Department of Sustainability and Environment

Establishing new populations for fire-affected Barred Galaxias (*Galaxias fuscus*): site selection, trial translocation and population genetics

Black Saturday Victoria 2009 – Natural values fire recovery program

Renae Ayres, Michael Nicol, Tarmo Raadik

Parks Victoria

Victoria
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Establishing new populations for fire-affected Barred Galaxias (*Galaxias fuscus*): site selection, trial translocation and population genetics

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Front cover image: Adult Barred Galaxias, *Galaxias fuscus* (S.J. Nicol).

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Summary

Barred Galaxias (*Galaxias fuscus*) is a small, nationally endangered freshwater fish endemic to the upper headwaters of the Goulburn River catchment in central Victoria, Australia. Its habitat, from Lake Mountain to Mount Disappointment, was burnt during the 2009 Black Saturday bushfires. This represents 45 % of the known range of the species and many populations in this area suffered from direct and indirect post-fire affects.

This study aimed to improve the recovery of post-fire affected Barred Galaxias populations and reduce their overall extinction risk by establishing new populations in suitable streams within their former range. We identified and assessed potential Barred Galaxias translocation sites and chose two suitable catchments as recipients for trial translocations. Fish from two source populations were reintroduced into these catchments and post-translocation monitoring occurred twice in the following six month period to determine the short-term success of the translocation.

Two hundred and sixteen potential sites were identified and mapped, mostly within upper reaches of the Yea, Acheron-Taggerty, Rubicon and Big River systems. Of these sites, 61 were surveyed and assessed for suitability based upon obvious habitat threats (e.g. fire and logging impacts, hydrology), the presence of fish, the existence of a physical barrier to fish movement, and catchment size. Only six sites in four upland catchments were immediately suitable for the translocation of Barred Galaxias. Two of these, namely Shaw Creek and the upper Taponga River, were selected for trial translocations. Of the remaining surveyed sites, 49 require further work before being suitable for Barred Galaxias translocation (e.g. further surveys to identify the location of physical barriers or installation of a physical barrier and upstream removal of trout). Six other surveyed sites were deemed unsuitable for translocation because they had no water or contained native fish species. Information collected during these surveys will be useful to inform future translocations of Barred Galaxias.

In late November/early December 2010, Barred Galaxias sourced from Luke Creek were translocated to Taponga River and Barred Galaxias sourced from Kalatha Creek were translocated to Shaw Creek. The source and translocated Barred Galaxias populations were monitored in March and June 2011 to determine their survival and, for translocated populations only, range expansion. The source populations appeared to be unaffected by the removal of individuals because fish were present within all reaches in similar abundances and size classes to those recorded during the initial collection period, and individuals displayed signs of reproductive development. In the short-term, we considered that the translocations were successful because fish survived and expanded in range at the translocated sites, they were in good physical condition and their maturation stages suggested they are likely to reproduce during the 2011 breeding season.

Post-fire management of Barred Galaxias highlighted the need to improve our understanding of the population genetics of the species across its range to effectively manage, conserve and recover populations. As a sub-project to establishing new populations for fire-affected Barred Galaxias, we conducted a comprehensive population genetic analysis throughout its current distribution to determine population genetic diversity and differentiation, and gene flow.

Tissue samples were collected from Barred Galaxias for genetic analysis and forty microsatellite markers were identified and trialled, of which, 12 were selected for use in the population genetic assessments. In total, 568 Barred Galaxias individuals were genotyped at 12 polymorphic microsatellite loci. Genetic variation within populations was found to be extremely low, with some displaying no genetic variation

between individuals. Genetic variation between populations was high, indicating limited gene flow over broad distances within and between river systems. Also, 94 Barred Galaxias individuals, collected from 26 locales, were studied in a phylogeographic assessment. Twelve haplotypes were revealed with most individuals belonging to two common haplotypes. Two major evolutionary clades were found. However there were weak phylogeographic patterns.

This population genetic study provides a valuable spatial framework for effective conservation management of Barred Galaxias and a foundation for future population genetic monitoring. The genetic factors identified, including low levels of genetic variation, high population structuring and limited gene flow, coupled with small population sizes and ongoing threats, such as trout predation and competition and stochastic environmental impacts, suggest Barred Galaxias populations are at high risk of extinction without effective management intervention. Translocations, genetic augmentation and supplementing populations with captive bred individuals are essential recovery actions that can be undertaken using this genetic data to strategically guide these management actions with the overall aim of enhancing the genetic diversity of populations, whilst maintaining the species' genetic integrity.

Future management actions and research are recommended to facilitate additional Barred Galaxias translocations and ensure the long-term persistence of the translocated populations, including:

- Install a second physical barrier at Taponga River upstream of the 15 Mile Road crossing to secure the translocated Barred Galaxias population;
- Conduct a cost/benefit analysis on the expense of conducting management works (i.e. physical barrier installation, trout removal) compared to further surveys for physical barriers at identified fishless sites and/or new translocation sites;
- Perform physical barrier surveys on the 24 identified fishless sites to potentially add to the list of sites suitable for immediate translocation;
- Install physical barriers and remove trout from upstream reaches at 19 sites to render them suitable for immediate Barred Galaxias translocation;
- Any future assessment of translocation site suitability should include measurements of water permanency, assessment of the amount of appropriate spawning habitat, and site accessibility;
- Continue monitoring the translocated Barred Galaxias populations to assess their long-term survival, establishment (breeding and recruitment), range expansion and genetic viability. This data will inform whether future population supplementation or habitat modification is necessary to sustain the population or aid successful natural recruitment;
- Undertake genetic analysis of translocated populations one, five and ten years post-establishment, with comparison with the genetic diversity of the founder individuals;
- Undertake captive breeding of Barred Galaxias to supplement natural populations when required, e.g. to boost population sizes and enhance population diversity;
- Undertake ongoing monitoring of all populations, particularly those with small populations sizes, those where translocations have occurred, those containing captive bred individuals and those under direct threat e.g. from drought and fire impacts, and trout predation;

- Mitigate threats to all populations, including trout prodations, sedimentation, dewatering, etc.
- Undertake additional translocations, guided by the results of the population genetics analysis, by mixing populations and creating new populations; and,
- Continue engaging and educating stakeholders regarding the significance of Barred Galaxias conservation and their key threats, including potential impacts of human-assisted trout dispersal.

1 General introduction

1.1 Barred Galaxias

Barred Galaxias (*Galaxias fuscus*) (Figure 1) is a small freshwater fish belonging to the family Galaxiidae. It is endemic to upper headwaters (above 400 m altitude) of the Goulburn River catchment in central Victoria. It is non-migratory and inhabits cool, clear, flowing streams with cobble or sandy substrate and their diet consists mainly of aquatic and terrestrial invertebrates (Raadik *et al.* 1996; 2010). Barred Galaxias are scaleless and have a yellow-orange body colour with up to 10 black, vertical stripes along the sides. They are relatively long-lived, have low fecundity and are slow growers (Raadik *et al.* 2010).

Barred Galaxias is listed nationally as Endangered under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* and is also listed under the Victorian *Flora and Fauna Guarantee Act 1988.* The species has suffered extensive decline in range and abundance. Several Barred Galaxias populations are extinct and only twelve small, isolated populations are known to remain.

Raadik *et al.* (2010) identified several key threats to Barred Galaxias:

- Predation by and competition from the introduced predatory salmonid species Rainbow Trout (*Oncorhynchus mykiss*) and Brown Trout (*Salmo trutta*), hereon referred to as 'trout'
- Surface water loss during drought
- Siltation/sedimentation
- Bushfire impacts
- Water regime changes
- Genetic isolation

The National Recovery Plan for Barred Galaxias (Raadik *et al.* 2010) details the species' distribution, ecology, threats, recovery objectives and the actions necessary to ensure their long-term survival.

1.1.1 Fire impacts on Barred Galaxias

Barred Galaxias habitat from Lake Mountain to Mt Disappointment was burnt during the 2009 Black Saturday fires. This area represents 45% of the known range of the species. Barred Galaxias from several populations occurring within the 2009 fire boundary were rescued soon after the fires and maintained temporarily in captive facilities at Department of Sustainability and Environment (DSE) Arthur Rylah Institute (ARI) until habitat conditions improved, following which they were returned to their natal streams. This included Barred Galaxias from S Creek, Luke Creek, Robertson Gully, Keppel Hut Creek, Rubicon River, Little Rubicon River, Upper Taggerty River and Torbreck River South Branch (Raadik *et al.* 2009). Some Barred Galaxias populations were affected by fires in 2006/07 and all populations have experienced a decade of low flows due to drought conditions.

Figure 1. Barred Galaxias, *Galaxias fuscus* (Image: Tarmo Raadik).

Fire poses direct and immediate or indirect and sustained impacts on aquatic ecosystems (Gresswell 1999). Its complex and diverse effects, however, depend on the extent and severity of the fire, post-fire weather conditions (e.g. rainfall intensity and duration), and the physical, chemical and biological characteristics of the catchment (e.g. geomorphology, geology) (Gresswell 1999).

During a fire event there is immediate loss of vegetation including that covering and shading streams. Fire burning adjacent to or crossing streams can increase water temperature. During the 2009 Black Saturday fires water temperatures in streams and ponds reached 55 °C (DSE 2009). Also the water chemistry and quality of streams can be altered by inputs of smoke, ash and fire retardants (Gresswell 1999; Dunham *et al.* 2003; Gimenez *et al.* 2004). The direct impacts of fire can result in immediate death or displacement of small numbers of fish to entire fish populations (Rieman and Clayton 1997; Lyon and O'Connor 2008).

Following fire events, changes in the surrounding landscape indirectly influence aquatic systems. Increases in water temperature occur primarily because of lack of riparian shading, but also due to channel simplification, topographic shading, and hydrologic changes (Gresswell 1999; Dunham *et al.* 2007). Loss of vegetation and changes in soil structure increase the likelihood of soil erosion, as well as greater and more variable stream flows (Gresswell 1999; Rieman and Clayton 1997). Rainfall events flush sediment and ash into streams, smothering instream habitat and causing fluxes in nutrient levels and reductions in water quality (Gresswell 1999; Lyon and O'Connor 2008; Raadik *et al.* 2009). Additionally, anthropogenic activities, such as post-fire salvage logging and road construction, can exacerbate post-fire impacts on aquatic systems (Rieman and Clayton 1997). Indirect effects of fire can cause local extirpation, displacement or reductions in the abundance and distribution of fish (Rinne and Neary 1996; Gresswell 1999; Lyon and O'Connor 2008). Adverse indirect impacts on fish include reduced water quality, loss of available habitat and food resources, and dramatic changes in flow conditions (Gresswell 1999). The time taken for fish populations to recover is variable and largely influenced by their life history and dispersal ability as well as habitat connectivity (Gresswell 1999; Lyon and O'Connor 2008). Fish can recolonise impacted reaches by migrating from local refugia or upstream or downstream sources (Rieman and Clayton 1997; Dunham *et al.* 2003). Small, isolated fish populations, particularly sedentary species, are more vulnerable to disturbance and have limited recolonisation potential because of reduced habitat connectivity (Gresswell 1999).

Barred Galaxias populations are small, fragmented and isolated; therefore they are highly susceptible to the effects of fire. Management actions are necessary to ensure the survival and recovery of threatened Barred Galaxias populations within fire-impacted areas.

1.2 Translocations

Translocation is the movement of living organisms from one area with free release in another (IUCN 1987) and can be undertaken in three different ways:

- *Introduction* is the deliberate or accidental translocation of a species into the wild in areas where it does not occur naturally.
- *Re-introduction* is the deliberate or accidental translocation of a species into the wild in areas where it was indigenous in historic times but is no longer present.
- *Re-stocking* is the translocation of an organism into the wild into an area where it is already present.

Translocations are becoming a popular management tool for conserving and restoring biodiversity and maintaining ecosystem function. Translocation programs aim to enhance the persistence and resilience of species by establishing or maintaining viable populations and mainly target threatened or keystone species. There are numerous examples of translocations of various taxa throughout the world (reviewed in: Griffith *et al.* 1989; Fischer and Lindenmayer 2000; Seddon *et al.* 2007), including plants (Stewart 2003), mammals (Pinter-Wollman *et al.* 2009; Poole and Lawton 2009), birds (Reese and Connelly 1997; Parker and Laurence 2008), amphibians (Hambler 1994; Griffiths and Pavajeau 2008), reptiles (Nelson *et al.* 2002; Germano and Bishop 2008), fish (Minckley 1995; Shute *et al.* 2005; Rakes and Shute 2006) and invertebrates (Wynhoff 1998). Many species translocations have occurred within Australia, for example, Short (2009) compiled 380 translocations of 102 threatened native vertebrate species focusing on mammals, birds, reptiles and amphibians.

Managers designing translocation events must make several critical and occasionally difficult decisions, particularly when population growth and future funding is uncertain (Haight *et al.* 2000). These include: where to source individuals for translocation; the number, age and sex ratio of individuals to translocate; the location, number and timing of releases; methods of collection, transportation and release; post-monitoring procedure and frequency; and the allocation of limited budget, staffing and resources among these activities. Translocations are an inherent part of captive breeding programs for species recovery (e.g. Tweed *et al.* 2003; Shute *et al.* 2005). Captive breeding techniques allow the production of large numbers of individuals to begin new populations with minimal detrimental effects on small source populations. However, there are often difficulties with establishing wild populations from captive-bred stock (Griffith *et al.* 1989; Snyder *et al.* 1996).

Increasing the number of Barred Galaxias populations and individuals is a recovery objective of the National Recovery Plan for Barred Galaxias (Raadik *et al.* 2010). Its associated recovery actions include investigating captive breeding techniques for Barred Galaxias, as well as planning and conducting translocations and maintaining these new populations. Translocations of Barred Galaxias are anticipated to reduce reliance on expensive captive management and improve the resilience and conservation of Barred Galaxias under changing environmental conditions.

1.3 Project objectives

This study aimed to improve the recovery of post-fire affected Barred Galaxias populations and reduce their overall extinction risk by establishing new populations in suitable streams within their former range.

Specifically, we:

- Developed guidelines for the translocation of Barred Galaxias for conservation purposes (separate document, see Ayres *et al.* 2012).
- Identified potential translocation sites for Barred Galaxias and assessed their suitability.
- Conducted two trial translocations of Barred Galaxias and assessed their short-term success.
- Analysed the population genetics of Barred Galaxias across their existing range.

This project delivers several high priority actions consistent with the species' National Recovery Plan (Raadik *et al.* 2010), the Victorian Flora and Fauna Guarantee Act Action Statement (Koehn and Raadik 1996; Raadik in review) and the Actions for Biodiversity Conservation (ABC) management system.

1.4 Report structure

This report is organised into a summary, a general introduction chapter (this chapter), followed by three research chapters.

The summary provides a brief overview of the project, research methods, key outcomes, and management recommendations. Chapter 2 presents research on identifying potential Barred Galaxias translocation sites and assessing their suitability. Chapter 3 describes the trial translocations of two Barred Galaxias populations and Chapter 4 investigates the genetic diversity of Barred Galaxias populations across the species' range. These research chapters are intended to be published as articles in leading scientific journals because they have broader scientific value and management applicability.

Guidelines for the translocation of Barred Galaxias for conservation purposes are provided in a separate publication in this series (Ayres *et al.* 2012). Briefly, they describe the methodology for translocating Barred Galaxias from a wild source population to a release site in public waters, with translocations subject to compliance with Victorian and Commonwealth policies and protocols, and are intended to guide development of translocation plans for specific Barred Galaxias translocation events. General principles regarding preparation and pre-planning for translocation, implementing the translocation and post-release monitoring are discussed.

2 Identifying suitable translocation sites for the reintroduction of Barred Galaxias

2.1 Introduction

An important aspect to facilitate a successful translocation is releasing individuals into a suitable location. It is important that release sites have appropriate habitat available to support the survival and establishment of translocated individuals into the foreseeable future and threats to the species are eliminated or mitigated.

Identifying suitable translocation sites for Barred Galaxias is urgently required to enable reintroduction and establishment of new populations within the species native range to aid recovery and reduce the overall extinction risk of impacted populations. Currently, the majority of sampled reaches in upland streams within the natural range of Barred Galaxias contain predatory trout (Figure 2) and are therefore unsuitable as translocation sites, though the suitability of unsampled reaches or streams is unknown. Having a readily available list of suitable Barred Galaxias translocation sites will benefit planning of future translocation events, particularly if urgent translocations are required.

