level of sensitivity analysis. Further, as eDNA detection is still in a development phase, a minimum of three replicate filtered samples should be taken per sampling site (spaced about 100–200 m apart, taken along a transect in still or flowing water), and a minimum of 20% of positive detections in the laboratory to be sequenced using PCR to verify the detection.

Establishing a wide and dense network of surveillance sites in each monitoring catchment would improve an eDNA surveillance program. This approach would increase the likelihood of detecting pest fish incursions which may occur anywhere in the monitoring catchments from other potential incursion pathways separate to the Snowy 2.0 scheme connection (see Section 2.2.2, above).

Table 5. Target pest fish and surveillance methods, potential sampling equipment, and sampling details for specific surveillance locations

PRE – pre-scheme connection, POST – post-scheme connection, eDNA – environmental DNA detection, Physical – physical sampling methods (EF/BP – backpack electrofishing, EF/B – boat electrofishing, LLT – larval light trap, GN – gill nets, FN – fyke nets).

Surveillance Location	Connection stage	Target pest fish	Primary method	Comments
Talbingo Reservoir near tunnel inlet	Pre & Post	Redfin Perch Climbing Galaxias Eastern Gambusia	eDNA	– monitor continuing presence of pest fish species – ongoing positive eDNA control for three pest fish species (undertake physical sampling (EF/B, LLT, consider GN, FN and other less rigorous methods listed above) if no positive eDNA detection)
Tantangara Reservoir	Pre	Redfin Perch Climbing Galaxias Eastern Gambusia	eDNA & Physical	– establishing a baseline of presence/absence – physical sampling (EF/B, LLT, consider GN, FN and other less rigorous methods listed above), to verify and complement eDNA results
	Post		eDNA & Physical	– shift eDNA sites into tributary streams above FSL – ongoing eDNA surveillance of pest fish – physical sampling (gear as above) to verify and complement eDNA results
Upper Murrumbidgee R and tributaries, including Goodradigbee R and Sallys Flat Ck)	Pre	Climbing Galaxias	eDNA	– establishing a baseline of presence/absence – physical sampling (EF/BP) to verify and complement eDNA results
	Post	(Eastern Gambusia and Redfin Perch if required in lower reaches)	eDNA & Physical	– ongoing eDNA surveillance of pest fish – physical sampling (EF/BP) to verify and complement eDNA results
Mid-Murrumbidgee R and tributaries	Pre	Redfin Perch Eastern Gambusia	eDNA	– establishing a baseline of presence/absence; Eastern Gambusia only at selected upper sites – one-off, wider eDNA catchment screen for target pest-fish with physical sampling (EF/B, LLT, consider GN, FN and other less rigorous methods listed above) to verify positive eDNA detections
	Post		eDNA & Physical	– shift eDNA sites into tributary streams or undertake mainstream sampling during periods when releases from the dam have not occurred for 1 month – ongoing eDNA surveillance of pest fish – physical sampling (gear as above) to verify and complement eDNA results (sites remain at pre-connection locations)
Lake Eucumbene	Pre	Redfin Perch	eDNA	– establishing a baseline for presence/absence – ongoing positive eDNA control for Climbing Galaxias in Gang Gang Creek (Pre and Post) (undertake physical sampling if no positive eDNA detection)
	Post		eDNA & physical	– ongoing eDNA surveillance for Redfin Perch – physical sampling (EF/B, LLT, consider GN, FN and other less rigorous methods listed above) to verify and complement eDNA results

3.1.1 Monitoring sites and frequency

A wide network of monitoring sites is proposed in each of the five surveillance catchments to cover high risk (high incursion probability) from these multiple areas (e.g. not just around tunnel outlets). This approach would maximise the potential detection of target pest fish as early as possible (i.e. at low abundance) after their introduction given the multiple incursion pathways,.

The following monitoring sites are proposed in each surveillance catchment: Talbingo Reservoir (Table 6), Tantangara Reservoir (Table 7), upper Murrumbidgee River upstream of Tantangara Reservoir, including two nearby catchment sites (Table 8), Lake Eucumbene (Table 9), and Mid Murrumbidgee catchment (Table 10). The sites are indicative only and will require ground-truthing and adaptive sampling with respect to the adequacy of vehicle or boat access (water level, tracks, boat ramps, etc.) during pre/and or post connection periods, including weather and safety constraints for boat access. Sampling locations in reservoirs have been selected based on geographic spread and areas of potential incursion via tunnel outlets (greater density around outlets), and popular fishing locations. The locations however will require localised refinement towards areas containing favourable habitat for the target species which is best determined in the field during sampling.

A frequency of surveillance is proposed in Tables 6–10 within each surveillance catchment (e.g. annual or biennial). Is should be noted that within a surveillance 'year', one or more surveillance trips may be required, depending on the surveillance technique(s) (e.g. eDNA, physical sampling) and the life-history phase of the pest fish species being targeted (e.g. larvae, juveniles, adults), which will be influenced by season. Further, depending on the review of the program if a pest fish incursion occurs, greater frequency of surveillance may be warranted (e.g. biannual)

Indicative locations for sites in the Upper Murrumbidgee, Eucumbene and Talbingo catchments are provided in Figure 1 (pre-connection) and Figure 2 (post connection), and for the Mid Murrumbidgee catchment in Figure 3.

Table 6. Indicative pest fish surveillance sites in Talbingo Reservoir, including target species, connection stage, method and frequency

CGal – Climbing Galaxias; EGam – Eastern Gambusia; RedF – Redfin Perch.

Site	Specific location	Species	Stage	Methods	Frequency	Justification
TL1 Ya1	Talbingo Reservoir around water intake infrastructure and in lower Yarrangobilly River	CGal EGam RedF	Pre & Post	– eDNA, Physical	Annual.	Positive control sites where target species are present – annual monitoring for each target taxa, until each established in Tantangara Reservoir a minimum of three months Note: A single detection of each species per year using any method is considered sufficient

Table 7. Indicative pest fish surveillance sites in Tantangara Reservoir, including target species, connection stage, method and frequency

CGal – Climbing Galaxias; EGam – Eastern Gambusia; RedF – Redfin Perch.

Site	Specific location	Species	Stage	Methods	Frequency	Justification
TR1 to 13	Around edge of reservoir in areas of potential habitat	CGal EGam RedF	Pre	– eDNA, Physical	Year 1, then biennially	Annual monitoring for incursion, establishment and expansion – eDNA sites along edge in reservoir.
TR1, 3 to 5, 7 to 10, 12 to 13	Sites into lower reaches of nearby stream (sites 2, 6, 11 excluded)		Post	– eDNA – Physical – at pre connection locations		Annual monitoring for incursion, establishment and expansion – eDNA sites shift to above supply level at the mouth of inflowing tributaries where possible or occur within the reservoir if no pumping has occurred for a period of 1 month.

Table 8. Indicative pest fish surveillance sites in Upper Murrumbidgee and surrounding catchments, including target species, connection stage, method and frequency

CGal – Climbing Galaxias; EGam – Eastern Gambusia; RedF – Redfin Perch.