This chapter presents research behind identifying and assessing potential translocation sites for two trial reintroductions of Barred Galaxias.

The specific objectives were to:

1. Identify and assess numerous upland and remote catchments to develop a list of potentially suitable translocation sites
2. Prioritise identified catchments with respect to their suitability against defined site criteria
3. Choose two suitable catchments to conduct immediate trial translocations, and
4. Identify potential sites that require further management actions before acceptance as suitable for Barred Galaxias translocations.

2.2 Methods

In Victoria, the translocation of live aquatic organisms into and within inland waters requires approval under the *Fisheries Act 1995*. As part of this process, the 'Protocols for the translocation of fish in Victorian inland public waters' (Department of Primary Industries 2005) provides guidance regarding the identification and assessment of potential translocation sites.

The following general criteria were considered when identifying and assessing the suitability of potential Barred Galaxias translocation sites:

- Confined to public waters;
- The waters are within the known former range of the species (> 400 m altitude, in the upper Goulburn River catchment, Victoria, Australia);
- Fish will not be translocated into waters for conservation purposes where they will be exposed to previous causes of decline;
- No fish, including resident populations of Barred Galaxias, will be present. This will eliminate predation, competition, disease spread, hybridisation and possible reductions in genetic integrity and diversity;
- Sites will have an effective instream physical barrier, hereon referred to as a barrier, present downstream to prevent upstream movement of other fish species into the site. Effective barriers:
  - Consist of a solid, long-lasting material such as rock;
  - Are vertical or near vertical with a height of 2.0 m or greater;
  - Are within a V-shaped valley so that higher flows remain directed to the centre of the channel (to minimise likelihood of fish moving upstream across recently inundated land along the stream bank); and,
  - Do not have a pool immediately below of significant depth or size which could aid fish in jumping over the barrier.
- Availability of suitable habitat to sustain Barred Galaxias into the foreseeable future. The waters should have sufficient capacity to sustain survival and growth of the translocated population (e.g. > km^2 in catchment area) and support a viable population in the long term. The catchment should have high water security and minimal or no disturbance from, for example, bushfire, drought, or timber harvesting activities;
- The translocation will not pose an unacceptable risk to another threatened species or community (e.g. listed under FFG Act or EPBC Act); and,
- The release site should, in part, be selected based on remoteness or human inaccessibility to reduce the likelihood of adverse anthropogenic effects, e.g. human introduction of predator species.

2.2.1 Study area

The study area was confined to public waters of the Goulburn River catchment above 400 m altitude, within the suspected former range of Barred Galaxias (Raadik *et al.* 2011). In particular, the upper catchments of the following systems were targeted for sampling: Sunday Creek; Yea River; Acheron/Taggerty River system; Rubicon River, and Big River. Greater priority was given to the Big River catchment as the aquatic fauna of its headwater reaches was considered less surveyed than the other catchments, was less impacted by the 2009 bushfires and consequently was considered to have a higher probability of harbouring suitable translocation sites. It was also in an area with higher streamflow (i.e. from snowmelt) and therefore may provide greater water security.

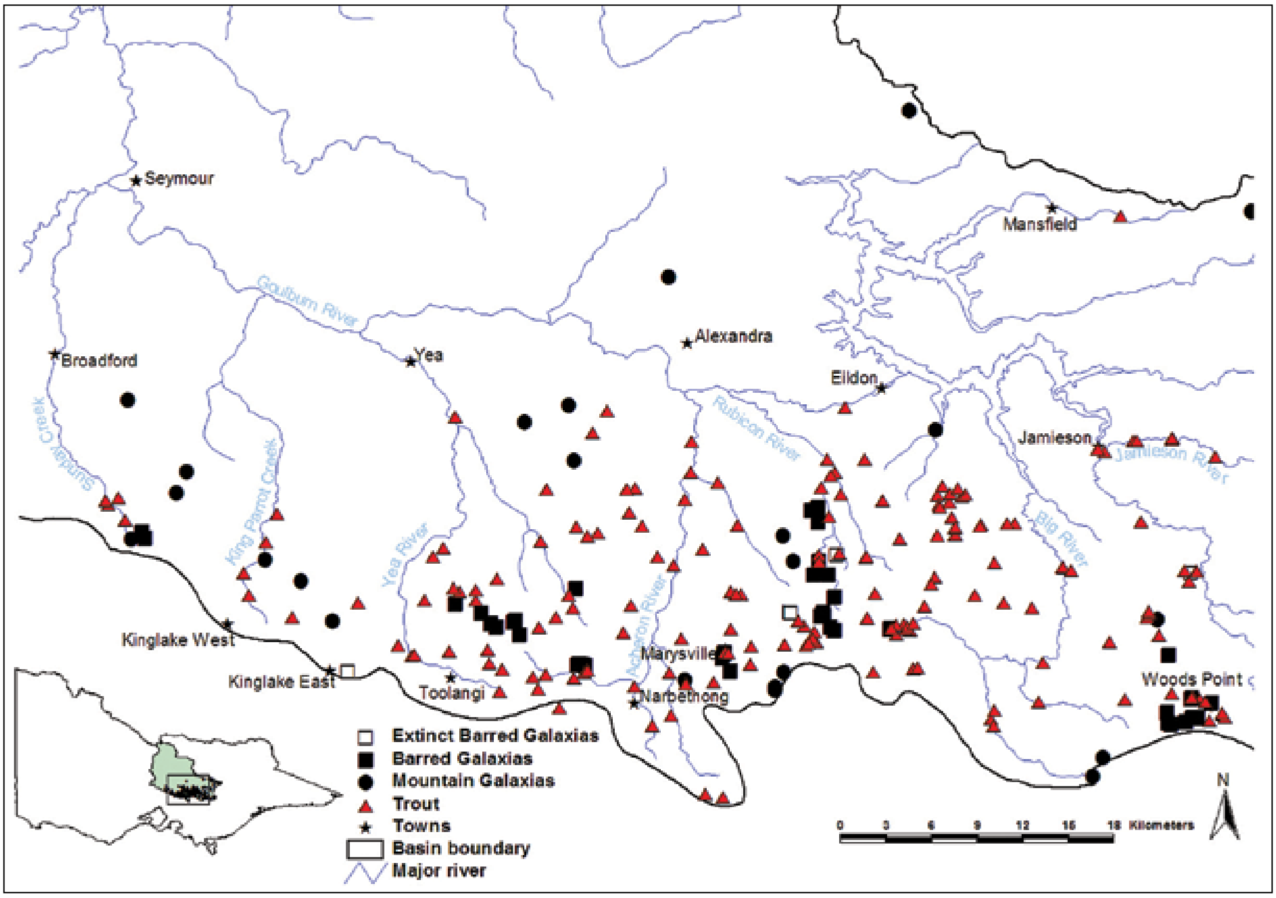
The Goulburn River catchment is part of the Murray-Darling Basin, covering an area of approximately 16 192 km^2 in north-central Victoria, Australia. The Goulburn River begins in the mountainous Great Dividing Range near Woods Point (1325 m altitude) flowing north-west to its confluence with the Murray River near Echuca (100 m altitude). Land use in the headwaters of the catchment varies from national park, state forest, forestry, agriculture, recreation and rural living. The main forms of disturbance are bushfire, drought and forestry practices.

Other than Barred Galaxias, Mountain Galaxias (*Galaxias olidus*) is the only native fish species likely to be found in the very upper reaches of the catchment. Brown Trout (*Salmo trutta*) and Rainbow Trout (*Oncorhynchus mykiss*), which are a key threat to Barred Galaxias (Raadik *et al.* 2011), may also inhabit these small headwater streams.

2.2.2 Selection of survey sites

Preferably, Barred Galaxias translocation sites would be located in streams above 400 m altitude, relatively remote from human access, have no fish species present, be within larger catchments (>1 km^2), and be upstream of a natural barrier, such as a waterfall (see 2.2 above), to provide a contained area free from the incursion of predators (i.e. trout). Sites identified as meeting these criteria required field assessment to verify their suitability as translocation sites.

Figure 2. Distribution of Barred Galaxias, Mountain Galaxias and trout within the study area. (Data source: Victorian Biodiversity Atlas).



Initially, high resolution aerial images and topographic maps covering the study area were examined to locate known waterfalls or potential instream barriers. Also regional DSE staff were queried about the location of known barriers. Additional potential sites, which met the majority of the above criteria, were selected from topographic maps, with emphasis given to sites located upstream of steep gradients which possibly indicated the presence of waterfalls, particularly those on streams which flowed across a high elevation plateau before plummeting down a steep valley. Site selection was further refined by overlaying fish distribution data (Figure 2) and rejecting sites which contained fish.

2.2.3 Assessment of translocation site suitability

Sites provisionally identified above were further assessed for suitability by onground inspection. The rationale for assessment is summarised in Figure 3 and is based on the general criteria noted at the beginning of the methods section, and involved a detailed inspection of catchment condition, streamflow, aquatic fauna and presence and structure of instream barriers. Sites were assessed as either suitable and short-listed as translocation sites, or unsuitable according to the selection criteria, and rejected.

Fish assessment

Fish surveys were conducted at potential translocation sites using a portable backpack electrofishing unit (Figure 4) with the aim to confidently verify the presence or absence of fish species in the small headwater streams. The operator fished all accessible habitats in an upstream direction, followed by an assistant to collect stunned fish. The stream distance surveyed varied from 1–150 m depending on whether fish were found (i.e. fishing ceased once fish were recorded), site conditions, and operator judgement. A 100 m reach was sampled if no fish were collected, though a minimum of 25 m was surveyed at sites with very low streamflow and lack of suitable habitat (e.g. pools, undercut banks, etc.).

Figure 3. Decision support framework for assessing the suitability of potential Barred Galaxias translocation sites.

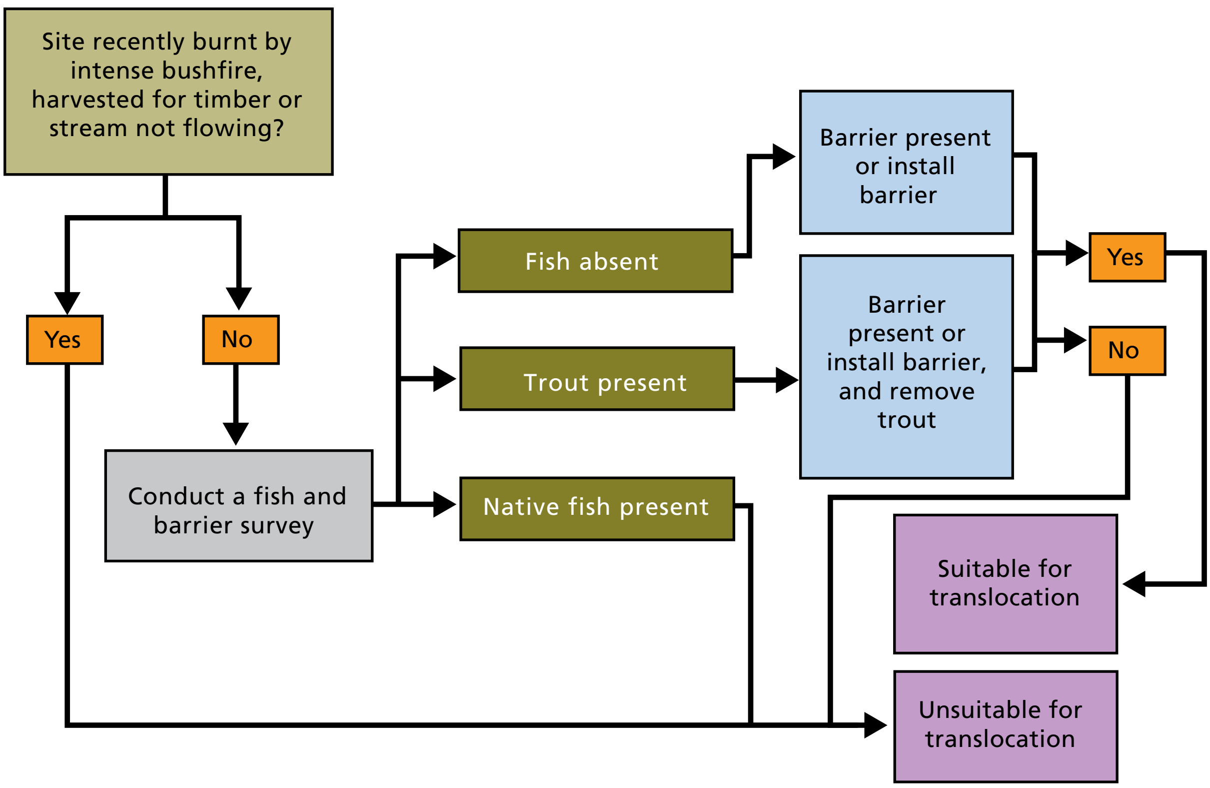


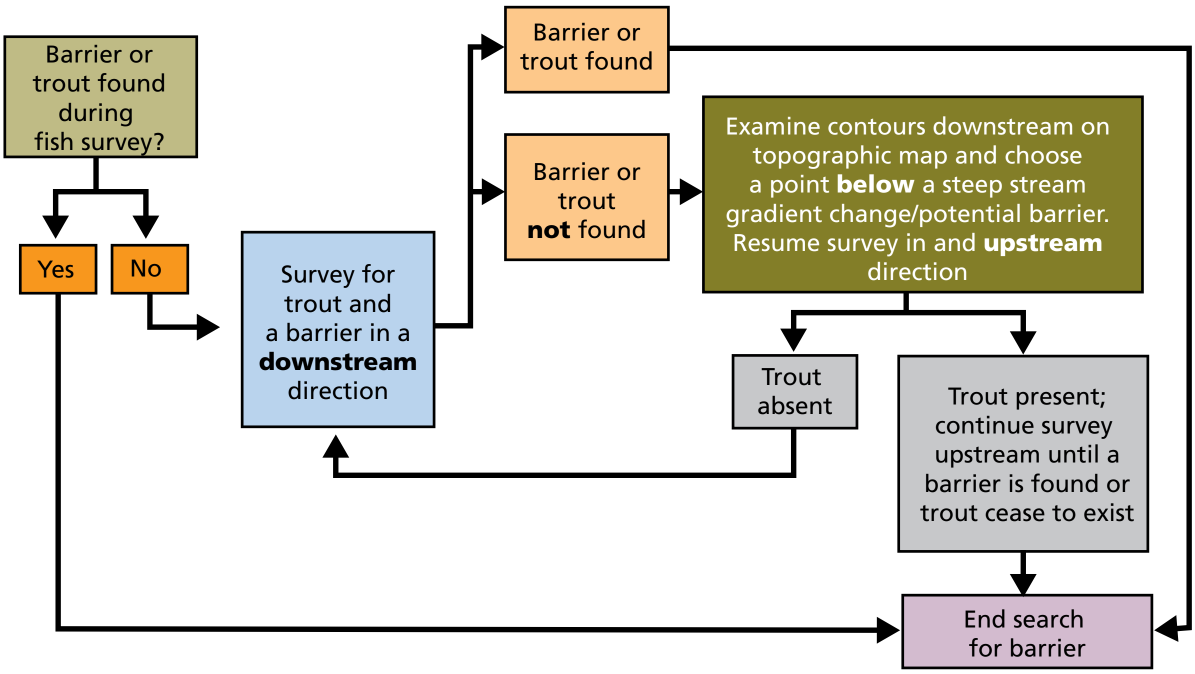
Figure 4. Electrofishing the upper Goulburn River (left) and a Rainbow Trout found in Spring Creek (site FT025) (right) (Images: left – Renae Ayres, right – Michael Nicol)

All fish captured were identified and, at the majority of sites, measured for length (length to caudal fork (LCF) (mm)), weighed (g) and examined for condition (presence of parasites, lesions, body colour, fin damage etc.) prior to release. The following water quality parameters were also recorded at most sites prior to fish surveys using a field-laboratory analyser (TPS 90-FLT: TPS, Brisbane, Australia): dissolved oxygen (mg/L); temperature (° C); pH; turbidity (NTU); and, electrical conductivity (µS/cm) (Appendix 1).