Site	Specific location	Species	Stage	Methods	Frequency	Justification
UMU1 to 7	Murrumbidgee River upstream of Tantangara Reservoir, to headwaters	CGal EGam RedF				Monitor for incursion, establishment and expansion upstream to Tantangara Creek and catchment divide to Stocky Galaxias population in Sally's Flat Creek
			Pre	– eDNA, Physical	Year 1, then biennially	– single eDNA run to set baseline, then biennially
			Post	– eDNA, Physical	Annual	– monitoring at UMU1 to 3, expanded to all sites for CGal if target taxa detected in Tantangara Reservoir
TA1 to 6, Tat1, BC1	Tantangara Creek system (includes one site on Boggy Plain Creek)	CGal				Monitor for incursion, establishment and expansion upstream towards, and into, the Stocky Galaxias population
			Pre	– eDNA, Physical	Year 1, then biennially	– single eDNA run to set baseline, then biennially
			Post	– eDNA, Physical	Annual	– annual eDNA monitoring at TA1, expanded to all sites if detected in Tantangara Reservoir
SF1	Sally's Flat Creek, below trout barrier (Goodradigbee system)	CGal				Monitor below Stocky Galaxias population for incursion and establishment
			Pre	– eDNA, Physical	Year 1, then biennially	– single eDNA run to set baseline for target taxa
			Post	– eDNA, Physical	Annual	– annual eDNA monitoring if CGal detected at UMU3, 4 or 5
GR1 to 3	Goodradigbee River headwaters, around diversion inlet	CGal				Monitor for incursion and establishment
			Pre	– eDNA, Physical	Year 1, then biennially	– single eDNA run to set baseline for target taxa
			Post	– eDNA, Physical	Annual	– annual eDNA monitoring if CGal detected in Tantangara Reservoir
GU1	Gurrangorambla Creek tributary, at outlet of Goodradigbee River aqueduct	CGal EGam RedF				Monitor for incursion and establishment
			Pre	– eDNA, Physical	Year 1, then biennially	– single eDNA run to set baseline for target taxa
			Post	– eDNA, Physical	Annual	– annual eDNA monitoring if detected or established in Tantangara Reservoir

Table 9. Indicative pest fish surveillance sites in Lake Eucumbene, including target species, connection stage, method and frequency

RedF – Redfin Perch; CGal – Climbing Galaxias.

Site	Specific location	Species	Stage	Methods	Frequency	Justification
GG1	Gang Gang Creek, at Snowy Mountain Highway	CGal	Pre &Post	– eDNA	Annual	Positive control site where target species is present – Annual monitoring for target taxa, until established in Tantangara Reservoir a minimum of three months Note: A single detection of the species per year using any method is considered sufficient
LE1 to 16	Eucumbene Inlet; Providence Flats; Hughes Creek Inlet; Long Plain Inlet; Wattledale Inlet; Wangrabelle Bay; Adaminaby Bay; Springwood Bay; White Rocks Inlet; Frying Pan Arm; Buckenderra Arm; Wainui Bay; Cobrabald Bay; Coppermine Bay; Braemer; Big Tolbar Inlet	RedF	Pre	– eDNA, Physical	Year 1, then biennially	Monitor for incursion, establishment and expansion – eDNA sites along edge of reservoir – single eDNA run to set baseline for target taxa, then biennially
LE1a to 5a, 11A to 2a, 15a to 16a	Eucumbene River; Swamp Creek; Hughes Creek Long Plain Creek; Little Plain Creek; Fryingpan Creek; Buckenderra Creek; Andys Creek (Braemer); Big Tolbar Creek		Post		Annual	– eDNA sites shift to above full supply level at the mouth of inflowing tributaries - Annual eDNA monitoring of sites between Eucumbene River Inlet and Wangrabelle Bay (sites LE1–5), expand to all other sites if target taxa detected in Tantangara Reservoir

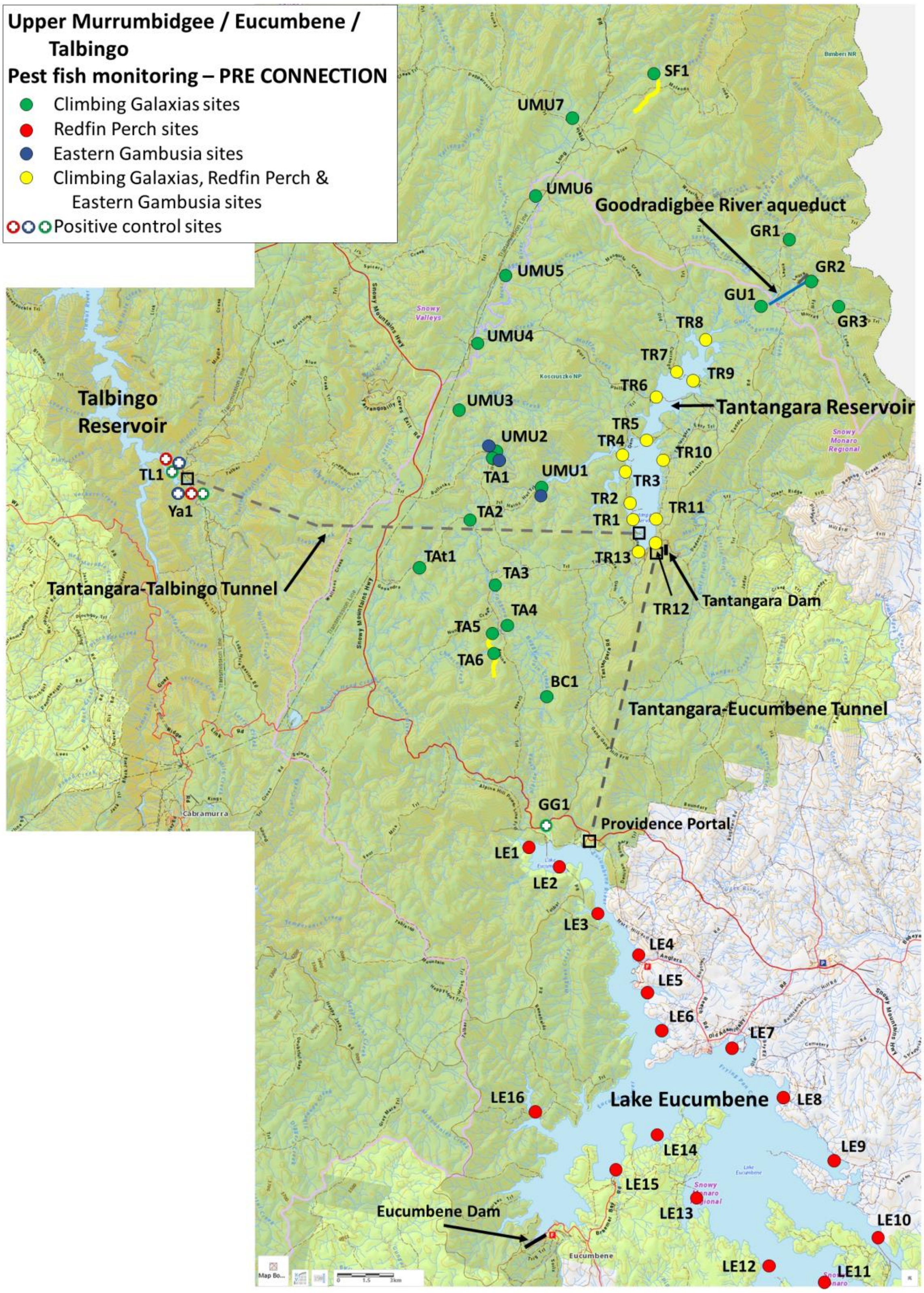

Figure 1. Indicative pre-connection pest fish surveillance sites in the upper reaches of the Tumut, Eucumbene, Upper Murrumbidgee and Goodradigbee River catchments.

Circles: Blue – Eastern Gambusia surveillance; Green – Climbing Galaxias surveillance; Red – Redfin Perch surveillance; Yellow – Redfin Perch, Climbing Galaxias and Eastern Gambusia surveillance. **Open square**: tunnel outlet/inlet. **Lines:** yellow – Stocky Galaxias distribution. **Site Numbers:** BC – Boggy Plain Ck; GG – Gang Gang Ck; GR – Goodradigbee River; GU – Gurrangorambla Creek; LE – Lake Eucumbene; SF – Sallys Flat Creek; TA – Tantangara Creek; TAt – Tantangara Creek tributary; TL – Talbingo Reservoir; TR – Tantangara Reservoir; UMU – upper Murrumbidgee River; Ya – Yarrangobilly River.

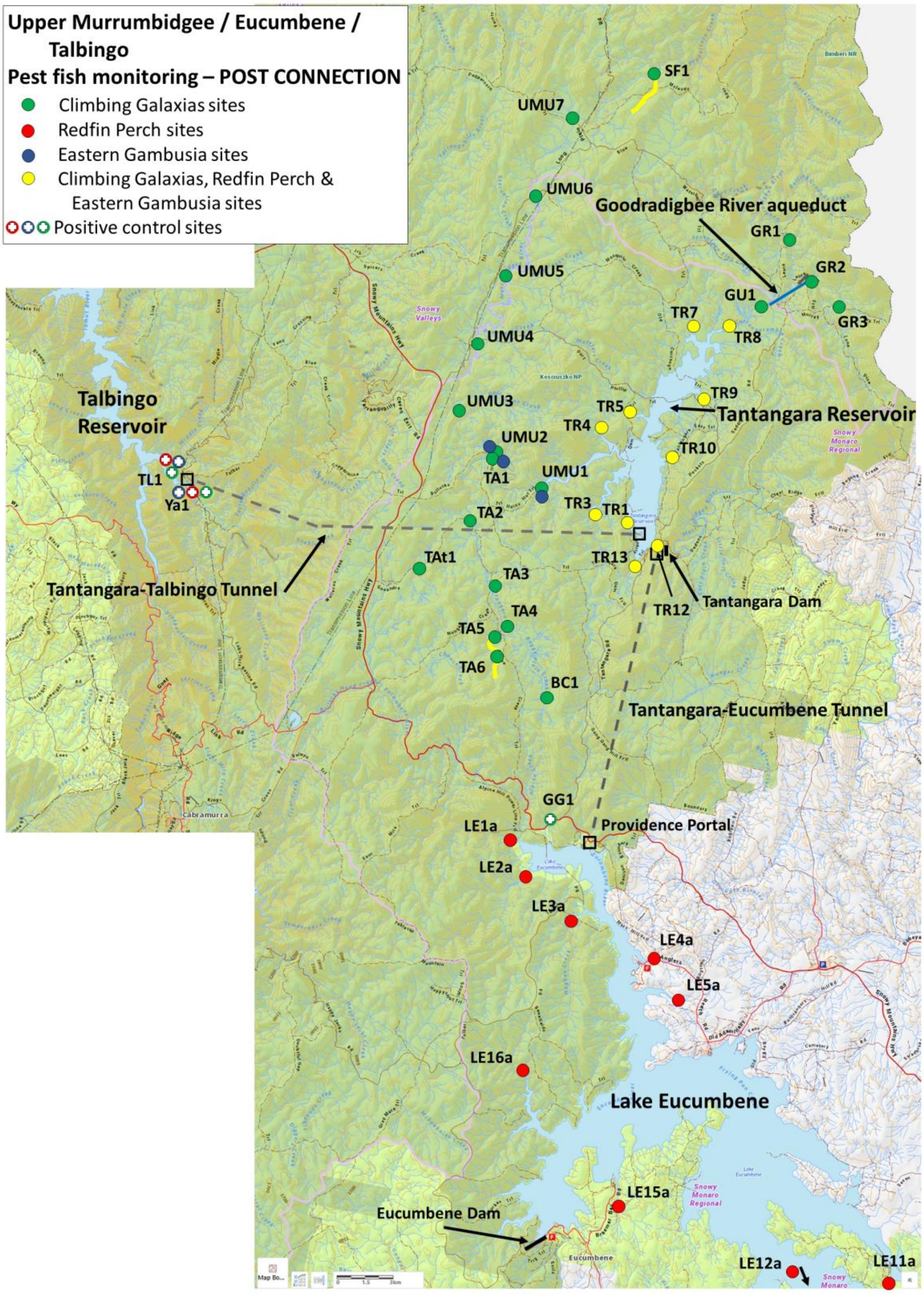

Figure 2. Indicative post-connection pest fish surveillance sites in the upper reaches of the Tumut, Eucumbene, Upper Murrumbidgee and Goodradigbee River catchments.

Circles: Blue – Eastern Gambusia surveillance; Green – Climbing Galaxias surveillance; Red – Redfin Perch surveillance; Yellow – Redfin Perch, Climbing Galaxias and Eastern Gambusia surveillance. **Open squares:** tunnel outlet/inlet. **Lines:** yellow – Stocky Galaxias distribution. **Site Numbers:** BC – Boggy Plain Creek; GG – Gang Gang Creek; GR – Goodradigbee River; GU – Gurrangorambla Creek; LE – Lake Eucumbene; SF – Sallys Flat Creek; TA – Tantangara Creek; TAt – Tantangara Creek tributary; TL – Talbingo Reservoir; TR – Tantangara Reservoir; UMU – upper Murrumbidgee River; Ya – Yarrangobilly River.

Table 10. Indicative pest fish surveillance sites in the Mid Murrumbidgee catchment (Figure 3), including target species, connection stage, method and frequency

A – annual; EGam – Eastern Gambusia; RedF – Redfin Perch.