Physical barrier assessment

Known barriers were inspected on the ground to reconfirm their locality and to assess their physical structure for suitability in preventing the upstream movement of trout. At all other potential translocation sites where the presence of a downstream barrier was uncertain, surveys for potential barriers were undertaken during and following fish assessments and the rationale is summarised in Figure 5. If trout were found during fish assessments it was assumed that an effective barrier was absent downstream.

Figure 5. Decision support framework for assessing the presence of instream barriers.



Selection of two trial translocation catchments

Following site assessments and the identification of a number of suitable translocation sites, two sites were provisionally selected for use in the trial translocation component of this study (see Chapter 3). Before final acceptance of suitability, these sites were resurveyed for fish to ensure the absence of trout and the presence of suitable conditions and habitat for Barred Galaxias.

2.3 Results

2.3.1 Site identification

A total of 217 survey sites were selected for field inspection. None of these were identified from post-fire aerial images as stream-flows at the time of photography were low and barriers could therefore not be identified by the presence of white water. Also, unburnt riparian vegetation and the local topography shaded and inhibited the view of streams in many areas. One site was marked on a topographic map as a waterfall (Figure 6), and the location of an additional barrier (on Sylvia Creek) was provided by an officer from the Toolangi DSE Office. The remaining 215 survey sites were selected from topographic maps.

Figure 6. Evelyn Falls on Koala Creek, which prevents upstream movement of trout (Image: David Bryant).

2.3.2 Site assessment

The list of 217 potential sites were prioritised by the presence of known barriers, geographic location and catchment area and 61 of the sites, including those with larger upstream catchment areas, were surveyed between May 2010 and March 2011 (Figures 7 & 8). Of these, none had recently been heavily burnt by bushfire or had sustained timber harvesting within their upstream catchment in the past 20 years. Three (5%) sites were found to be dry and 36 (59 %) sites had no fish (Table 1; Figure 8). Of the 22 where fish were present, trout (Brown Trout, Rainbow Trout, or both species) were recorded from 20 (33%) sites, one (2%) site (FT045) contained a previously unknown population of Barred Galaxias, and one (2%) site (FT023) contained a population of Mountain Galaxias (Table 1; Figure 8).

Of the 36 fish-less sites, 7 (19%) had instream barriers located further downstream, equating to five barriers in total as two streams had two sites each (Table 1). These included: Koala and Silvia creeks (FT001, FT005, FT018) and Torbreck River (FT061), where the presence of barriers was previously known but their locations were reconfirmed and physical structure investigated; Taponga River (FT033), where a barrier was found within the fish survey reach; and, Shaw Creek (FT024, FT043), where a barrier was identified by surveying further downstream after examining contour lines on a topographic map. All barriers were considered effective as trout were not detected upstream. Conversely, barriers were assumed either absent or ineffective at the 20 sites which contained trout in the assessment reach. The presence of barriers at the remaining 29 fish-less sites was undetermined as none were found during fish assessments and time constraints prevented additional downstream investigation at these sites (Table 1).

Water quality parameters measured at all sites where similar to those at known Barred Galaxias sites (Raadik 2000, 2002, Raadik, T.A. unpublished data).

Figure 7. Examples of survey sites: tributary of Big River (left); and Shaw Creek (right) (Images: Michael Nicol).

Figure 8. Distribution of the 61 sites surveyed including their assessment result and fish species recorded. Blue squares indicate suitable sites. Note that multiple symbols overlap in some areas.

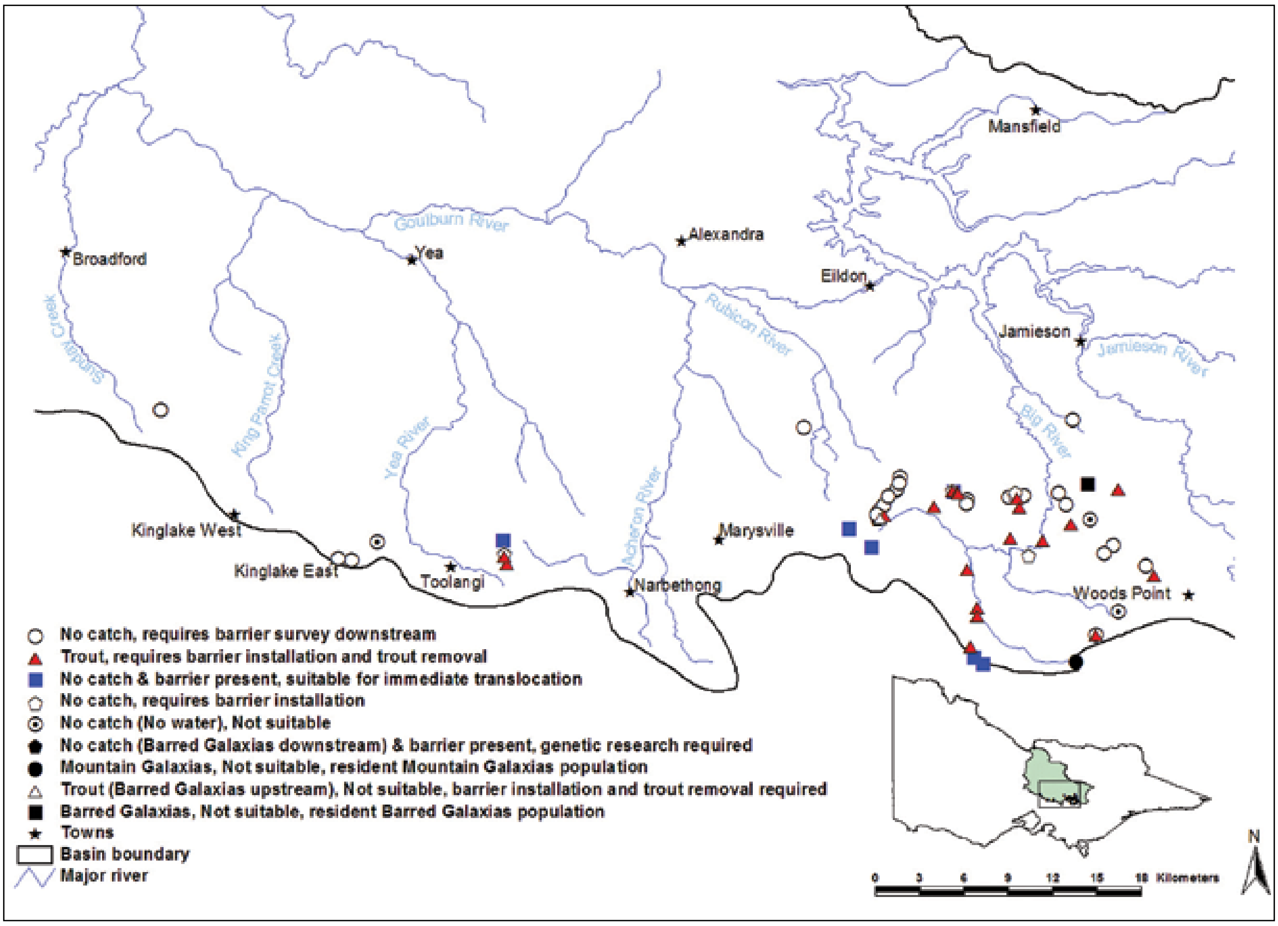


Table 1. Details of the 61 potential Barred Galaxias translocation sites surveyed and their translocation suitability. BG – Barred Galaxias; Brch – branch; BT – Brown Trout; Crk – creek; d/s – downstream; MG – Mountain Galaxias; Nth – north; R – river; RT – Rainbow Trout; Sth – south; Trib – tributary; TSp – unidentified trout species; ? – undetermined.

Site no.	Waterbody (Catchment)	Date sampled	Latitude (°S)	Longitude (°E)	Altitude (m)	Catchment Area (km^2)	Fish species present	Barrier present downstream	Translocation suitability
FT001	Koala Crk (Big R)	17/05/2010	-37.5176	145.9122	910	8.3	No fish	Yes	Yes – immediate
FT005	Koala Crk (Big R)	18/05/2010	-37.4982	145.8878	1200	2.5	No fish	Yes	Yes – immediate
FT024	Shaw Crk (Big R)	28/07/2010	-37.6348	146.0216	830	3.5	No fish	Yes	Yes – immediate
FT043	Shaw Crk (Big R)	7/10/2010	-37.6417	146.0308	960	1.1	No fish	Yes	Yes – immediate
FT033	Taponga R (Big R)	11/08/2010	-37.4578	145.9998	620	1.4	No fish	Yes	Yes – immediate
FT018	Sylvia Crk Nth Brch (Yea R)	16/07/2010	-37.5104	145.5197	810	0.1	No fish	Yes	Yes – immediate
FT061	Torbreck R Sth Brch, trib. (Big R)	23/03/2011	-37.4873	145.9190	960	2.4	No fish (BG ds)	Yes	Potentially – genetic research required
FT014	Westcott Crk (Sunday Crk)	6/07/2010	-37.3717	145.1555	630	5.1	No fish	?	Potentially– barrier survey required d/s
FT017	Number One Crk (Yea R)	9/07/2010	-37.5308	145.3589	510	2.7	No fish	?	Potentially– barrier survey required d/s
FT013	Comet Crk (Sunday Crk)	6/07/2010	-37.3717	145.1555	560	2.1	No fish	?	Potentially– barrier survey required d/s
FT046	Big R, trib. (Big R)	27/10/2010	-37.4584	146.1110	600	2	No fish	?	Potentially– barrier survey required d/s
FT031	Big R, trib. (Big R)	11/08/2010	-37.4635	146.0579	640	1.7	No fish	?	Potentially– barrier survey required d/s
FT015	Number One Crk (Yea R)	9/07/2010	-37.5290	145.3445	540	1.6	No fish	?	Potentially– barrier survey required d/s
FT060	Tom Burns Crk (Rubicon R)	1/02/2011	-37.3888	145.8398	1010	1.3	No fish	?	Potentially– barrier survey required d/s

Site no.	Waterbody (Catchment)	Date sampled	Latitude (°S)	Longitude (°E)	Altitude (m)	Catchment Area (km^2)	Fish species present	Barrier present downstream	Translocation suitability
FT022	Dungaree Crk (Big R)	27/07/2010	-37.6100	146.1508	1060	1	No fish	?	Potentially– barrier survey required d/s
FT044	Fryer Crk (Big R)	26/10/2010	-37.3812	146.1258	840	1	No fish	?	Potentially– barrier survey required d/s
FT051	Twenty Five Mile Crk, trib. (Big R)	14/12/2010	-37.4712	146.1188	680	1	No fish	?	Potentially– barrier survey required d/s
FT036	Taponga R, trib. (Big R)	12/08/2010	-37.4658	146.0137	650	0.9	No fish	?	Potentially– barrier survey required d/s
FT050	Wombat Crk (Goulburn R)	13/12/2010	-37.5146	146.1697	1010	0.9	No fish	?	Potentially– barrier survey required d/s
FT035	Wild Dog Crk (Big R)	12/08/2010	-37.4700	146.0125	710	0.8	No fish	?	Potentially– barrier survey required d/s
FT003	Torbreck R Sth Brch, trib. (Big R)	17/05/2010	-37.4777	145.9203	930	0.8	No fish	?	Potentially– barrier survey required d/s
FT004	Torbreck R Sth Brch, trib. (Big R)	17/05/2010	-37.4718	145.9241	960	0.8	No fish	?	Potentially– barrier survey required d/s
FT058	Ryan Crk (Goulburn R)	1/02/2011	-37.5237	146.1596	1050	0.7	No fish	?	Potentially– barrier survey required d/s
FT048	Goulburn R (Goulburn R)	29/10/2010	-37.5362	146.2036	1010	0.6	No fish	?	Potentially– barrier survey required d/s
FT029	Big R, trib. (Big R)	10/08/2010	-37.4617	146.0736	690	0.5	No fish	?	Potentially– barrier survey required d/s
FT010	Second Crk (Big R)	18/05/2010	-37.4420	145.9418	1080	0.4	No fish	?	Potentially– barrier survey required d/s
FT007	Torbreck R Nth Brch, trib. (Big R)	18/05/2010	-37.4559	145.9377	1040	0.4	No fish	?	Potentially– barrier survey required d/s
FT008	Torbreck R Nth Brch, trib. (Big R)	18/05/2010	-37.4548	145.9387	1040	0.4	No fish	?	Potentially– barrier survey required d/s

Site no.	Waterbody (Catchment)	Date sampled	Latitude (°S)	Longitude (°E)	Altitude (m)	Catchment Area (km^2)	Fish species present	Barrier present downstream	Translocation suitability
FT009	Torbreck R N Brch, trib. (Big R)	18/05/2010	-37.4479	145.9415	1080	0.4	No fish	?	Potentially– barrier survey required d/s
FT006	Deep Crk (Big R)	18/05/2010	-37.4621	145.9301	1050	0.3	No fish	?	Potentially– barrier survey required d/s
FT002	Torbreck R Sth Brch, trib. (Big R)	17/05/2010	-37.4818	145.9171	950	0.3	No fish	?	Potentially– barrier survey required d/s
FT056	Big R, trib. (Big R)	15/12/2010	-37.5254	146.0791	530	4	No fish	No	Potentially – barrier installation required
FT020	Sylvia Crk Nth Brch (Yea R)	20/07/2010	-37.5236	145.5214	680	2.1	No fish	No	Potentially – barrier installation required
FT040	Taponga R, trib. (Big R)	22/09/2010	-37.4561	145.9975	620	0.7	No fish	No	Potentially – barrier installation required
FT032	Big R, trib. (Big R)	11/08/2010	-37.4605	146.0644	600	0.5	No fish	No	Potentially – barrier installation required
FT030	Big R, trib. (Big R)	10/08/2010	-37.4576	146.0654	620	0.3	No fish	No	Potentially – barrier installation required
FT039	Taponga R, trib. (Big R)	22/09/2010	-37.4560	145.9976	620	0.8	RT	No	Potentially – barrier installation and trout removal required
FT041	Dudley Crk (Big R)	23/09/2010	-37.4729	145.9787	640	1.4	RT	No	Potentially – barrier installation and trout removal required
FT038	Taponga R (Big R)	21/09/2010	-37.4573	146.0029	580	1.9	TSp	No	Potentially – barrier installation and trout removal required
FT021	Sylvia Crk Sth Brch (Yea R)	20/07/2010	-37.5337	145.5237	700	2.3	BT	No	Potentially – barrier installation and trout removal required
FT019	Sylvia Crk Nth Brch (Yea R)	16/07/2010	-37.5261	145.5214	660	2.8	BT	No	Potentially – barrier installation and trout removal required

Site no.	Waterbody (Catchment)	Date sampled	Latitude (°S)	Longitude (°E)	Altitude (m)	Catchment Area (km²)	Fish species present	Barrier present downstream	Translocation suitability
FT034	Wild Dog Crk (Big R)	11/08/2010	-37.4591	146.0040	600	3.3	RT	No	Potentially – barrier installation and trout removal required
FT053	Goulburn R (Goulburn R)	14/12/2010	-37.5462	146.2128	880	3.3	BT	No	Potentially – barrier installation and trout removal required
FT059	Little Crk (Big R)	1/02/2011	-37.5397	146.0141	550	3.4	RT	No	Potentially – barrier installation and trout removal required
FT025	Spring Crk (Big R)	29/07/2010	-37.6103	146.1511	1110	3.6	RT	No	Potentially – barrier installation and trout removal required
FT055	Big R, trib. (Big R)	15/12/2010	-37.5068	146.0600	580	3.7	RT	No	Potentially – barrier installation and trout removal required
FT026	Boundary Crk (Big R)	30/07/2010	-37.5805	146.0242	600	3.7	RT	No	Potentially – barrier installation and trout removal required
FT037	Big R, trib. (Big R)	21/09/2010	-37.4640	146.0663	560	4.4	TSp	No	Potentially – barrier installation and trout removal required
FT054	25 Mile Crk (Big R)	15/12/2010	-37.4918	146.1244	620	5.2	RT	No	Potentially – barrier installation and trout removal required
FT042	Shaw Crk (Big R)	5/10/2010	-37.6228	146.0170	710	5.7	BT RT	No	Potentially – barrier installation and trout removal required
FT028	Big R, trib. (Big R)	10/08/2010	-37.4741	146.0696	550	5.8	BT RT	No	Potentially – barrier installation and trout removal required
FT011	Torbreck R Sth Brch (Big R)	19/05/2010	-37.4829	145.9256	790	6.6	BT	No	Potentially – barrier installation and trout removal required

Site no.	Waterbody (Catchment)	Date sampled	Latitude (°S)	Longitude (°E)	Altitude (m)	Catchment Area (km^2)	Fish species present	Barrier present downstream	Translocation suitability
FT052	Moonlight Crk (Goulburn R)	14/12/2010	-37.4542	146.1750	650	9.6	RT	No	Potentially – barrier installation and trout removal required
FT057	Dear Hound Crk (Big R)	28/10/2010	-37.5091	146.0938	460	13	RT	No	Potentially – barrier installation and trout removal required
FT027	Shaw Crk (Big R)	30/07/2010	-37.5897	146.0243	610	17.5	BT	No	Potentially – barrier installation and trout removal required
FT012	Torbreck R Sth Brch, trib. (Big R)	19/05/2010	-37.4857	145.9209	880	2.5	BT (BG upstream)	No	No – barrier installation and trout removal required
FT045	Moonlight Crk, trib. (Goulburn R)	27/10/2010	-37.4500	146.1423	910	1.3	BG	?	No – barrier survey required d/s
FT023	Oaks Crk (Big R)	27/07/2010	-37.6394	146.1299	1150	1.1	MG	?	No
FT016	Number Three Crk (Yea R)	9/07/2010	-37.5113	145.3859	400	3.8	DRY	?	No
FT047	Ryan Crk, trib. (Goulburn R)	28/10/2010	-37.4873	146.1443	820	1.3	DRY	?	No
FT049	Frenchmans Crk, trib. (Big R)	13/12/2010	-37.5852	146.1744	1130	0.4	DRY	?	No

2.3.3 Selection of suitable translocation sites

Based on assessment criteria, six (10%) sites assessed were found suitable for the immediate translocation of Barred Galaxias (Table 1; Figure 8). These are located in the Koala Creek (FT001, FT005), Shaw Creek (FT024, FT043), Sylvia Creek north branch (FT018), and upper Taponga River (FT033) catchments (Table 1). In particular, these sites were located upstream of a suitable instream barriers, did not have a resident fish fauna, had good catchment and streamflow conditions and ranged in catchment area from 0.1–8.3 km^2.

A further 24 (39%) sites lacked fish but require further assessment of the presence and condition of potential downstream barriers before further assessment against the suitability criteria and possible inclusion as potential translocation sites (Table 1; Figure 8). Five (8%) other sites (FT020, FT030, FT032, FT040, FT056) lacked fish but also lacked instream barriers (Table 1; Figure 8). These sites, in catchments varying from 0.3–4 km^2, require the installation of an appropriate instream barrier to prevent the upstream movement of trout before they can be included as potential translocation sites.

The upper reaches of a tributary of the Torbreck River, south branch (FT061) lacked fish and had a barrier downstream, however a small population of Barred Galaxias currently exists below this barrier. This site may be included as a potential translocation site once the genetic structure of the resident Barred Galaxias population is determined.

Nineteen sites are currently unsuitable for translocation as they contain trout and do not have an effective downstream barrier. The remaining six sites were rejected as suitable for translocation because they were dry (three sites), or contained populations of native fish species (three sites: FT012, Torbreck River south branch tributary; FT023, Oaks Creek; and, FT045, Moonlight Creek) (Table 1, Figure 8).

Selection of two trial translocation catchments

Four catchments were initially identified as suitable for use in the trial translocation of Barred Galaxias (Table 1). They ranged in upstream catchment area from 0.1 km^2 (Sylvia Creek north branch) to 8.3 km^2 (Koala Creek). Koala Creek was rejected from use in the translocation trial because smaller catchments were preferable to keep the scale of the trial manageable. It was also considered more valuable to be set aside for a large and critical future translocation of Barred Galaxias once procedures for effective translocations had been established.

Consequently, Shaw Creek and the upper Taponga River (catchment areas of 3.5 and 1.4 km^2 respectively) were chosen as the trial translocation catchments (see Chapter 3), being the next two consecutively smaller catchments (Table 1).

The upper Taponga River site (FT033) was surveyed a second time over an 800 m reach upstream from the instream barrier, targeting habitat suitable for trout (i.e. pools). Again no trout were found and suitable conditions and habitat were confirmed for Barred Galaxias. Similarly, Shaw Creek was surveyed a second time, at approximately 1.75 km's upstream of the original survey location (FT043). No trout were found in a 100 m reach and suitable conditions and habitat for Barred Galaxias also existed.

2.3.4 Distribution of Mountain Galaxias

An unexpected outcome of the field assessments was the lack of populations of Mountain Galaxias recorded within the study area, with the species found at only one of the 61 sites (Table 1; FT023, Oaks Creek). This species is widespread in foothill to montane areas of north-eastern Vitoria (Raadik 2011). Comparison of Figure 2 with Figure 8 indicates that Mountain Galaxias are almost as rare in distribution as Barred Galaxias in the upland reaches of the south-east portion of the Goulburn River system, particularly in the Big River catchment.

2.4 Discussion and recommendations

Careful site selection is a pivotal step in planning translocation events (Minckley 1995). Translocation sites should satisfy the species' habitat requirements to facilitate their survival, establishment and range expansion (Minckley 1995; George *et al.* 2009), however, not all areas within the species former range will contain suitable habitat. Differences in stream and catchment condition could make some sites more preferable than others. Preferably all known threats to the species (e.g. predation, dewatering, hybridisation, etc.) should be absent or mitigated at the translocation site. For example, instream sedimentation, commonly occurring in catchments impacted by bushfire and forestry activities, reduces water quality and degrades instream habitat, which inturn influences the survival and recovery of fish (Lyon and O'Connor 2008). Thus, sites impacted by sedimentation would be less suitable for Barred Galaxias translocation than those unaffected. Furthermore, the total stream area above an instream barrier should be large enough to support long-term population growth and range expansion of the translocated fish population, as well as being large enough to maintain the natural diversity and condition of the stream itself (Moyle and Sato 1991).

This project highlighted the scarcity of available potential translocation sites within a portion of the natural range of Barred Galaxias, due mainly to the widespread distribution of trout, extending upstream into small headwater streams. This is likely to reflect the situation in other areas within the range of Barred Galaxias in the Goulburn River system, as trout are prevalent and widespread throughout all of the cooler, upland reaches of the system. Many of these streams are likely to have historically harboured Barred Galaxias populations, which are unable to co-occur with trout (Raadik *et al.* 2010, Raadik T.A. unpublished data). Trout predate on, and out-compete, the galaxiids for food and habitat (McDowall 2006, Raadik *et al.* 2010), and as such, the remaining Barred Galaxias populations are restricted to headwater streams were trout do not occur (Raadik *et al.* 2010), usually upstream of instream barriers.

Identifying potential Barred Galaxias translocation sites in predator-free catchments was also difficult as very few existing natural physical instream barriers were known within the study area that did not already have Barred Galaxias upstream of them. As the conservation of distinctive native fish taxa is a prime objective in conservation translocation of threatened species (Minckley 1995) selection of potential translocation sites also excluded stream reaches known to contain populations of Mountain Galaxias as the two galaxiid species are known to naturally hybridise (Raadik 2011). Consequently, of the 217 potential sites identified for assessment the majority were in headwater reaches and small in catchment area. Only 61 of these were able to be assessed for suitability during this study as many were remote and difficult to access. The remaining potential sites are therefore available to be ground-truthed in the future if required.

Of the 61 sites visited, only four separate catchments, each without resident fish and with an effective barrier downstream, were considered appropriate for the immediate translocation of Barred Galaxias. We selected two of these that were in catchments of medium size for the forthcoming trial translocation of Barred Galaxias (see Chapter 3), to ensure that the monitoring of the trial translocation would be manageable relative to the small number of fish to be translocated. A suitable translocation site in a larger catchment (Koala Creek) was set aside for future use when translocating a larger population.

As expected, given their wide distribution, a high proportion of potential sites assessed contained trout, further extending their known range in headwater reaches. Without future management intervention, including installation of a barrier and upstream removal of trout, these sites are currently unsuitable for Barred Galaxias translocation. Many other potential translocation sites appeared to lack fish but the presence of an effective barrier downstream could not be determined. Further surveys for barriers downstream of these sites is necessary, and if none are found, the installation of a barrier is required to improve the security of the upstream reach from trout invasion before these sites can be considered suitable for translocation. That only a small number of suitable catchments were found is indicative of the difficulty of locating suitable translocation sites and the lack of remaining catchments that do not first require potentially expensive management works (i.e. removal of resident fish, installation of instream barrier) to make them suitable.

The cost of undertaking surveys for effective barriers to locate catchments suitably protected from upstream predator invasion is considered to be less than that of barrier installation in steep and remote catchments, particularly if the complete removal of any upstream resident trout is also required. Therefore undertaking barrier surveys to identify suitable translocation sites for Barred Galaxias appears more acceptable than the more costly and difficult second option. However, this is predicated on the assumption that suitably large upland catchments are not limited across the target landscape. This assumption is incorrect given the wide distribution of trout in headwater reaches and therefore barrier construction and fish removal may need to be considered in the future as a conservation management option if additional and larger sites are needed for Barred Galaxias translocations.

The success of upstream colonisation of trout is influenced by physical parameters (barrier type and height), hydrological conditions (flow and pool depth), and their jumping and darting abilities. Meixler *et al.* (2009) calculated the maximum jumping height and darting speed of Brown Trout (average length = 52 cm) to be 1.10 m and 4.64 m/s respectively, whilst Rainbow Trout were similar (average length 50 cm, maximum jumping height = 1.03 m, maximum darting speed 4.50 m/s). Therefore, we subjectively rated barriers as 100% effective if they were greater than 2 m in height, with no plunge pool downstream. Also the barrier needed to be effective in all flow conditions, particularly high flows. The location of a barrier in a v-shape landscape would direct water to the centre of the stream channel, even during periods of high flow, and prevent lateral water movement through which trout may migrate. Ideally, a barrier would also consist of solid rock for permanency.

The seven barriers identified in this study were greater than 2 m in height and were natural rock waterfalls, except on the upper Taponga River, where the barrier was a fall in height from the downstream side of a road culvert. There is a risk of this culvert being washed out in high flow, thus allowing the trout residing downstream to easily migrate upstream into the translocation zone. Installation of a second barrier upstream of the road culvert would further secure this site from trout invasion.

During site assessments, no sites were found that suffered from sedimentation impacts. However, three sites surveyed were dry and were therefore considered unsuitable for Barred Galaxias translocation because water security is a threat. Furthermore, upstream catchments of sites varied in size, with many relatively small in area (< 4 km^2). The degree of water permanency in a small headwater catchment, to sustain a population of Barred Galaxias, is difficult to determine and may vary over time. It could be measured by annual site visits during the low flow period or be subjectively assessed by checking for the presence of in-stream bryophytes (an indicator of water permanency).

Stoessel *et al.* (2012) found that Barred Galaxias lay clusters of eggs on the underside of cobbles positioned in flowing water. The presence and abundance of instream cobbles, and possibly smaller boulders, is therefore needed to facilitate breeding of Barred Galaxias. Observing and/or mapping the presence and abundance of potential spawning substrates should therefore be incorporated into the site assessment process to identify future translocation sites. The deliberate release of trout into areas upstream of barriers is also an issue, particularly at sites easily accessed by road, such as Koala Creek and Taponga River. Site inaccessibility is therefore important to decrease the likelihood of human-assisted dispersal of trout. This can be assessed by the location of the proposed site to existing roads and trails within a catchment.

To further improve the suitability of potential translocation sites for Barred Galaxias, future site assessments should therefore also incorporate evaluation of the presence of suitable spawning habitat throughout the site, degree of site accessibility, and a more detailed assessment of water permanency. Community engagement and education activities will also be important to help mitigate human-assisted trout dispersal.

Another important outcome of this project is the development of a list of secondary sites, not immediately suitable for Barred Galaxias translocation, which have been preliminarily assessed and require varying levels of works to improve their suitability. As the location and issues with these sites is already known, their suitability may be improved in the future in a relatively short time and with more strategic investment if the need arises. This is particularly pertinent if a stochastic event occurs requiring urgent translocation of a Barred Galaxias population. However, all potential translocation sites should be reassessed before a translocation event occurs in case habitat conditions have changed since the initial assessment, for example, trout may have since colonised the stream rendering the site unsuitable.

Given the scarcity of available potential translocation sites within the natural range of Barred Galaxias, due mainly to the widespread distribution of predatory trout, there is a need to create and manage trout-free zones in headwater catchments for future translocation events to ensure the long-term survival and recovery of this species. This would readdress to some extent the current imbalance in the total area occupied by indigenous native fish species in headwater reaches compared to that of trout. This issue is also pertinent for Mountain Galaxias, a species also impacted by trout (Lintermans 2000, Jackson *et al.* 2004, McDowall 2006, Raadik 2011), which were found to be restricted to a few, small and isolated populations in the study area. This current distribution is considered to be a fragmentation of a former widespread range.

The following actions are required to improve the success of the trial translocations and to improve the success of future Barred Galaxias translocations:

- Install a second barrier at Taponga River upstream of the 15 Mile road crossing to secure the translocated Barred Galaxias population;
- Perform barrier surveys on the 24 identified fishless sites to potentially add to the list of sites suitable for immediate translocation;
- Install barriers and remove trout from the 19 identified small headwater catchments to render them suitable for immediate translocation;
- Undertake a more rigorous assessment of site accessibility, water permanency and include assessment of the presence of suitable spawning habitat (see Stoessel *et al.* 2012) throughout each site in future assessment of translocation site suitability; and,
- Undertake stakeholder engagement and education regarding Barred Galaxias conservation and the impacts from human assisted dispersal of trout.

3 Trial translocations of post-fire impacted Barred Galaxias

3.1 Introduction

Numerous native freshwater fish species have been translocated within Australia (SKM 2008), including Freshwater Catfish (*Tandanus tandanus*) (Clunie and Koehn 2001), Murray Cod (*Maccullochella peelii*) (National Murray Cod Recovery Team 2010), Bluenose or Trout Cod (*Maccullochella macquariensis*) (Trout Cod Recovery Team 2008) and the Australian Lungfish (*Neoceratodus forsteri*) (Arthington 2009). Although most translocations of Australian native freshwater fish have occurred as part of stocking programs (SKM 2008), translocations are increasingly being considered or recommended as a conservation tool for threatened fishes in Australia.

Translocations of Barred Galaxias are recommended in the National Recovery Plan for the species (Raadik *et al.* 2010). This chapter documents trial reintroductions of two Barred Galaxias populations into two suitable subcatchments within their former range.

The specific objectives were to:

1. Identify two source populations and collect a subsample of Barred Galaxias for translocation.
2. Transport and release Barred Galaxias into two suitable, previously identified (see Chapter 2) release catchments.
3. Monitor the source and translocated populations to assess the short-term success of the reintroduction.

3.2 Methods

Guidelines for the translocation of Barred Galaxias (Ayres *et al.* 2011) were followed throughout the Barred Galaxias trial translocation.

3.2.1 Source and translocation sites

Annual monitoring of known Barred Galaxias populations has occurred since 2000 (T. Raadik, personal communication 1 July 2010). Kalatha Creek and Luke Creek (Figures 9 and 10), tributaries of the Yea River, were selected as sources of Barred Galaxias for the trial translocation because annual monitoring showed that these populations have a reasonable population size and have been reproductively stable over time. Therefore they were unlikely to suffer negative effects from the removal of some individuals for trial translocations.

Other considerations were:

- Luke and Kalatha creeks have less predictable water security than Bared Galaxias sites further eastward as the streams are not fed by snow melt, and were affected by recent drought conditions;
- The Luke Creek catchment was partly burnt during the 2009 Black Saturday bushfires and therefore the Barred Galaxias population was potentially suffering from post-fire impacts; and,
- Populations would benefit from creating secure 'replicate' populations elsewhere.

Figure 9. Site of the source population for the Barred Galaxias translocation trial in Kalatha Creek (left) and Luke Creek (right). (Images: left – Michael Nicol, right – Renae Ayres).