Site	Specific location	Species	Stage	Methods	Frequency	Justification
Angle Crossing	Murrumbidgee R, off Angle Crossing Rd	RedF	Pre Post	– eDNA, Physical – Physical	Annual Annual	Monitor for incursion, establishment, expansion. Continue post-connection physical sampling
Lawler Rd	Murrumbidgee R, off Lawler Rd	RedF	Pre Post	– eDNA, Physical – Physical	Annual Annual	Monitor for incursion, establishment and expansion. Continue post-connection physical sampling
D/s Bredbo	Murrumbidgee R, off Bumbalong Rd	RedF	Pre Post	– eDNA, Physical – Physical	Annual Annual*	Monitor for incursion, establishment and expansion. Continue post-connection physical sampling
Bredbo R 1	Monaro Highway	RedF	Post	– eDNA	Annual	D/s Bredbo and Billilingra Rd replacement sites for eDNA
Billilingra Rd	Murrumbidgee R, Billilingra Rd	RedF	Pre Post	– eDNA, Physical – Physical	Annual Annual*	Monitor for incursion, establishment and expansion
Numeralla R	Murrumbidgee R, off Monaro Highway	RedF	Pre Post	– eDNA, Physical – Physical	Annual Annual*	Monitor for incursion, establishment and expansion. Continue post-connection physical sampling only
Numeralla R	Monaro Highway	RedF	Post	– eDNA	Annual	Numeralla R (on Murrumbidgee) site replacement for eDNA
Mittagang Crossing	Murrumbidgee R, Mittagang Rd	RedF	Pre Post	– eDNA, Physical – Physical	Annual Annual*	Monitor for incursion, establishment and expansion. Continue post-connection physical sampling only
Kissops Flat	Murrumbidgee R, private road off Dry Plains Road	RedF	Pre Post	– eDNA, Physical – Physical	Annual Annual*	Monitor for incursion, establishment and expansion
Bridle Ck	Dry Plains Rd	RedF	Post	– eDNA	Annual	Kissops Flat site replacement for eDNA
Alum Creek	Murrumbidgee R off Jones Plain Rd	RedF, EGam	Pre Post	– eDNA, Physical – Physical	Annual Annual*	Monitor for incursion, establishment and expansion. Continue post-connection physical sampling. *Positive control for Eastern Gambusia.*
Alum Ck	Jones Plain Rd	RedF, EGam	Post	– eDNA	Annual	Alum Creek site replacement for eDNA. *Positive control for Eastern Gambusia*
Bolaro	Murrumbidgee R, Bolaro Rd	RedF, EGam	Pre Post	– eDNA, Physical – Physical	Annual Annual*	Monitor for incursion, establishment and expansion. Continue post-connection physical sampling only. *Positive control for Eastern Gambusia*

Site	Specific location	Species	Stage	Methods	Frequency	Justification
Yaouk	Murrumbidgee R, Yaouk Rd	RedF, EGam	Pre Post	– eDNA, Physical – Physical	Annual Annual	Monitor for incursion, establishment and expansion. Continue post-connection physical sampling
Yaouk Creek	Yaouk Rd	RedF, EGam	Post	– eDNA	Annual	Yaouk site replacement for eDNA
Murrumbidgee R Firetrail	At Murrumbidgee R	RedF, EGam	Pre Post	– eDNA, Physical – Physical	Annual Annual	Monitor for incursion, establishment and expansion. Continue post-connection physical sampling
Paytens Ck	Off Murrumbidgee River Firetrail	RedF, EGam	Post	– eDNA	Annual	Murrumbidgee Firetrail site replacement for eDNA
Tantangara Road	At Murrumbidgee R	RedF, EGam	Pre Post	– eDNA, Physical – Physical	Annual Annual	Monitor for incursion, establishment and expansion. Continue post-connection physical sampling
Gulf Plain Creek	Pedens Hut Firetrail	RedF, EGam	Post	– eDNA	Annual	Tantangara Rd site replacement for eDNA

* Annual sampling in these locations is only to occur if the relevant species presence in Tantangara reservoir or other Mid-Murrumbidgee catchment locations is confirmed.

To improve baseline knowledge of the distribution of Redfin Perch more broadly in the Mid Murrumbidgee catchment, a detailed pre-connection eDNA survey is proposed (Table 11, Figure 3). This would also increase understanding of other potential sources of incursion into Macquarie Perch habitat other than via downstream or upstream colonisation in the Murrumbidgee River.. This should involve eDNA sampling, with positive detections confirmed by physical sampling.

Table 11. Indicative sites for pre-connection, one-off wider catchment scan for Redfin Perch using eDNA in the Mid Murrumbidgee catchment (Figure 3)

Site designation	Specific location
Strike-A-Light R	Jerangle Road
Bredbo R 2	Dowling Firetrail
Bredbo R 3	Peak View Road
Cowra Ck 1	Dowling Firetrail
Cowra Ck 2	Peak View Road
Murrumbucca Ck	Nightingale Road
Numeralla R 2	Numeralla Road, Numeralla
Numeralla R 3	Off Carlaminda Road
Big Badja R	Badja Road
Kybeyan R 1	Warrens Corner Road
Kybeyan R 2	Tuross Road
Cooma Ck 1	Monaro Highway (downstream of Cooma)
Cooma Ck 2	Monaro Highway (upstream of Cooma)
Cooma Ck 3	Myalla Road
Rock Flat Ck 1	Numeralla Road
Rock Flat Ck 2	Monaro Highway
Cooma Back Ck 1	Snowy Mountains Highway
Cooma Back Ck 2	Maffra Road
Slacks Ck	Dry Plains Road
Wambrook Ck	Snowy Mountains Highway
Bulga Ck	Track off Shannons Flat Road
Caddigat Ck 1	Caddigat Road
Caddigat Ck 2	Snowy Mountains Highway
Wild Mares Ck	Bolaro Road
Goorudee R	Yaouk Road