Figure 10. Location of Kalatha, Luke and Shaw creeks and Taponga River in the Goulburn River catchment, Victoria. Same shape (square or circle) indicates translocated source (closed) and recipient (open) creeks.

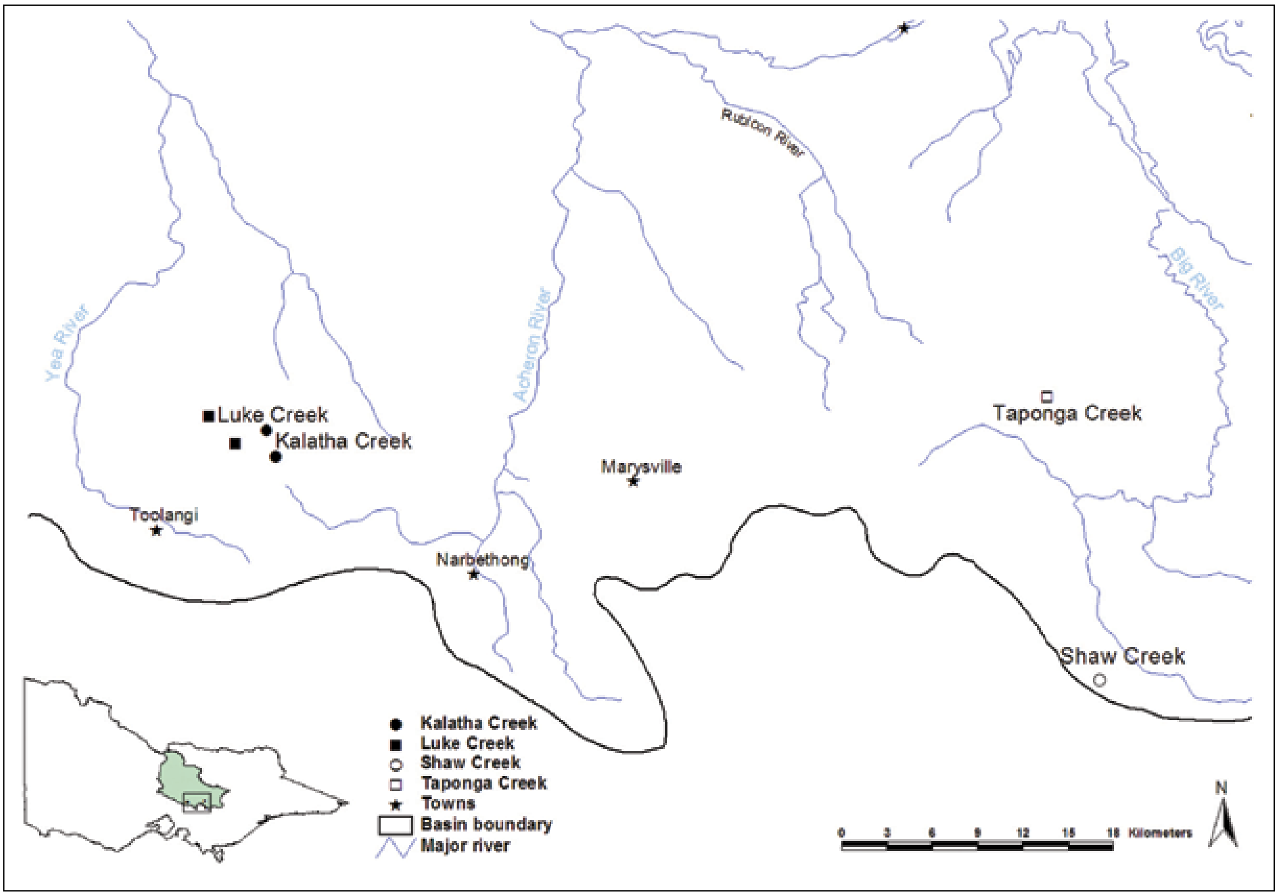


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Figure 11. Barred Galaxias translocation site on Shaw Creek (site FT024) (left) and Taponga River (site FT033) (right). (Images: Renae Ayres).

Taponga River and Shaw Creek (Figures 10 and 11), tributaries within the Big River catchment, were selected as recipient sites for translocated Barred Galaxias from six suitable Barred Galaxias translocation sites identified (Chapter 2). These locations satisfy the release site criteria in the Barred Galaxias translocation guidelines (Ayres *et al.* 2011).

3.2.2 Translocation

Approval was gained from the Victorian Department of Primary Industries' Translocation Evaluation Panel to conduct two trial translocations of Barred Galaxias from: 1) Kalatha Creek to Shaw Creek; and, 2) Luke Creek to Taponga River (Figure 10). The translocation and post-monitoring schedule is outlined in Table 2.

Collection of fish

Adult collection from source sites

To reduce localised impacts, adult Barred Galaxias were collected from two separate reaches each within Kalatha and Luke creeks (Table 2). Fish were sampled at each location using backpack electrofishing (Figure 12). At each reach an operator electrofished all accessible habitat in an upstream direction followed by an assistant to collect stunned fish. All Barred Galaxias collected were measured for length (caudal fork length (LCF) mm) and weight (g), a subset was selected for translocation, and the remainder were returned to their point of capture. Individuals selected for translocation were adults (> 55 mm LCF), healthy (with no visible lesions, disease etc.) and represented all size classes above 55 mm collected. This ensured that all individuals translocated were potentially capable of breeding and producing offspring at the next spawning season to maximise the establishment of the species at the new sites.

A small amount of caudal fin (5 mm^2) was clipped from each individual selected for translocation, to be used for population genetic analysis (see Chapter 4).

Table 2. Translocation and post-monitoring schedule.

Event	Date	Detail
Translocation	30 Nov 2010	Collect adult fish from Kalatha Creek
	1 Dec 2010	Translocate Kalatha Creek fish to Shaw Creek
	2 Dec 2010	Collect adult fish from Luke Creek
	3 Dec 2010	Translocate Luke Creek fish to Taponga River
3 month Post-monitoring	15 March 2011	Luke Creek source reaches
	16 March 2011	Taponga River translocation site
	17 March 2011	Shaw Creek translocation site
	18 March 2011	Kalatha Creek source sites
6 month Post-monitoring	14 June 2011	Luke Creek source reaches
	15 June 2011	Taponga River translocation site
	16 June 2011	Shaw Creek translocation site
	17 June 2011	Kalatha Creek source reaches

Figure 12. Collecting Barred Galaxias for translocation from Luke Creek using electrofishing. (Image: Dean Hartwell).

The following additional information was collected at each sampling reach in Kalatha and Luke creeks:

- Geographic location, using a hand-held GPS unit;
- Water quality (temperature, EC, pH, turbidity and dissolved oxygen), using a TPS 90-FLT Field laboratory analyser;
- Electrofishing settings (volts, amps, hertz, duty cycle, electrofishing seconds);
- Distance electrofished (m);
- Average stream width (m);
- Maximum and average stream depth (m);
- Flow (flood, high, medium, low); and,
- Digital images of habitat.

Larval fish collection

As part of a separate fire recovery project, wild spawned Barred Galaxias eggs, collected earlier from Kalatha and Luke creeks, were hatched in secure aquarium facilities at ARI (Stoessel *et al.* 2012). Larvae were reared ex situ for approximately three months and 120 from each source population were translocated into Shaw Creek and Taponga River respectively, coinciding with the adult Barred Galaxias translocations.

Fish transportation

Following collection, adult Barred Galaxias were placed into sterilised plastic drums (80 L) (Figure 13), filled with creek water from the source location, and transported overnight to the translocation sites. Larval Barred Galaxias were transported overnight to the translocation sites in sterilised plastic buckets (20 L) containing water from ARI's aquarium facilities. Water in drums and buckets was continually aerated and chilled using ice bags to maintain temperature (Figure 14) and salt was added to water at a concentration of 2.5 g/L to calm fish and treat any infections. Fish were not fed and regularly monitored during transportation.

Once at the translocation site, water quality (temperature, EC, pH, turbidity and dissolved oxygen) was measured within the fish transportation medium, as well as in the translocation streams. Fish were acclimatised over a lengthy time period (10–15 minutes) by gradually mixing water from the translocation site into the transport medium, with care taken to ensure that decanted water did not flow into the waterways. Fish behaviour and health was visually monitored. Once fish had acclimatised and their holding water was completely changed to the translocation site water, fish release commenced.

Figure 13. ARI staff transporting Barred Galaxias. (Image: Renae Ayres).

Figure 14. Adult Barred Galaxias were transported in sterilised drums containing creek water from the source site and 2.5g/L salt solution to calm fish. Water was continually aerated and cooled using bags of ice. (Image: Renae Ayres).

Fish release into translocation sites

At Shaw Creek, adult fish from Kalatha Creek were released into pools over a 175 m reach approximately 235 m upstream of the physical barrier (Shaw Creek waterfall) (Figure 15). Larval Kalatha Creek fish were released over 100 m into still, pool habitats and slowly flowing backwaters, beginning 185 m upstream from the end of the adult release reach.

At Taponga River, adult fish from Luke Creek were released into slowly flowing pools over a 130 m reach approximately 200 m upstream of the physical barrier (Taponga River Road culvert) (Figures 15 and 16). Larval Luke Creek fish were released over 100 m into still, pool habitats and slowly flowing backwaters, beginning 100 m upstream from the end of the adult release reach (Figures 15 and 16).

Figure 15. Adult and larval Barred Galaxias release and range expansion (RE) reaches at Shaw Creek (upper) and Taponga River (lower).

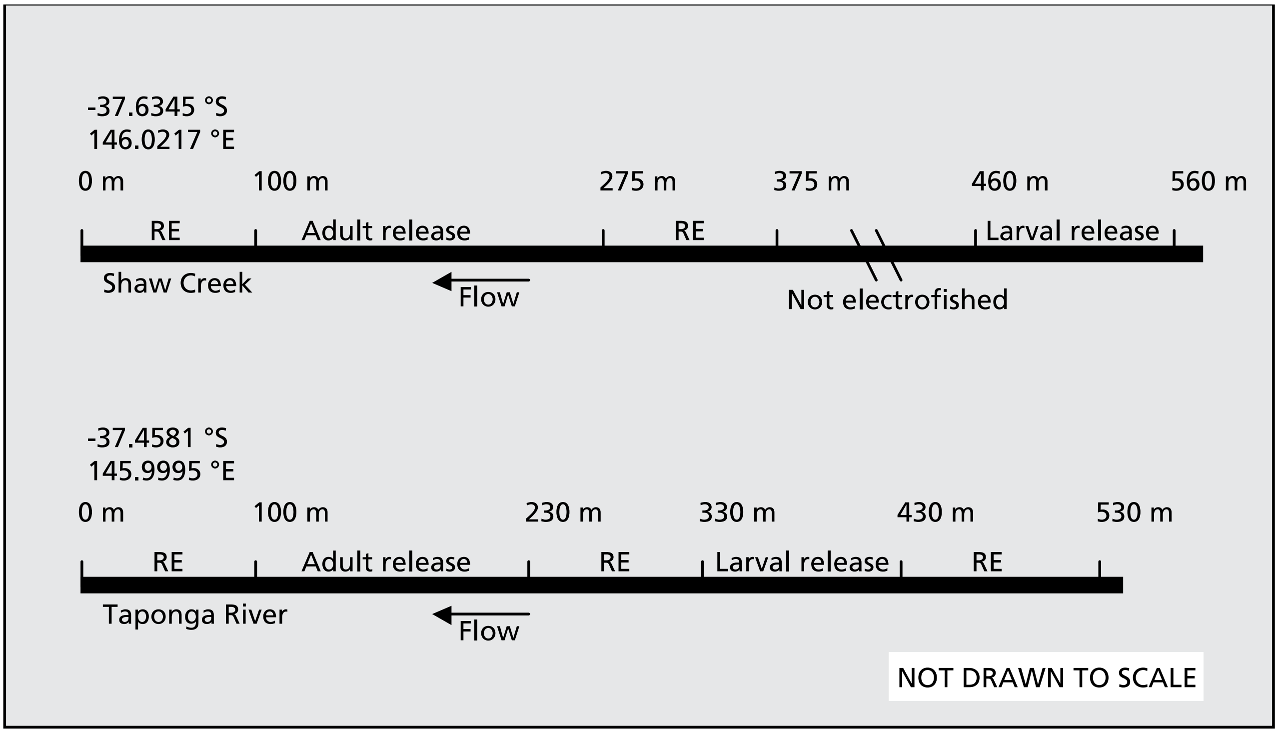


Figure 16. Releasing translocated adult (left) and larval (right) Barred Galaxias into Taponga River. (Images: left – Fern Hames, right – Renae Ayres).

3.2.3 Post-translocation monitoring

Post-translocation monitoring of Barred Galaxias at source and release sites was undertaken three and six months after the translocation event. At Kalatha and Luke creeks, each reach from where Barred Galaxias were collected for translocation was electrofished to confirm the number and size range of fish present. At Shaw Creek and Taponga River, the reaches where adults and larval fish were released, including a 100 m section upstream and downstream of these areas, was electrofished to determine if translocated individuals had survived and increased in range (Figure 15), and if they were reproductively developing. Survival of fish was measured simply as presence, whilst developing gonads (noted only during the 6 month post-monitoring event) would suggest reproductive development. Range expansion was measured as individuals present in the 100 m sections directly upstream or downstream of the adult or larval release reaches.

Fish sampled at each location followed the methodology described for fish collection (see section 3.2.2). Similar additional information was also collected, except general fish condition was also subjectively noted, and the specific location where Barred Galaxias were collected during post-monitoring was noted, i.e. measured as distance from starting point (0 m). Gonad maturity stages were determined by visually examining gonads through the body wall and classifying development according to stages modified from Pollard (1972) (Table 3).

Table 3. Barred Galaxias maturation categories (modified from Pollard 1972).

Maturation stage	Stage Description
I	*Immature virgin* – Applies to all fish less than 45 mm TL, and to males less than 55 mm TL. Smallest length of females not yet determined. Testes and ovaries not visible.
II	*Developing virgin and recovering spent* – Sex of fish cannot be determined, particularly in smaller individuals (usually 75 mm or less). Testes and ovaries indistinguishable, but can be seen that some reproductive tissue is present, filling less than 0.25 of body cavity. Eggs or lobes of testes not clearly distinguishable. (Stage 2 is difficult for some fish by external examination only)
III	*Developing* – Testes thickening, fill more than 0.25 of body cavity. Ovaries fill less than 0.25 of body cavity and are opaque/slightly yellowish, eggs very small and granular in appearance.
IV	*Maturing* – Testes enlarged, whitish and lobes clearly visible, filling less than 0.5 of body cavity. Ovaries small, filling less than 0.5 of body cavity, opaque/yellowish, eggs small but distinctly visible to naked eye.
V	*Mature* – Testes fill 0.5 or more of body cavity (can be a little less), lobes visible and white, no milt extruded by gentle pressure. Ovaries fill 0.5 to 0.75 of body cavity, eggs large but body cavity not distended, and eggs not extruded by gentle pressure (but may be by stronger pressure). Spawning vent in males and females enlarged.
VI	*Ripe* – Testes fill 0.5 or more of body cavity (can be a little less), lobes clearly visible and creamy-white, milt extruded by gentle pressure on body wall. Ovaries fill almost all of body cavity, eggs large and body cavity clearly distended, eggs extruded by gentler pressure on body wall. Spawning vent in males and females enlarged and extended.

3.3 Results

3.3.1 Translocation

A total of 92 Barred Galaxias were collected from a combined distance of 374 m from the two reaches in Kalatha Creek (Table 4) with adult fish (> 55 mm in LCF) accounting for 91% of all fish collected. The smallest fish recorded was 40.0 mm in length and weighed 0.5 g. Fifty adults were selected for translocation to Shaw Creek and the remainder were returned to their point of capture (Table 4). The average length of adult Barred Galaxias translocated to Shaw Creek was 78.1 mm, ranging from 55–106 mm. One hundred and twenty larval Barred Galaxias, averaging 14.0 mm in length (range: 11–17 mm), were released at Shaw Creek.

Seventy two Barred Galaxias were collected from a combined distance of 570 m from two reaches in Luke Creek (Table 4) with all classified as adult fish (> 55 mm in LCF). The smallest fish recorded was 58 mm in length and weighed 1.5 g. Forty adults were selected for translocation to Taponga River (Table 4) and the remainder were returned to their point of capture. The average length of the translocated fish was 86.3 mm, ranging from 58.0–114.0 mm. One hundred and twenty larval Barred Galaxias, averaging 13.8 mm in length (range: 11–18 mm), were released at Taponga River.