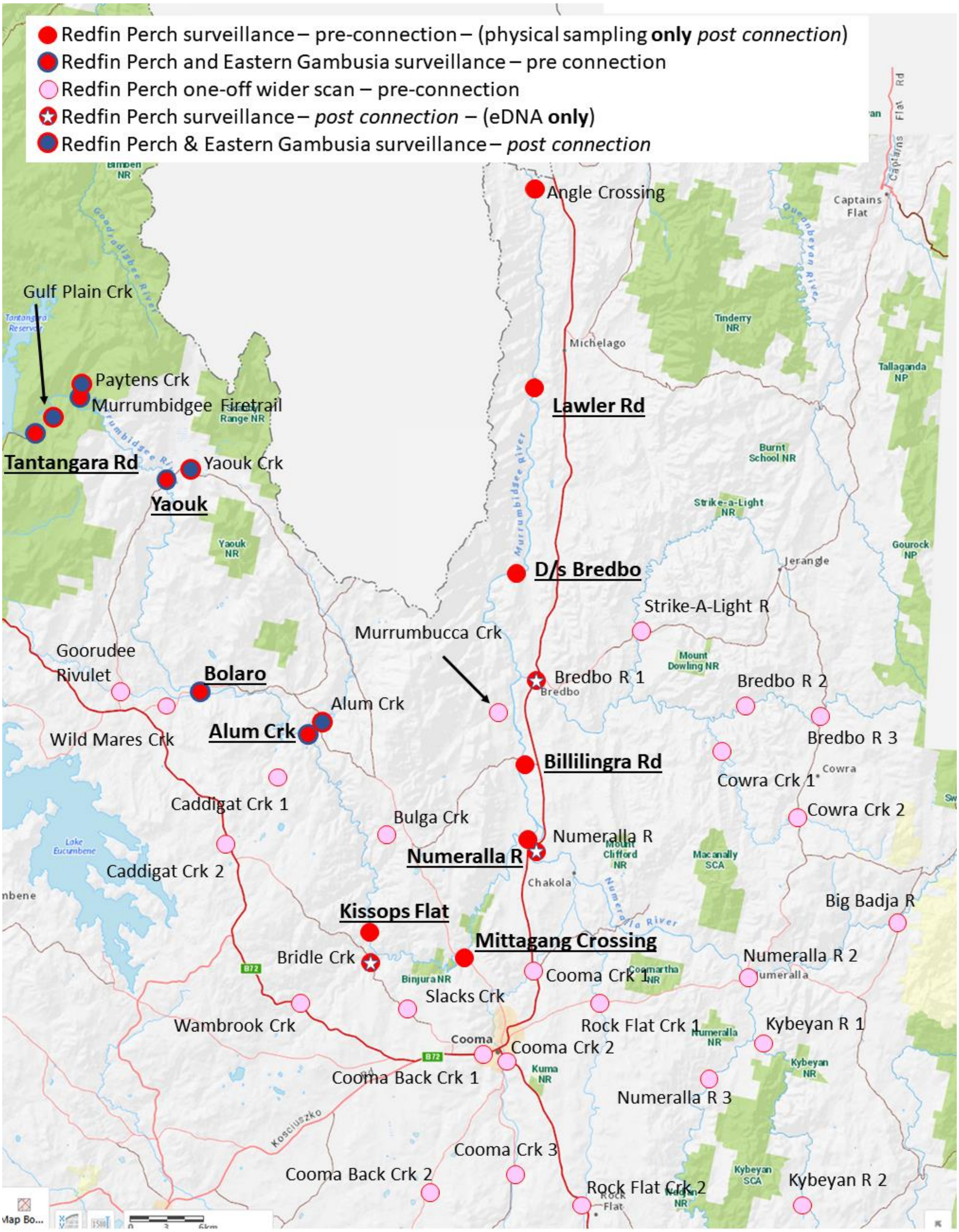

Figure 3. Indicative pre- and post-connection pest fish surveillance sites in the Mid Murrumbidgee catchment, including one-off Redfin Perch wider catchment scan.

Annual monitoring, as per surveillance targets and methods specified for each catchment and site (above), including the one-off broader catchment scan for Redfin Perch, is considered appropriate during the pre-connection phase. However, the detection, or unconfirmed report of, any pest fish at novel sites should trigger 1) a detailed investigation of the incursion (see 3.2, below) and 2) a review of the surveillance program, particularly with respect to any modifications required to maintain or improve the surveillance program efficacy. This may involve appropriate changes in the number or distribution of surveillance sites for target pest species, variation to detection methods employed, and/or overall surveillance effort at sites. This can also be informed by the outcomes of the incursion investigation and any follow-up pest fish management undertaken.

Whilst the initial period post connection poses a risk of transfer and establishment of pest fish into Tantangara Reservoir, transfer and establishment to other secondary locations is unlikely to occur as a direct result of the project unless pest fish first establish in Tantangara Reservoir. It may, however, happen due to human mediated dispersal though this is considered of low likelihood. In the case of Lake Eucumbene and the mid-Murrumbidgee River, fish screens on the outlets to these locations are intended to prevent transfer as far as is reasonably practicable. Similarly, the fish barrier on the upper Tantangara Creek is intended to prevent access to this catchment by Climbing Galaxias. As such, although a baseline level of sampling should occur in all catchments following connection of the Snowy 2.0 pumped hydroelectric station and tunnel, the full suite of annual sampling of all locations is not required unless evidence of establishment is obtained in Tantangara Reservoir or other sentinel locations.

If establishment of pest fish occurs within Tantangara Reservoir following connection of the Snowy 2.0 scheme, there should be an expectation that greater flexibility may be required in the surveillance plan with respect to reviewing plan specifics to maintain efficacy, particularly in the years immediately following pest fish establishment in Tantangara Reservoir. This will obviously be triggered by pest fish reports or detections, but also by the operating schedule of the scheme (when, and how long water is transferred, etc.), with the need for reviews of surveillance activities, and possible increased frequency of surveillance (e.g. biannual) potentially decreasing as the operation of the scheme stabilises over time, the efficacy of fish screens is tested, and any pest fish incursions are managed.

3.1.2 Timing of surveillance

The surveillance program also needs to consider the timing of activities, as these depend on factors such as the target species, their life-history stages and habitats, and sampling efficiency, which can change with seasons and water levels. This may also differ pre- and post- scheme connection.

A key consideration is sampling efficiency, as the focus of surveillance is early detection to allow for early management, which means the ability to detect a small number of individuals (e.g. such as in a founder population). Therefore, undertaking sampling (physical or eDNA) when detection probability may be very low (e.g. elevated water levels and coincident very fast flows during winter to early spring, or following large storm events), should be avoided. However, this may have a trade-off with respect to the optimal time for detecting a particular life-history stage (e.g. larvae) or delaying early detection by months if the incursion occurred during winter.

Other, finer-scale (within season) considerations for timing of surveillance include avoiding sampling during or immediately following large flow events. eDNA sampling, if not upstream of Tantangara Reservoir or connected waterways, may be targeted towards periods when no pumping or water transfers have occurred to minimise the possibility of false positives arising from DNA transferred from Talbingo Reservoir.

Based on the above considerations, indicative surveillance periods are provided below (Table 12).

3.2 Response to positive detection

The success of a response to a pest fish incursion often relates to how swiftly the response is initiated, followed by prolonged intensive and rigorous effort; quick, concerted and sustained (Ayres and Clunie 2010). An integral, early step to achieve a rapid response to an incursion is the development of a response plan prior to incursions occurring. Such a plan should clearly detail roles and responsibilities, relevant legislation, and address common challenges such as policy, capacity, resources and agency coordination (Ayres and Clunie 2010). A more specific response plan to a pest fish incursion can then developed.