All fish selected for translocation were in good health before and after transportation, and following 10–45 minutes of acclimatisation. During acclimatisation water quality of fish holding tanks had equilibrated to conditions measured in the respective translocation sites and fish were behaving normally and actively swimming. Adult fish were released across a distance of 175 m and 130 m at Shaw Creek and Taponga River respectively and larval fish over a 100 m reach in each recipient stream (Figure 15).

3.3.2 Post-monitoring

During the three-month post-monitoring event (Table 2), 125 Barred Galaxias were collected each from the source reaches within Kalatha and Luke creeks (Table 5). At Kalatha Creek, fish averaged 69.6 mm in length (range 35.0–114.0 mm) and 70 % were adults. At Luke Creek, fish averaged 76.4 mm in length (range 36.0–119.0 mm) and 80 % were adults. Fish densities (fish/100 m stream length) were higher than when fish were collected for translocation and size and weight ranges did not indicate a negative impact from fish removal as they had not reduced (Table 4, Table 5).

Table 4. Number (*n*) and size (average length/weight (range) mm/g) of Barred Galaxias collected from Kalatha and Luke Creeks and those translocated to Shaw Creek and Taponga River. Distance (m) indicates the total reach length from which fish were collected from within source creeks and the reach length that adult fish were released into at translocation streams.

Waterbody	Distance surveyed (m)	*n*	Fish/100 m stream length	Proportion of adults – > 55 mm (%)	Average length (range) (mm)	Average weight (range) (g)
Source						
Kalatha Creek	374	92	25	91	74.6 (40.0–106.0)	4.1 (0.5–12.2)
Luke Creek	570	72	13	100	86.1 (58.0–114.0)	6.1 (1.5–14.2)
Translocation						
Shaw Creek	175	50	29	100	78.1 (55.0–106.0)	4.7 (1.0–12.2)
Taponga River	130	40	31	100	86.3 (58.0–114.0)	5.9 (1.5–14.2)

Table 5. Number (*n*) and size (average length/weight (range) mm/g) of Barred Galaxias collected at source and translocation creeks during three and six month post-monitoring events.

Water body	Distance surveyed (m)	*n*	Fish/100 m stream length	Proportion of adults – >55 mm (%)	Average length (range) (mm)	Average weight (range) (g)
3 month post-monitoring						
Source						
Kalatha Creek	177	125	70	70	69.6 (35.0–114.0)	4.1 (0.3–15.8)
Luke Creek	257	125	49	80	76.4 (36.0–119.0)	4.8 (0.4–16.7)
Translocation						
Shaw Creek	475	13	3	100	84.5 (55.0–110.0)	8.1 (1.0–17.9)
Taponga River	530	14	3	100	96.4 (58.0–112.0)	10.6 (1.4–17.4)
6 month post-monitoring						
Source						
Kalatha Creek	177	66	37	85	76.6 (41.0–106.0)	4.9 (1.5–14.3)
Luke Creek	300	61	20	84	79.1 (46.0–117.0)	5.1 (0.8–16.3)
Translocation						
Shaw Creek	475	6	1	100	82.0 (55.0–98.0)	7.6 (1.1–13.1)
Taponga River	530	6	1	100	85.6 (58.0–119.0)	7.0 (1.5–13.3)

Only adult Barred Galaxias were collected at the translocated sites during the three-month post-monitoring event (Table 5). Twenty six percent (*n* = 13) of fish translocated to Shaw Creek were recaptured, and 35 % (*n* = 14) were recaptured at Taponga River, representing individuals across the length range of fish translocated to each stream. Fish density in each system had declined by approximately 10 fold from 29–31 fish/100 m of stream length to a density of three. Fish recaptures demonstrate that a proportion of translocated Barred Galaxias survived at each site, however the decline in fish density may indicate mortality, fish moving from the site of release to occupy new habitat, or both. Inherent sampling inefficiencies may also account for a decrease in fish numbers, but sampling was undertaken in March when stream conditions were optimal for sampling (clear water and low water levels).

Fish were absent from the range expansion reach downstream of the adult release reach in both streams, though a 55 mm LCF individual and two individuals, 55 and 105 mm in length, were found in the upstream range expansion reaches in Shaw Creek and Taponga River respectively. These fish had migrated minimum distances upstream of 35 m and 88 m respectively, indicating that some fish had undertaken upstream movement and that each translocated population had expanded in range within its recipient stream.

No larval fish were detected, though this was not surprising given the difficulty in detecting small sized individuals and the amount of habitat into which they were released. If the larval fish survive and grow, this cohort should be able to be detected in subsequent sampling events as they increase in size, particularly during the 12 month post-monitoring event as they should be approximately 30–40 mm in length (T. Raadik, unpublished data).

During the six month post-monitoring event, 66 and 61 Barred Galaxias were collected from Kalatha and Luke creeks respectively (Table 5). At Kalatha Creek, fish averaged 76.6 mm in length (range 41.0–106.0 mm) and 80 % were adults. At Luke Creek, fish averaged 79.1 mm in length (range 46.0–117.0 mm) and 84 % were adults. Fish densities (fish/100 m stream length) were slightly higher than when fish were collected for translocation and size and weight ranges did not indicate a negative impact from fish removal as they had not reduced (Table 4, Table 5).

During the six-month post-monitoring event only adult Barred Galaxias were again collected at the translocated sites (Table 5). Twelve percent (*n* = 6) of adults translocated to Shaw Creek were recaptured and 15 % (*n* = 6) were recaptured at Taponga River, again representing individuals almost across the length range of fish translocated to each stream. This further demonstrates that a proportion of translocated Barred Galaxias had survived at each site. Fish density in each system had declined further, from three fish/100 m of stream length to a density of one/100 m. Fish recaptures demonstrate that a proportion of translocated Barred Galaxias survived at each site for six months post-release, however the further decline in fish density is considered related to potential mortality and fish movement, as discussed above, and to increased sampling inefficiency due to higher flows during the mid-June monitoring period. Consequently, the density of fish in the translocation reaches is considered to be higher than that recorded.

Barred Galaxias were again absent from the range expansion reaches downstream of the adult release reaches in both streams. Continued upstream movement of fish was demonstrated by the collection of five fish each in the upstream adult release reaches at Shaw Creek and Taponga River, and one fish each (55 mm LCF and 56 mm LCF) in the upstream larval release reach in each system. These two fish had migrated minimum distances upstream of 139 m and 214 m respectively in 6 months.

The gonad maturation stage of adult fish collected at source and translocated sites during the six month post-monitoring event varied between Stage 2 – Developing virgin and recovering spent to Stage 6 – Ripe (data not shown), with the majority of individuals at Stage 3 – developing or Stage 4 – maturing. Only a few individuals were at Stage 6 – ripe (Figure 17). This indicates that fish in source and translocation streams were undertaking reproductive development, which was typical for that time of year when compared with previous monitoring data for the species (T. Raadik, unpublished data). Importantly, reproductive development was occurring in translocated fish which may lead to some spawning at these new sites in the first year of establishment.

No fish collected during both post-monitoring events displayed signs of adverse physical condition (i.e. fin damage, lesions, starvation) and were therefore considered healthy.

Figure 17. Barred Galaxias collected during the six month post-monitoring event: (A) Gravid female from Shaw Creek; (B) Milt produced by a mature male from Kalatha Creek; (C) Smallest adult Barred Galaxias translocated at Shaw Creek (55 mm LCF). (Images: A– Michael Nicol, B– Renae Ayres, C– Michael Nicol).

A

B

C

3.4 Discussion and recommendations

We successfully undertook a trial translocation of two separate populations of Barred Galaxias into streams within their former range uninhabited by other fish species, with translocated individuals surviving and maintaining condition for at least six months post release. Post-monitoring of source populations showed they appeared unaffected by removal of individuals as Barred Galaxias were present within all sample reaches, had a similar size range and were recorded at a higher abundance to those recorded during the initial collection period, and individuals displayed signs of normal reproductive development. Monitoring of the translocated populations demonstrated fish survival and range expansion, maintenance of a similar size range and physical condition to the initial stocked fish, and reproductive development which suggests each population is likely to spawn during the first year of establishment (during the 2011 breeding season). This suggests at least short-term translocation success, however a decrease in fish density at translocated sites was noted and appeared to be partly explained by the redistribution of fish in each system, but may indicate fish mortality.

Longer term (> 5 years) success of translocation will be evident by indications of successful spawning and recruitment (collection of 0+ age cohorts) and an overall increase in population size and distribution, including maintenance of levels of population genetic diversity close to those of the founder individuals.

This was the first time trial reintroductions of Barred Galaxias have occurred and significant ground-truthing and planning was undertaken to provide the best opportunity for successful outcomes. Several potential Barred Galaxias translocation sites were identified and assessed, with Shaw Creek and Taponga River being selected for the trial (Chapter 2). Experts were consulted to provide information and advice on past fish translocation events and transportation methods (P. Fairbrother, personal communication 2 August 2010; M. Lintermans, personal communication 12 August 2010; I. Ellis, personal communication 30 August 2010; Neil Hyatt, personal communication 14 September 2010). Literature describing previous terrestrial and aquatic species translocations was reviewed to learn from experience and apply best-practice (see Ayres *et al.* 2012), and general guidelines on the translocation of Barred Galaxias were developed using this information as a foundation (Ayres *et al.* 2012) and the guidelines were implemented for the trial Barred Galaxias reintroductions.

The timing of the trial reintroductions considered the breeding season of Barred Galaxias, seasonality and project timelines. Reintroducing Barred Galaxias populations in late November 2010 allowed sufficient time to identify and assess potential sites before translocation and to conduct two post-translocation monitoring events before the end of the project to determine the short-term success of the reintroductions. The trial translocations were conducted in November following the August to September spawning season (Shirley and Raadik 1997) to prevent potential adverse impacts on the breeding and recruitment at source populations. It also allowed time for translocated individuals to establish and begin feeding in their new environment, to improve the chance of successful gonad development and possible spawning in the first year of establishment. Also, undertaking the reintroductions outside the hot summer period likely reduced stress to individuals during collection, transportation and release. Ideally, monitoring of translocated Barred Galaxias populations would be ongoing, however this is restricted by the project timeframe and possibly by future funding opportunities.

For Barred Galaxias populations to be viable and self-sustaining, the survival and recruitment of individuals is imperative. To maximise capture of the natural genetic diversity in the source population, we translocated a selected subsample of individuals collected from multiple locations within the source population's range and ensured that the translocated population size was sufficient without adversely impacting the source population. Higher genetic diversity and population size is positively correlated with population fitness, which is necessary to allow population adaptability and minimise the risk of extinction (Reed and Frankham 2003). Adult Barred Galaxias representing various size cohorts, and therefore presumably different ages, were chosen to allow the translocated populations to naturally reproduce and persist for many years. Furthermore, translocating a mixture of size cohorts allows naive individuals to learn social behaviours, such as anti-predator behaviour, migration, foraging and mate choice, from experienced conspecifics (Brown and Laland 2003). These behaviours may be crucial for adaptation and survival.

Post-translocation monitoring revealed that adult Barred Galaxias had survived the translocation event; however the fate of the larval fish remains uncertain. Possibly the larvae did not survive. Several studies have shown that fish survival is greater when individuals are released at a larger size (Stork and Newman 1988; Szendrey and Walh 1996; Sutton and Ney 2001). Alternatively, the larval Barred Galaxias survived translocation but, due to their small size and the large expanse of available habitat, the electrofishing technique applied was unsuccessful at detecting them. Small fish, including larvae and juveniles, are less vulnerable than larger fish to electrofishing techniques (Reynolds 1996). As such, electrofishing for larvae has generally been considered ineffective and is not commonly used (Copp 1989). Our data supports Reynolds (1996) theory, as most Barred Galaxias collected across all sampling occasions were larger fish (average lengths greater than 69 mm) and the smallest sized Barred Galaxias collected was 35 mm LCF, larger than the size of stocked individuals (11–18 mm).

This is also supported by results from annual monitoring, where individuals less than approximately 35 mm in length are not recorded in streams where populations successfully recruit yearly and smaller larvae must be present (T. Raadik unpublished data).

Changes in habitat conditions between sampling events also likely influenced electrofishing efficiency and the number of Barred Galaxias collected. For example, during the six month post-translocation monitoring in mid June we experienced increased flows and stream widths, as well as reduced light intensity, which made it difficult to sample all available habitats and to see into the water to collect stunned fish. Reduced numbers of Barred Galaxias were also collected at source sites during this sampling period.

Standard electrofishing units can be modified to target and increase capture of juvenile and larval fish (King and Crook 2002; Copp 2010). Such modifications may be warranted in future monitoring events to improve detection of larval Barred Galaxias.

Ongoing monitoring of the Taponga River and Shaw Creek translocated populations is required to assess population establishment and survival and longer term range expansion and genetic viability. If a smaller sized cohort (40–55 mm length) is detected during a monitoring event at one year post-translocation, this would indicate that the translocated larval Barred Galaxias have survived and are now at a size detectable via electrofishing. If a smaller cohort is also detected, or cohorts < 55 mm in length in subsequent years, this is evidence that adult Barred Galaxias are successfully reproducing and their offspring are surviving.

If there is no evidence of natural recruitment at translocated sites, possible causes may be that spawning habitat is insufficient, requiring evaluation and augmentation, or the adult translocated fish were distributed over too large a stream distance or have dispersed too widely, to locate each other during the breeding season. Population supplementation may therefore be required. Post-monitoring results indicated that smaller adults were more likely to migrate upstream, whilst larger adults were sedentary and remained in pools in which they were originally released. Ongoing monitoring would verify this trend of smaller sized individuals predominately driving upstream range expansion.

Assessing the genetic viability and effective population size of translocated Barred Galaxias will also be important to identify potential founder effects and inbreeding. Collection of tissue samples from each of the new populations five and 10 years post-translocation, should be analysed and the genetic diversity compared with that of the original founder populations. If diversity is found to be low population supplementation may be necessary to mitigate the loss of genetic diversity and boost population abundances. At minimum, ongoing monitoring would allow compilation of basic data on the translocated populations themselves, and increase our knowledge on translocation as a conservation tool (Minckley 1995). Ongoing monitoring of the translocated populations at Taponga River and Shaw Creek should be incorporated into the broader annual monitoring program of all known Barred Galaxias populations.

It is important to continue engaging and educating stakeholders on the significance and progress of the translocated populations and Barred Galaxias conservation in general. The local community, user groups (e.g. anglers, four wheel drivers and hikers), resource managers and other stakeholders should be informed about Barred Galaxias and how their actions may pose an impact. Regional staff need to be aware of the location of the new Barred Galaxias populations when considering management zoning and works. Fostering ownership for conserving Barred Galaxias will help to implement a comprehensive and integrated management approach to protect and recover populations.

Documenting this trial reintroduction of Barred Galaxias is also important as it will provide valuable insights when planning similar translocations. Documenting this trial translocation and the post-translocation monitoring events has permitted better evaluation of the outcomes of the reintroduction against the objectives, and identification of areas for improvement and future management.

The following actions are required to further evaluate the success of the trial translocation and to further improve Barred Galaxias conservation management:

- Continue monitoring the translocated Barred Galaxias populations to assess their long-term survival, establishment, range expansion and genetic viability. This data will inform whether future population supplementation or habitat modification is necessary to sustain the population or aid successful natural recruitment;
- Undertake genetic analysis of the translocated populations one, five and ten years post-establishment, with comparison with the genetic diversity of the founder individuals;
- Assess and select additional potential translocation sites for future use, particularly in larger headwater catchment areas. This may necessitate the construction of instream barriers and the removal of predators in upstream reaches; and,
- Continue engaging and educating stakeholders regarding the significance of Barred Galaxias conservation and their key threats, including potential impacts of human-assisted trout dispersal.

4 Understanding the genetics of Barred Galaxias populations

4.1 Introduction

Genetic diversity is one of three fundamental levels of biodiversity (genetic, species and ecosystem) (Frankham 1995). Threats to species that cause small, isolated and fragmented populations may also result in the loss of genetic variation and restrict gene flow between populations. Species with low genetic diversity have higher risk of extinction and reduced adaptability to environmental stochasticity and catastrophes (Frankham 1995). Conserving genetic diversity is therefore important to the overall resilience and health of populations and species.