Table 12. Indicative surveillance periods at surveillance catchments for pest fish species, pre- and post-connection phase

Surveillance catchment	Scheme connection phase	Target Pest fish	Timing of surveillance (and life history stage if applicable)
Talbingo	Pre and Post (positive control)	Redfin Perch Climbing Galaxias Eastern Gambusia	{– summer-autumn (adults)
Tantangara Reservoir	Pre (one-off benchmark survey)	Redfin Perch Climbing Galaxias Eastern Gambusia	{– summer-autumn (adults)
	Post	Redfin Perch Climbing Galaxias	{– spring (larvae) – late spring/early summer (juveniles) [2] – summer-autumn (adults)
		Eastern Gambusia	– summer-autumn (adults)
Upper Murrumbidgee	Pre (one-off benchmark survey)	Climbing Galaxias Redfin Perch Eastern Gambusia	{– summer-autumn (adults)
	Post	Climbing Galaxias Redfin Perch [1]	{– spring (larvae) [2] – late spring/early summer (juveniles) [2] – summer-autumn (adults)
		Eastern Gambusia [1]	– summer-autumn (adults)
Mid Murrumbidgee	Pre (one-off benchmark survey)	Redfin Perch Eastern Gambusia	{– summer-autumn (adults)
	Post	Redfin Perch	{– spring (larvae) [2] – late spring/early summer (juveniles) [2] – summer-autumn (adults)
		Eastern Gambusia	– summer-autumn (adults)
Lake Eucumbene	Pre (one-off benchmark survey)	Redfin Perch	– summer-autumn (adults)
	Post	Redfin Perch	{– spring (larvae) [2] – late spring/early summer (juveniles) [2] – summer-autumn (adults)

[1] – only in lower reaches of Murrumbidgee River downstream of Tantangara Creek to monitor upstream colonisation.
[2] – only required in locations other than Tantangara Reservoir if species presence is confirmed in Tantangara Reservoir.

Table 13. Criteria, trigger levels, and suggested response activities, for each alert level for pest fish surveillance and a positive detection in target catchments

Alert level	Action
1 (Normal)	Continue surveillance of target pest fish at following locations. – Tantangara Reservoir – Climbing Galaxias, Redfin Perch, Eastern Gambusia. – Eucumbene Reservoir – Redfin Perch. – Murrumbidgee River downstream of Tantangara – Redfin Perch – Murrumbidgee River upstream of Adaminaby – Eastern Gambusia
2 (Alert / investigate)	*Trigger*: target pest fish detected by eDNA, angler/public report, unverifiable but suspect BRUV image. *Response:* – undertake rapid pest fish confirmation sampling in relevant catchment(s).
3 (High alert / rectify)	*Trigger:* Pest fish detection confirmed by physical sampling or verifiable BRUV image(s). *Response:* Immediately notify NSW DPI and enact management/control procedure (see 3.3).

The response to a positive detection of a pest species in the surveillance catchments can be framed in the context of a Trigger Action Response Plan (TARP) (Lintermans et al 2022b; Raadik and Lintermans 2022a). Criteria, trigger levels and suggested response activities for a positive pest fish detection in a surveillance catchment where they are currently considered absent are provided in Table 13. The first step involves verification of the incursion (validating the detection) followed by a range of potential control/management measures (see Section 3.3). The target pest fish for each surveillance catchment are those defined above in Table 5 (Section 3.1) and Section 3.1.1.

Given the constraints of eDNA detection (see 2.2.3, 3.1), it is assumed that positive detections have been verified in the laboratory by sequencing validation of 20% of positive samples, and that the location of the detection may not be the exact location of the pest fish, as DNA is transported by, and spreads throughout, the water column. A specimen is required to confirm an eDNA detection, an unclear but suspect image from a BRUV and general reports of fish sightings or captures, which elevates the response to level 2. A clear image from a BRUV, which allows positive identification by an expert familiar with the species, immediately raises the response to level 3 (Table 13).

Physical sampling to verify a pest fish detection is best undertaken by active methods such as electrofishing (backpack or boat) or larval trawls, as they are faster, and can be more efficient than passive techniques such as netting (fyke nets, gill nets, bait traps or larval light traps); rapid verification is important to enable control/management measures to commence, if needed. Consequently, consideration should be given to how verification sampling can be rapidly achieved (e.g. within 1–2 days of possible detection), as the control/management phase should commence as soon as possible to improve the probability of success. This could be achieved by a rapid-response team which could be deployed within this timeframe, with appropriate training for sampling with the selected gear types and with appropriate equipment.

The capture of at least one specimen of target pest fish is enough for species verification, and to trigger the next phase of notification and enactment of control/management activities.

3.3 Pest fish management/control

Following verification of a report, or positive eDNA detection, of a target pest fish (see Section 3.2, above) in the surveillance catchments a range of actions should be rapidly implemented to enable management of the incursion. This response will vary based on the area of the incursion and the aquatic asset being protected from the pest fish (Macquarie Perch in the Mid Murrumbidgee catchment, Stocky Galaxias in the upper Murrumbidgee River, and trout in Lake Eucumbene), and the pest fish species detected. However, it should be acknowledged that a key obstacle to managing risk in pest species control is uncertainty and whilst this requires flexibility in the management strategy (Kopf *et al.* 2017), outcomes may still be unexpected (e.g. unsuccessful, minimal, etc.).

To reduce risk, a key consideration in pest incursion control is the time since invasion and the spatial extent of the pest fish distribution (Kopf *et al.* 2017), as this can influence control success. Therefore, before specific management actions are identified, the following activities are required to provide additional data to support management decisions and to minimise the incursion spread, where possible, to improve the effectiveness of any following actions:

- Assess incursion status – abundance, life-stage and distribution of individuals.
- Undertake containment of incursion, if possible, to prevent or reduce spread.

Once this preliminary data has been obtained, and the incursion possibly contained, the following broad options for management of the incursion are available and a flexible management plan can be prepared:

1. Eradication – complete removal of pest fish.
2. Control – i.e. population size reduction – partial removal of pest fish to reduce density or particular size class strength.
3. Beneficial complimentary actions – other measures to reduce impacts from pest fish on the aquatic assets (Stocky Galaxias, Macquarie Perch, trout in Lake Eucumbene), for example, installation of instream barriers, habitat augmentation to reduce aggressive/predatory interactions, reduction in preferred pest fish habitats (i.e. spawning, juvenile or adult habitat, etc.), bolstering the populations of aquatic assets by translocation or stocking, etc..

One option, or a combination of options, may be appropriate for an incursion, and these may change depending on the length of time the incursion occurs, the outcome of earlier options, and as data on pest fish demographics, behaviour, plasticity of life-history traits, or seasonal habitat choice are gathered to refine control or eradication methods (e.g. Sabetian *et al.* 2014, Yick *et al.* 2021). Importantly, the level of response and response options, which are related to the level of concern about the impact to the aquatic asset from a pest fish incursion, will potentially differ based on the location of the incursion and the aquatic asset and pest fish species involved.

Considerations important in determining the level of risk from an incursion to an aquatic asset are:

- The fish species being protected.
- The pest fish species with respect to its potential impact on the aquatic asset (e.g. rapid or slow major to minor impact, species or population level impact).

Once the level of risk is assessed, available management options (1–3 above) can be prioritised, and assessed for degree of difficulty in implementation/success, based on the following considerations:

- The location of incursion (e.g. waterbody size, remoteness, complexity of habitat, proximity to the fish asset, etc.).
- The incursion status (e.g. early, or late (i.e. established), low of high abundance, etc.).

This will then influence the selection of the initial management response: eradication, population size reduction, or beneficial measure.