Population genetics is the study of the genetic diversity, gene dispersal and genetic composition of biological populations. Genetic tools can be used to study genetic variation within and between populations to determine, for example, genetic diversity and differentiation, gene flow, population size, mating systems, and other factors that influence population fitness (Conner and Hartl 2004). Information gained from genetic research can assist identifying and prioritising actions needed to effectively manage, conserve and enhance populations and species.

We currently have no knowledge on the population genetics of Barred Galaxias, a species which has undergone significant population fragmentation and is now isolated to small headwater streams (Raadik *et al.* 2010). These populations are under risk of extinction from predators and stochastic events, and more recently from impacts from drought and bushfire. Post-fire management of Barred Galaxias included research on their reproduction and possible spawning habitat augmentation in impacted streams (Stoessel *et al.* 2012), temporary captive maintenance (Raadik *et al.* 2009), translocation programs (see Chapters 2 and 3), and trialling captive breeding (Stoessel *et al.* in prep). These projects highlighted the need to improve our understanding of the species' population genetics. Also, 'undertaking an assessment and monitoring of the population structure (genetic diversity) of Barred Galaxias throughout the range' is a recovery action listed in the National Recovery Plan for the Barred Galaxias (Raadik *et al.* 2010).

This chapter presents research on the genetics of Barred Galaxias populations. Their population genetic structure and diversity, and historical and contemporary patterns of gene flow, were investigated using mitochondrial and nuclear microsatellite markers.

The specific project objectives were to:

1. Collect fin-clip samples from all known populations.
2. Isolate and characterise nuclear microsatellites markers.
3. Characterise the genetic variability and differentiation of populations using nuclear microsatellites.
4. Characterise phylogenetic relationships among populations using a mitochondrial DNA marker.
5. Identify management units for conservation purposes.

Information included in this chapter is summarised from an unpublished confidential client report provided by **Cesar** Pty Ltd to DSE ARI. Research herein will be published in detail as articles in scientific journals because of their broader scientific and management applicability. Two manuscript papers are currently in preparation: Miller *et al.* (2012) and Ayres *et al.* (in prep).

4.2 Methods

Barred Galaxias were collected from 28 locations within their existing range via back pack electrofishing (Figures 18, 19 and 20). A small clip of the caudal fin was taken from up to 30 individuals per location, prior to release. Additional fin-clip and muscle tissue samples were taken from preserved Barred Galaxias specimens held at ARI. Tissue samples were used for genetic analysis.

Cesar, an environmental consultancy based in Melbourne, were contracted to develop nuclear microsatellite markers for Barred Galaxias and to conduct the genetic analysis. DNA was extracted from each fin clip sample using a Chelex® extraction method modified from Walsh *et al.* (1991). DNA extractions were stored at -20°C until required.

4.2.1 Microsatellite analysis

Microsatellite markers were characterised for Barred Galaxias using 454-sequencing by the Australian Genome Facility, Adelaide. **Cesar** identified and designed microsatellite markers using genetic computer programs and forty microsatellite markers were trialled in polymerase chain reactions (PCR). To investigate contemporary population genetic patterns, 568 Barred Galaxias samples were genotyped at 12 of these microsatellite loci and statistical measures of population structure, genetic diversity and gene flow were calculated using various computer programs, including FSTAT (Goudet 1995), Genepop (Raymond and Rousset 1995), and STRUCTURE (Pritchard *et al.* 2000).

4.2.2 Mitochondrial DNA analysis

To investigate the phylogeography of Barred Galaxias populations, a partial sequence of the mitochondrial cytochrome b gene (580 base pairs) was amplified in selected samples via PCR using the primers: CytB_GalFus_F 5' GAT GTC GTT TTGAGG GGC TA 3' and CytB_GalFus_R 5' ATC GGC TAC CAA AGC TCA GA 3'. PCR products were sequenced in forward and reverse directions and sequences were aligned, edited and analysed using various computer programs, including Sequencher (Genecodes), MEGA (Tamura *et al.* 2007) and PAUP (Swofford 2002).

Figure 18. The 28 locations in the Goulburn River basin from which Barred Galaxias samples were collected for genetic analysis. Location codes are shown in Table 7. Locations with the same symbols represent population clusters according to STRUCTURE analysis.

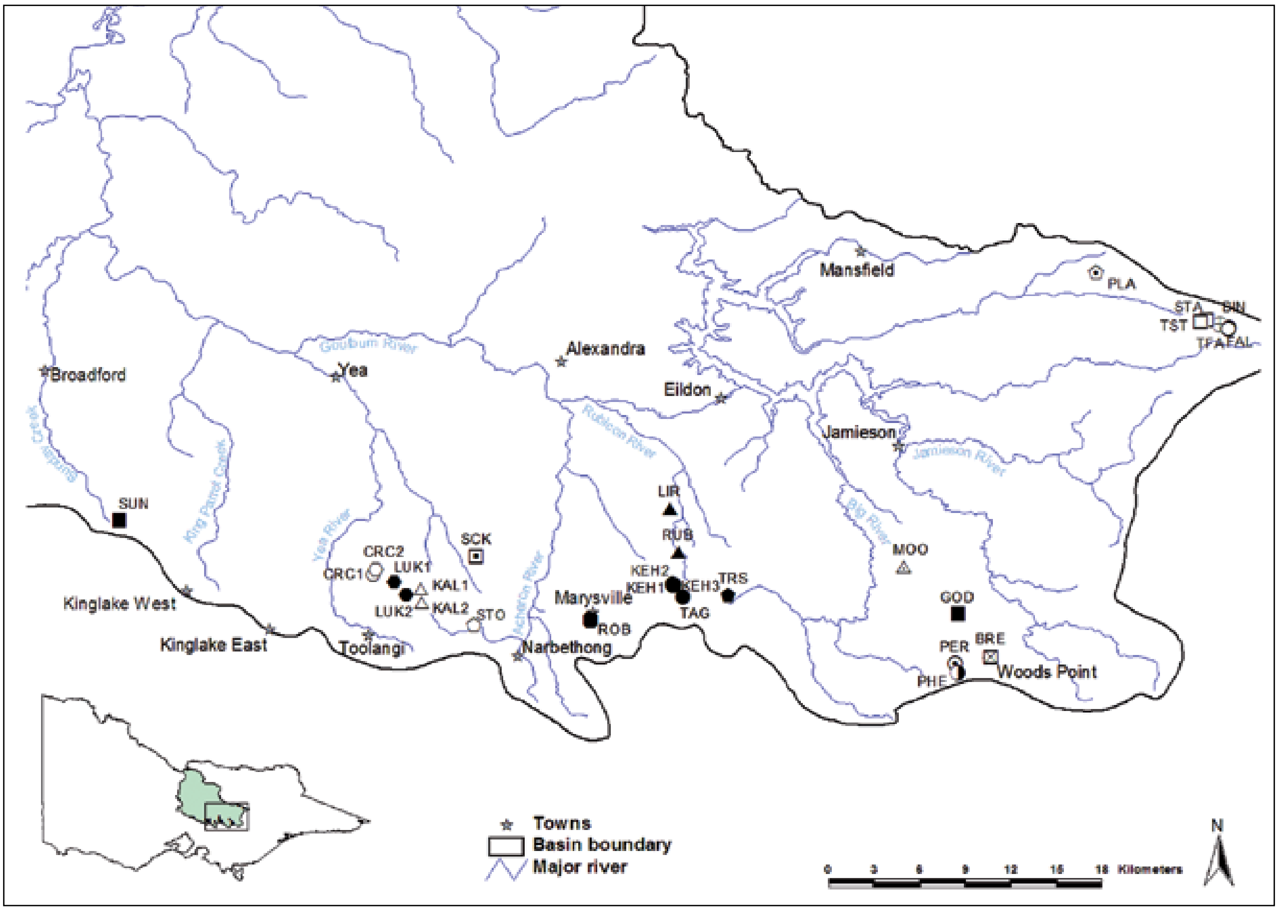


Figure 19. Examples of locations where Barred Galaxias were collected: Taggerty River (left); Rubicon River (right). (Images: Peter Fairbrother).

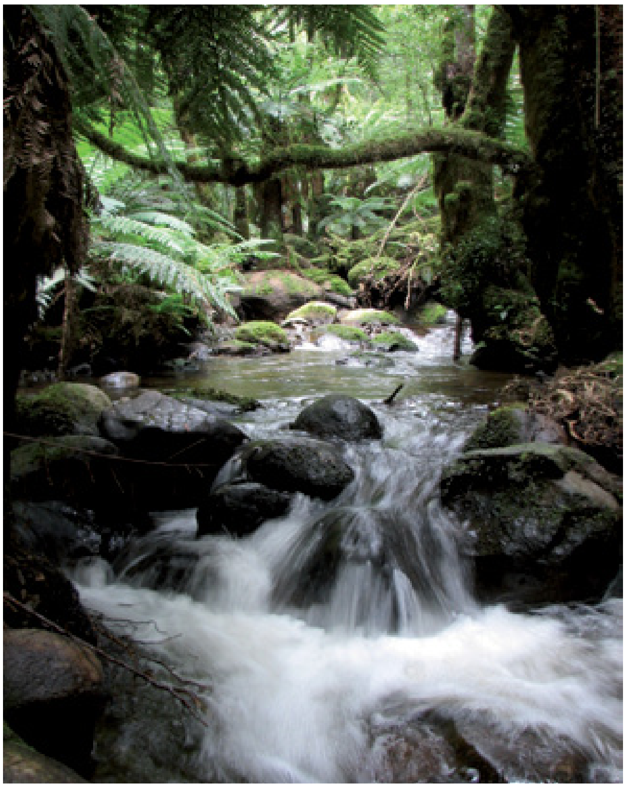


Figure 20. Electrofishing for Barred Galaxias in Robertsons Gully near Marysville. (Image: Simon Nicol).

4.3 Results

In total, 774 Barred Galaxias tissue samples were taken from wild caught individuals or preserved specimens.

4.3.1 Microsatellite analysis

Forty microsatellite markers were identified and trialled. Of these, 24 were found to be polymorphic and 12 were chosen to assess population genetics (Table 6). Samples not consumed in the genetic analysis are being stored in freezers at ARI.

Table 6. The 12 microsatellite markers used in population genetic assessments of Barred Galaxias.

Locus	Repeat motif	Number of alleles	Size range (base pair)
GF7	$(GA)^9$	4	178–184
GF8	$(AC)^9$	9	78–96
GF13	$(CAT)^{10}$	7	127–145
GF15	$(CA)^{10}$	4	87–99
GF18	$(CA)^{14}$	7	142–160
GF23	$(AC)^{11}$	5	81–89
GF25	$(GT)^9$	7	101–114
GF28	$(GT)^{13}$	5	164–172
GF32	$(AC)^{10}$	2	126–128
GF33	$(AC)^{13}$	5	110–118
GF38	$(AAT)^{14}$	7	131–152
GF40	$(TG)^{13}$	5	201–216

Genetic statistics for each location analysed, including location code and number of individuals sampled, is provided in Table 7. A total of 67 alleles were observed for 12 microsatellite loci in 568 Barred Galaxias individuals from 28 locales. Allelic richness averaged over loci was low in all locales (range = 1.000–1.865, average = 1.443) (Table 7). Observed and expected heterozygosities (not shown) ranged between 0 to 0.425 and 0 to 0.421 respectively in all locales. The microsatellite loci GF8 had the highest number of alleles, followed by GF13, GF18, GF25 and GF38 (Table 6). Significant departures from Hardy-Weinberg equilibrium (HWE) over all loci were found in two of the 28 locales (TRS and SUN) after corrections for multiple comparisons (Table 7). The small sample size from TRS may have influenced this result. The mitochondrial DNA results suggest individuals from SUN are Mountain Galaxias (*Galaxias olidus*), but SUN has strong overlap of microsatellite alleles with other Barred Galaxias populations, suggesting that individuals from this location may be Barred Galaxias with introgressed Mountain Galaxias mtDNA from a previous hybridisation event.

Pairwise F_{ST} estimates (not shown) indicated significant differentiation between populations in most cases with only 6 of the 378 pairwise comparisons (CRC 1 and 2; KEH 2 and 3; LUK 1 and 2; KAL 1 and 2; STA and TST; FAL and TFA) not significantly different after correction for multiple comparisons, indicating that gene flow occurs only between connected waterways within close distances.

An analysis of molecular variance showed significant genetic differentiation between river systems, between sample locales within river systems and within locales. The majority of the variance was found between sample locales within river systems (50%), with more variation within locales (26 %) than between river systems (24%).

GENELAND and STRUCTURE analyses each indicated that the number of populations within our data set was 17. Figure 18 shows these population clusters, locations with the same symbol depict a population. These population clusters are mostly consistent with pairwise F_{ST} estimates (not shown), with the following pairs CRC 1 and 2, LUK 1 and 2, KAL 1 and 2, STA and TST, and FAL and TFA, representing separate but nearby samples from the same stream, clustering as single populations.

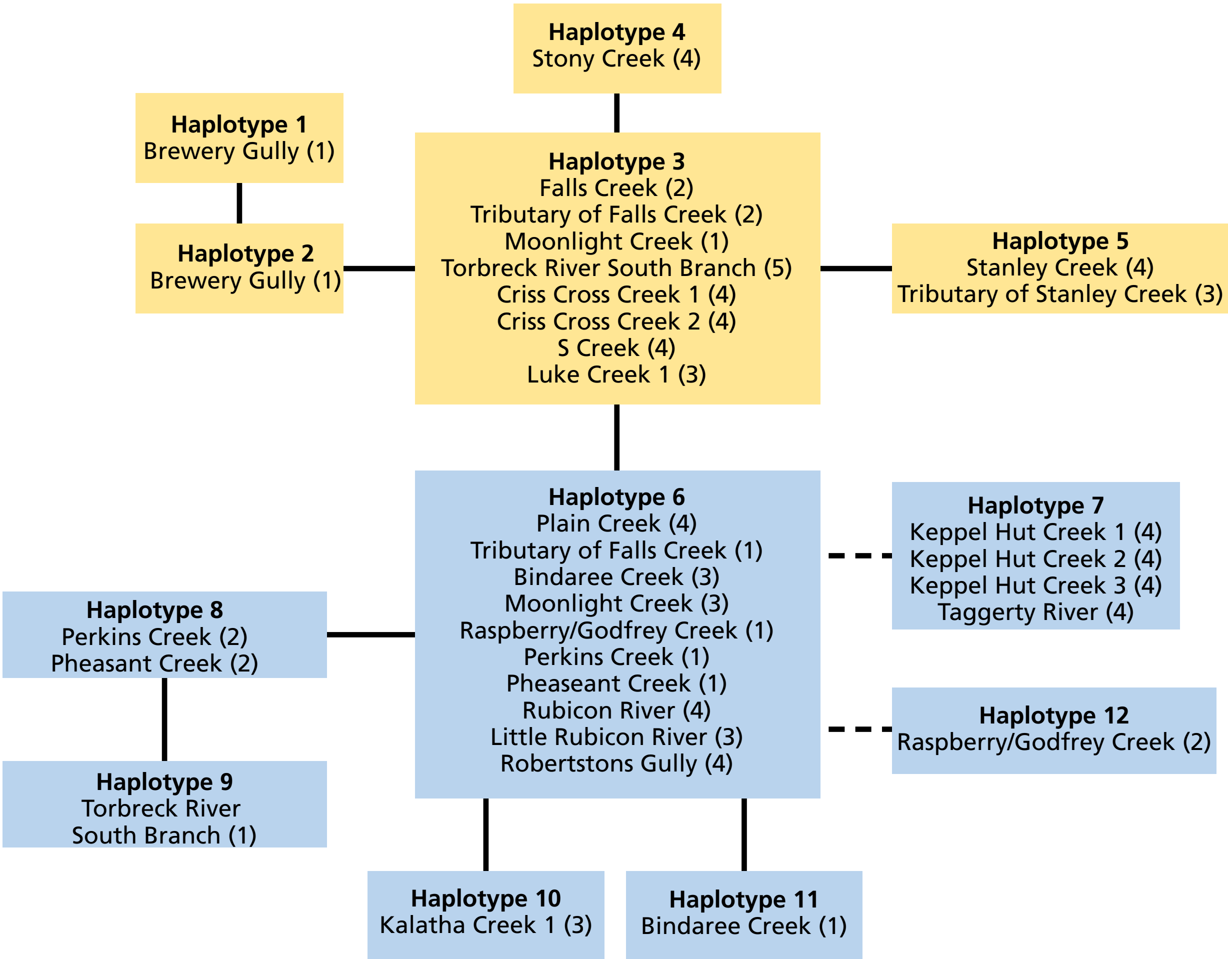
4.3.2 Mitochondrial DNA analysis

A 590 base pair region of the mitochondrial cytochrome *b* gene was successfully amplified in 94 Barred Galaxias individuals from 26 locations, of which a 513 base pair region was used in analysis. Twelve haplotypes, that reflect different ancestral origins, were identified and were distributed broadly throughout the range of Barred Galaxias (Table 7 and Figure 21). SUN individuals closely aligned with the outgroup species Mountain Galaxias, suggesting introgressed hybrids (see above). Most individuals belonged to two common haplotypes, haplotypes 3 and 6 (Table 7 and Figure 21) and analysis grouped haplotypes into two major evolutionary clades (Figure 21). Some locales have multiple haplotypes present, e.g. BIN, PER, GOD, whilst others only had one (Table 7 and Figure 21) and MOO, TRS and TFA had multiple haplotypes representing both clades (Table 7 and Figure 21). There were relatively weak phylogeographic patterns, with individuals from the Howqua, Upper Goulburn, Torbreck and Yea River systems spread across both clades, whilst fish from the Acheron River systems were exclusive to clade 1 and those from the Rubicon, Taggerty and Delatite River system exclusive to clade 2 (Table 7).