Whilst pest fish eradication is often seen as the preferred outcome , i.e. the pest species may be removed thereby long-term (legacy) management is not required, success can often be limited, and restricted to smaller waterbodies than large reservoirs or rivers (Closs *et al.* 2003; Lintermans and Raadik 2003; Beatty and Morgan 2017; Rytwiniski *et al.* 2019). Whether eradication is appropriate/feasible is likely to be situation specific, informed by the following considerations:

- The degree of risk to the aquatic asset (species, distinct genetic lineage, or subpopulations)
- Characteristics of the incursion location:
    - Remoteness (ease of access)
    - waterbody characteristics (width, depth, volume, lotic or lentic, system complexity (single stream reach, wetland or lake, stream system including tributaries, etc.), aquatic habitat complexity, etc.) (constraints in undertaking eradication).
    - Closeness to the target aquatic asset distribution (degree of urgency for action).
- Incursion characteristics (early, late (established), fish abundance and life history stages, degree of spread).
- Ability to contain current incursion.
- Available eradication methods and assessment of feasibility of use (e.g. non-target species impacts, efficiency, etc.).
- Likely effort/cost.
- Incursion pathway and potential for recurrence.

Eradication can still be a viable option, even in complex habitats and larger waterbodies, though it may need a high level of, and sustained, effort over many years (e.g. Yick *et al.* 2021). If the perceived risk of the incursion to the aquatic asset is high enough, it may be the best initial option. However, meeting the objectives for a flexible management plan, as eradication efforts progress and other situations unfold, a shift to pest fish population reduction or beneficial measures to the aquatic asset may be warranted. These two management options are not mutually exclusive, with beneficial measures often being valuable where pest fish population reduction is undertaken, and beneficial measures may also be valuable during eradication to counter pest fish impacts on the aquatic asset as pest fish are removed.

Importantly, relatively little information is available regarding pest fish eradication/control in freshwater, including for Climbing Galaxias, Redfin Perch and Eastern Gambusia (Ayres and Clunie 2010; Rytwiniski *et al.* 2019). Consequently, consideration should be given to identifying and resolving unexpected/novel factors influencing the success of pest fish management, such as plasticity in life history traits or reduction in cannibalism (Closs et al 2003; Ludgate and Closs 2003; Sabetian *et al.* 2014). The incorporation of adaptive management into pest fish management further exemplifies that pest fish management options, particularly the methods employed, will be location specific and will potentially require refinement for local conditions.

Therefore, documenting the management options undertaken, including monitoring the level of success, is critical to inform the adaptive management of the program, but also to contributing to the relatively poor literature on pest fish management in freshwater (Rytwiniski *et al.* 2019).

The range of methods used in previous eradication/control events in freshwater, or as potential for use, have been reviewed by Ayres and Clunie (2010), Knight (2010), Faulks *et al.* (2011), Ingram (2016), Gwin and Ingram (2018), and Rytwiniski *et al.* (2019), including the use of physical barriers for containment or preventing access (Ayres and Clunie 2010; Knight 2010; Raadik *et al.* 2015; Jones *et al.* 2021). These include, physical removal, chemical removal, harvest regimes, biological control, and barriers in aquatic environments.

In the context of Snowy 2.0, the following methods have potential for pest fish management:

- Physical removal – active (electrofishing) or passive (nets, traps, etc.). Can be effective for Redfin Perch and Climbing Galaxias, though limited for Eastern Gambusia.
  - Electrofishing can be selective for target species and can be rapid and effective, though this is reduced in turbid water and increasing water depth. Netting techniques, however, which can be effective in single-species systems (Pacas and Taylor 2015) may also capture non-target species and are therefore of limited value in multi-species systems. .
- Chemical application – the release of an ichthyocide to the target body of water. Can be effective for Redfin Perch, Climbing Galaxias and Eastern Gambusia.
  - Whilst relatively successful where applied, though more so in smaller waterbodies, it is non-selective and therefore impacts non-target species in multi-species systems. It is therefore of very limited use, possibly in the small upper tributaries of the Murrumbidgee River if invaded by Climbing Galaxias.
- Harvest regimes – the increase of harvest pressure on a species or selected size range, often by angling or selective netting. Possibly effective for Redfin Perch.
  - Relies on dedicated harvest pressure, netting is non-selective, and reduction rate in large waterbodies can be very slow. Due to the potential long-timeframe required for population reduction, this is considered of very limited use.
- Habitat manipulations (e.g. manipulating (decreasing) water levels and flows).
- Biological control (e.g. introducing predators or genetically modified organisms), traps and cages are not considered viable eradication or control options.

The selection of beneficial measure to improve the protection or resilience of the target aquatic assets(s), can also be location and species specific, and may also depend on the effectiveness of management outcomes. These measures may include, but are not restricted to:

- Barriers to prevent the incursion of pest fish into the distribution of the aquatic asset (e.g. instream barrier (physical or behavioural) to prevent the upstream incursion of Redfin Perch or Climbing Galaxias from Stocky Galaxias habitat).
- Structural habitat augmentation – to increase instream protection of aquatic assets by reducing negative interactions (e.g. increasing the amount of habitat complexity).
- Reducing or removing instream spawning habitat for specific pest fish species (e.g. manipulating flow to reduce quiet water habitat, removal of shallow water aquatic vegetation, preventing access to spawning habitat).
- Bolstering the population of an affected aquatic asset by translocation of individuals or stocking individuals from a hatchery.
- Reducing competition/predation on an affected aquatic asset by ceasing the stocking of another fish species already present within the catchment/location (e.g. ceasing stocking of salmonids into Macquarie Perch habitat following incursion/establishment of Redfin Perch or Eastern Gambusia).

Based on the above, indicative pest fish management options for the surveillance catchments in this program are proposed in Table 14.

Table 14. Indicative pest fish management options and beneficial measures for aquatic assets for the surveillance catchments

Surveillance catchment	Target Pest fish	Pest fish management	Justification	Beneficial measure
Tantangara Reservoir	Redfin Perch Climbing Galaxias Eastern Gambusia	Priority 1 – Eradication acknowledging that this may be very difficult and only likely to be worthwhile if live transfer from Talbingo is considered to be infrequent. Electrofishing, Fyke nets If established: Priority 2 – Population reduction – continue program of beneficial measures	Considered main pathway for pest fish incursion via Snowy 2.0 scheme Population management (and if practicable, eradication) is recommended to minimise risk of secondary incursion to upper and mid-Murrumbidgee R and Lake Eucumbene, increasing risk to aquatic assets and effort/cost for management	– Fish screens on Tantangara Dam and outlet to Eucumbene (planned) – Bolster trout by stocking if population impacted (planned) – Encourage recreational take of Redfin Perch for population reduction
Upper Murrumbidgee	Climbing Galaxias Redfin Perch	Priority 1 – Eradication Electrofishing, Chemical (limited) If established: – retain annual eradication to maintain buffer zones to Stocky Galaxias populations – continue program of beneficial measures	Protection of high value native conservation species Stocky Galaxias Protection of largest known population of Stocky Galaxias Protection of one of only two known populations of Stocky Galaxias	– Climbing Galaxias barrier on Tantangara Creek (planned) – Velocity barriers on upper Murrumbidgee R to contain Redfin downstream – Establish additional populations of Stocky Galaxias outside of upper Murrumbidgee catchment – Prevent Climbing Galaxias incursion outside of upper Murrumbidgee /Tantangara Reservoir catchments (i.e. to Goodradigbee catchment)
Mid Murrumbidgee	Redfin Perch Eastern Gambusia	Priority 1 – Eradication acknowledging that this may be very difficult. Electrofishing, Fyke nets Priority 2 (if established) – Population reduction – continue program of beneficial measures	Protection of high value native conservation Macquarie Perch population	– Regular bolstering of Macquarie Perch by stocking if population impacted – Habitat augmentation
Lake Eucumbene	Redfin Perch	Priority 1 – Eradication acknowledging that this may be very difficult. Electrofishing If established: – continue program of beneficial measures	Valuable recreational salmonid fishery	– Regular bolstering of trout by stocking if population impacted – Encourage recreational take of Redfin Perch for population reduction

3.4 Determining causality of pest fish incursion

Identifying the introduction pathway and the specific details of the incursion of any pest fish into and throughout the surveillance area is important for the assessment of efficiency of control measures to restrict incursion or spread (e.g. fish screens) and to inform the revision of existing, or development of additional, management strategies aimed at preventing incursion or spread. Likely pathways for incursion are via the new inter-catchment water connection created by the Snowy 2.0 Scheme, human-assisted dispersal, and natural colonisation (see Section 2.2.2, above). Of these, the first pathway has the highest probability but will only exists post scheme connection. The other two pathways are potentially always present, pre- and post-connection, with natural colonisation having the lowest probability overall.

More specifically, the pre-connection likely pathway for incursion of Redfin Perch into Tantangara Reservoir is via human-mediated dispersal as no direct upstream hydraulic pathway currently exists between Tantangara Reservoir and Talbingo Reservoir. The species is present in the Murrumbidgee River, but approx. 200 km downstream, and any upstream access into Tantangara Reservoir is blocked by the dam wall. Human-mediated dispersal is also likely, but less so, for Climbing Galaxias, as this species may possibly be spread as it is used as bait by recreational anglers. Multiple additional, though less likely, potential pathways also exist for natural colonisation of Climbing Galaxias, primarily the Murrumbidgee to Eucumbene (M-E) Tunnel from Tantangara Reservoir to Lake Eucumbene and the very narrow drainage divide between the Upper Murrumbidgee, Eucumbene and Yarrangobilly catchments (Raadik 2019). However no upstream incursion of this species from Lake Eucumbene via the M-E Tunnel has occurred since it was constructed in the 1960s (Raadik 2019), and cross-catchment natural colonisation has not been detected to date. Eastern Gambusia is considered very unlikely to be introduced by human-mediated dispersal as, it is a small, non-angling species. Further, whilst it is present in the Mid Murrumbidgee catchment to approximately 18 km upstream of Cooma (Lintermans 2019), it has not been recorded in the ~50–60 km of Murrumbidgee River between Adaminaby and Tantangara Dam (Lintermans unpublished data), is unlikely to naturally colonise over that distance, and would be blocked from the upper Murrumbidgee waters by the dam wall.

Post-connection, a pathway for the incursion of all three target species will exist from Talbingo Reservoir to Tantangara Reservoir, down the Murrumbidgee River, and across the catchment into the Lake Eucumbene, during operation of the Snowy 2.0 Scheme. Transfer of pest fish from Tantangara Reservoir to the mid-Murrumbidgee River and Lake Eucumbene will potentially be minimised through the installation of fish screens. Similarly, a fish barrier will be installed on the upper reaches of Tantangara Creek to prevent potential upstream transfer of Climbing Galaxias into the habitat of the Stocky Galaxias. As such, pest fish incursion into these locations may potentially only occur because of Snowy 2.0 if these controls fail or, in the case of the mid-Murrumbidgee River, if the reservoir spills.

Consequently, any pre-connection incursions will be highly unlikely to be directly related to the Snowy 2.0 Scheme, however, pest fish incursions post-connection are more likely to be due to the operation of the scheme, but also possibly via human-mediated dispersal: natural colonisation is also possible but very unlikely.

Distinguishing the pathway of post-connection pest fish incursion will be difficult between the different potential pathways, however, the following information will be important:

- Date/s and location/s of incursion (the earliest detected if possible).
- Life history stages present.
- Estimate of abundance.
- The geographic spread of the population.
- Tissue samples of each species for provenance testing via DNA analysis.

The first four may contribute to understanding the approximate date of incursion, initial location site, life-stage introduced, pathway of incursion, time since introduction, or timing of incursion with respect to Snowy 2.0 and dam operations. It is unlikely that pest fish will establish in the mid-Murrumbidgee and Lake Eucumbene, as a result of the operation of the Snowy 2.0 scheme unless they first establish in Tantangara Reservoir.

The provenance of the pest fish may also contribute to understanding the pathway of introduction (e.g. Brown and Stepien 2008; Riva Rossi *et al.* 2012; Hunter and Nico 2015; Bariche et al. 2017; Zhai *et al.* 2022), by matching the fish to known populations within, or nearby, to the surveillance catchments. For example, if Redfin Perch individuals from an incursion are genetically match only to those within Talbingo Reservoir post-connection, it is likely that the Snowy 2.0 Scheme was responsible for the introduction. Combining the five sets of information above may strengthen this conclusion. Genetic tracing in the context of this surveillance plan (e.g. within < 1 year of incursion) may be easier than if undertaken many years after an introduction but does rely on the populations of target pest fish being sufficiently genetically divergent.

Collection of the above five datasets should be part of the pest fish surveillance program and the response to a positive detection. With respect to provenance testing of pest fish, for each target species within the surveillance catchments and nearby catchments, existing genetic variation between populations should be determined by a DNA barcode analysis technique such as single nucleotide polymorphism (SNP), and differing populations, if they exist, should therefore be identified by unique barcodes. Pest fish can then be compared genetically to known populations to seek a genetic match.

Collection of target pest fish genetic population data should be undertaken in the pre-connection phase to build up a reference library of nearby species population diversity. This dataset should be augmented if different, additional populations are discovered during catchment sampling. Target river systems and catchments for tissue collection for the three target pest species are as follows, as these are likely source catchments for incursions:

- Snowy River system – Eucumbene, Snowy catchments.
- Upper Murray system – Geehi, Tooma catchments.
- Murrumbidgee system – Tumut, Goobarragandra, Goodradigbee, Cotter, Mid and Upper Murrumbidgee catchments.

Pest fish from incursions during the post-connection phase should be DNA barcoded and compared with the reference population library to check provenance, and together with the additional incursion data (listed above) used to attempt to define the incursion pathway.

It must be recognised, however, that there may remain a possibility of being unable to definitively determine the pathway of an incursion based on the above information. For example, the provenance of fish in an incursion in Tantangara Reservoir may match the species population in Talbingo Reservoir, however the fish had been actively translocated into that system and not transported by the scheme. Alternatively, pest fish cannot be matched confidently to a source population (e.g. no or little genetic diversity between populations, low genetic diversity in founder population, etc.).

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