Table 7. Genetic statistics for Barred Galaxias assessed per location. *N* denotes number of samples screened using 12 microsatellite markers. Mean values over all microsatellite loci are shown for allelic richness (*r*) and Hardy-Weinberg equilibrium (HWE) *P* value. Mitochondrial DNA (mtDNA) haplotypes identified at each site are shown, including the number (*n*) of samples in parenthesis.

River/Creek system Location	Code	*N*	*r*	HWE *P* value	mtDNA haplotype (*n*)
Delatite River System					
Plain Creek	PLA	30	1.707	0.0662	6 (4)
Howqua River System					
Stanley Creek	STA	29	1.363	0.7520	5 (4)
Tributary of Stanley Creek	TST	15	1.338	0.6470	5 (3)
Falls Creeks	FAL	14	1.802	0.9211	3 (2)
Tributary of Falls Creek	TFA	30	1.771	0.6700	3 (2), 6 (1)
Bindaree Creek	BIN	18	1.071	1.000	6 (3), 11 (1)
Upper Goulburn River System					
Moonlight Creek	MOO	14	1.498	0.9137	3 (1), 6 (3)
Raspberry/Godfrey Creeks	GOD	18	1.636	0.0777	6 (1), 12 (2)
Perkins Creek	PER	30	1.581	0.7391	6 (1), 8 (2)
Pheasant Creek	PHE	30	1.430	0.6038	6 (1), 8 (2)
Brewery Gully	BRE	30	1.825	0.8853	1 (1), 2 (2)
Torbreck River System					
Torbreck River South Brch	TRS	6	1.381	<0.001	3 (5), 9 (1)
Rubicon River System					
Rubicon River	RUB	30	1.189	0.9928	6 (4)
Little Rubicon River	LIR	15	1.209	0.8942	6 (3)
Taggerty River System					
Taggerty River	TAG	30	1.000	NA	7 (4)
Keppel Hut Creek 1	KEH1	4	1.081	NA	7 (4)
Keppel Hut Creek 2	KEH2	10	1.066	NA	7 (4)
Keppel Hut Creek 3	KEH3	16	1.071	NA	7 (4)
Robertsons Gully	ROB	30	1.865	0.7207	6 (4)
Acheron River System					
Stony Creek	STO	13	1.589	0.6195	4 (4)
Yea River System					
Criss Cross Creek 1	CRC1	10	1.536	0.9360	3 (4)
Criss Cross Creek 2	CRC2	20	1.709	0.0698	3 (4)
Kalatha Creek 1	KAL1	30	1.660	0.7222	10 (3)
Kalatha Creek 2	KAL2	18	1.674	0.7584	NA
Luke Creek 1	LUK1	30	1.411	0.5860	3 (3)
Luke Creek 2	LUK2	10	1.314	1.0000	NA
S Creek	SCK	26	1.184	0.9795	3 (4)
Sunday Creek System					
Sunday Creek	SUN	12	1.455	<0.0001	Mountain Galaxias (3)

Figure 21. Relationships of the 12 mitochondrial DNA haplotypes for Barred Galaxias. For each haplotype, the number of samples per location is shown in parenthesis. A solid line represents a single DNA base pair change between adjoining haplotypes, whilst a dashed line represents a two DNA base pair change between adjoining haplotypes. Yellow or blue coloured boxes represent haplotypes belonging to clade 1 and clade 2 respectively. Sunday Creek (SUN) not shown.



4.4 Discussion and recommendations

Twelve polymorphic microsatellite loci were isolated from Barred Galaxias specimens and used to assess the genetic structure of known populations across the species' geographic range in central Victoria. Microsatellite data showed strong genetic structuring and differentiation between Barred Galaxias populations, with 17 populations identified throughout the sampling area. Gene flow within the species' distribution was highly constrained and only occurred between locales in close proximity and often within the same creek, e.g. CRC 1 and 2, RUB and LIR. This indicates that long distance movement of Barred Galaxias within and between river systems is limited. The movement and dispersal patterns of adult and juvenile fish, including their climbing ability, are unknown (Raadik *et al.* 2010). The dispersal and gene flow of Barred Galaxias may be hindered by connectivity, such as the presence of barriers, or trout competition and predation. Alternatively, Barred Galaxias may generally be poor dispersers and largely sedentary, and hence the gene flow potential over large distances is unlikely.

Despite the high number of alleles detected at each loci, the genetic variation of Barred Galaxias within locales was extremely low, as indicated by measures of allelic richness and heterozygosity. At some locales there was almost no genetic difference between individuals e.g. TAG and KEH 1, 2, and 3, whilst most other locales displayed low genetic variation between individuals, e.g. BIN and SCK. Reductions in genetic diversity within populations have likely resulted from levels of breeding between closely related individuals greater than what is expected by chance, termed inbreeding, and random genetic drift. Extreme decreases in population size, called a bottleneck, increase the likelihood of genetic drift. We know that population sizes of Barred Galaxias have declined rapidly in the past due to fire, drought and trout impacts, and it is anticipated that effected populations have lost genetic diversity whilst enduring these impacts. Low genetic diversity is a major concern because genetic variation is vital for population persistence and resilience (Weeks *et al.* 2011), though lower levels than found in fish populations further downstream in the catchment may be normal for some headwater species.

Considering the mitochondrial and microsatellite data, patterns of the genetic structure of Barred Galaxias populations may be explained by their historical and contemporary connectivity. Historically, Barred Galaxias populations are likely to have established by a few individuals dispersing from a larger genetically diverse source population and colonising a new habitat. Flooding events and climatic periods may have facilitated the dispersal of founding individuals over large distances. This would explain the relatively weak phylogeographic patterns observed in the mitochondrial data as there is no clear relationship between haplotypes and river systems. Barred Galaxias populations have since suffered dramatic declines in range and abundance, becoming isolated and fragmented (Raadik *et al.* 2010). The remaining isolated populations are likely remnants of larger historical populations and have diverged over time due to founder events, inbreeding and genetic drift occurring as a result of imposing threats. Populations therefore share common ancestral origins, but are vastly different in respect to their contemporary genetic patterns.

Raadik *et al.* (2010) noted several Barred Galaxias populations that are known to have become extinct. Several localised extant Barred Galaxias populations face the same fate unless their population sizes and genetic variability increase. Management intervention is needed improve the genetic variability of Barred Galaxias populations because they are isolated and gene flow is restricted. Translocations are an effective tool for the restoration of isolated populations (Bouzat *et al.* 2009; Weeks *et al.* 2011) and are recommended as a recovery action for Barred Galaxias (Raadik *et al.* 2010). Ayres *et al.* (2011) presented guidelines for the translocation of Barred Galaxias for conservation purposes and the decision support process defined in Weeks *et al.* (2011) should also be considered when undertaking genetic translocations and identifying the associated risks.

Considering the current genetic data, known Barred Galaxias populations should be considered as a group of populations with some degree of gene flow when devising translocation strategies. Translocations may occur by transferring individuals between existing populations or creating new populations within the former range using founder individuals from selected extant populations (see Chapter 3). Population mixing should initially be confined to populations within the same river system, but more widespread mixing should be considered following Weeks *et al.* (2011). Some suggested translocations for initial consideration include genetic augmentation of S Creek with fish from Luke and Criss Cross creeks, Bindaree Creek with fish from Plain Creek, Keppel Hut Creek and Taggerty River with fish from Rubicon River, and Stony Creek with fish from Luke and Criss Cross creeks. Establishing a new population of Barred Galaxias using founder individuals from most extant populations could also be contemplated, possibly into the previously identified translocation site on Koala Creek (see Chapter 2).

Captive breeding of Barred Galaxias may also be warranted to boost population sizes or provide animals for translocation, particularly when extant populations are too small to supply founder individuals (Rakes *et al.* 1999). Captive breeding should consider the genetics of broodstock and their offspring, as well as the genetics of populations receiving the captive bred individuals and populations nearby. Undertaking translocations of Barred Galaxias, however, is more preferable compared to their captive breeding because of the unavoidable adverse effects of captive environments (Philippart 1995; Synder *et al.* 1996). Ongoing monitoring of populations that supply individuals for translocation or captive breeding, or receive translocated or captive bred individuals, is needed to measure the success of translocation or captive breeding programs and to identify future threats. Ongoing monitoring should also include genetic and phenotypic measures.

The following actions are required to improve the genetic variability and resilience of Barred Galaxias populations and conserve the genetic integrity of the species:

- Translocate Barred Galaxias by mixing existing populations and creating new populations within their former range. Population mixing should initially be confined to populations within the same river system (see above), but more widespread mixing should be considered, such as creating a new population using a few founder individuals from various existing populations (i.e. in Koala Creek);
- Undertake captive breeding of Barred Galaxias to supplement natural populations when required. The captive breeding programs should be targeted and strategic with the aim of boosting population sizes and enhancing population genetic diversity, whilst avoiding 'swamping' populations with particular genotypes;
- Undertake ongoing monitoring of all Barred Galaxias populations, particularly those with small population sizes, those where translocations have occurred, those containing captive bred individuals, and those under direct threat e.g. from drought and fire impacts. Genetic and phenotypic conditions should be monitored; and,
- Mitigate threats to Barred Galaxias populations. While actions can be undertaken to help recover populations, the key threats to Barred Galaxias, e.g. trout predation, sedimentation, dewatering (Raadik *et al.* 2010), etc. need to be addressed and reversed where possible, for effective and long-term species conservation.

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Appendix 1

Environmental variables recorded at sampling sites. Brch – branch; DO – dissolved oxygen; EC– electrical conductivity at 25 °C.

Site no.	Waterbody	Dry	EC (µS/cm)	Water temperature (°C)	DO (mg/L)	DO (% Saturation)	pH	Turbidity (NTU)
FT001	Koala Creek	No	–	–	–	–	–	–
FT002	Torbreck River South Brch, tributary	No	–	–	–	–	–	–
FT003	Torbreck River South Brch, tributary	No	–	–	–	–	–	–
FT004	Torbreck River South Brch, tributary	No	–	–	–	–	–	–
FT005	Koala Creek	No	–	–	–	–	–	–
FT006	Deep Creek	No	–	–	–	–	–	–
FT007	Torbreck River North Brch, tributary	No	–	–	–	–	–	–
FT008	Torbreck River North Brch, tributary	No	–	–	–	–	–	–
FT009	Torbreck River North Brch, tributary	No	–	–	–	–	–	–
FT010	Second Creek	No	–	–	–	–	–	–
FT011	Torbreck River South Brch	No	–	–	–	–	–	–
FT012	Torbreck River South Brch, tributary	No	–	–	–	–	–	–
FT013	Comet Creek	No	111.0	7.3	8.5	80.0	7.2	1.0
FT014	Westcott Creek	No	72.4	6.5	11.6	95.0	5.8	1.0
FT015	Number One Creek	No	75.0	7.9	9.9	83.0	6.0	5.3
FT016	Number Three Creek	Yes	–	–	–	–	–	–
FT017	Number One Creek	No	70.0	8.9	10.8	93.0	5.8	10.0
FT018	Sylvia Creek North Brch	No	35.0	10.4	8.5	75.0	6.0	1.0
FT019	Sylvia Creek North Brch	No	–	–	–	–	–	–
FT020	Sylvia Creek North Brch	No	–	–	–	–	–	–
FT021	Sylvia Creek South Brch	No	27.8	6.2	13.1	105.6	6.9	10.6
FT022	Dungaree Creek	No	15.9	4.1	14.2	113.0	6.9	2.8
FT023	Oaks Creek	No	13.2	6.8	10.1	79.0	5.6	5.0
FT024	Shaw Creek	No	17.9	7.0	11.8	97.6	7.0	0.8
FT025	Spring Creek	No	10.6	8.1	10.0	84.6	6.7	0.0
FT026	Boundary Creek	No	29.3	9.2	10.7	94.3	7.1	1.6
FT027	Shaw Creek	No	–	–	–	–	–	–
FT028	Big River, tributary	No	–	–	–	–	–	–
FT029	Big River, tributary	No	–	–	–	–	–	–

Site no.	Waterbody	Dry	EC (µS/cm)	Water temperature (°C)	DO (mg/L)	DO (% Saturation)	pH	Turbidity (NTU)
FT030	Big River, tributary	No	–	–	–	–	–	–
FT031	Big River, tributary	No	–	–	–	–	–	–
FT032	Big River, tributary	No	–	–	–	–	–	–
FT033	Taponga River	No	–	–	–	–	–	–
FT034	Wild Dog Creek	No	–	–	–	–	–	–
FT035	Wild Dog Creek	No	–	–	–	–	–	5.0
FT036	Taponga River, tributary	No	–	–	–	–	–	–
FT037	Big River, tributary	No	–	–	–	–	–	–
FT038	Taponga River	No	–	–	–	–	–	–
FT039	Taponga River, tributary	No	–	–	–	–	–	–
FT040	Taponga River, tributary	No	20.3	9.7	6.0	–	3.9	1.4
FT041	Dudley Creek	No	22.8	8.2	9.5	–	6.5	2.5
FT042	Shaw Creek	No	–	–	–	–	–	–
FT043	Shaw Creek	No	–	–	–	–	–	–
FT044	Fryer Creek	No	18.0	10.5	10.8	96.0	6.5	0.3
FT045	Moonlight Creek, tributary	No	17.4	10.2	11.7	105.4	6.5	0.3
FT046	Big River, tributary	No	30.9	10.4	11.4	102.0	7.2	5.2
FT047	Ryan Creek, tributary	Yes	–	–	–	–	–	–
FT048	Upper Goulburn River	No	15.0	–	–	–	–	–
FT049	Frenchmans Creek, tributary	Yes	–	–	–	–	–	–
FT050	Wombat Creek	No	10.5	10.4	11.0	99.0	6.7	5.0
FT051	Twenty Five Mile Creek, tributary	No	–	–	–	–	–	–
FT052	Moonlight Creek	No	19.5	12.0	11.1	103.2	7.0	0.9
FT053	Upper Goulburn River	No	–	–	–	–	–	–
FT054	25 Mile Creek	No	–	–	–	–	–	–
FT055	Big River, tributary	No	–	–	–	–	–	–
FT056	Big River, tributary	No	–	–	–	–	–	–
FT057	Dear Hound Creek	No	–	–	–	–	–	–
FT058	Ryan Creek	No	–	–	–	–	–	–
FT059	Little Creek	No	–	–	–	–	–	–
FT060	Tom Burns Creek	No	11.0	14.1	9.2	90.0	6.8	2.2
FT061	Torbreck River South Brch, tributary	No	11.6	11.5	9.7	99.0	6.7	3.0

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