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Improved fish passage along the Nepean River as a result of retrofitting weirs with vertical-slot fishways

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Non-technical summary

Improved fish passage along the Nepean River as a result of retrofitting weirs with vertical-slot fishways

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Objectives

- To compare fish passage in the Nepean River before and after construction of new fishways.
- To determine if the new fishways are complying to design specification.
- To ascertain whether the environmental flows program enhances fish movement through the fishways.
- To determine whether the fishway construction program has increased gene-flow in the river.

Key words

Nepean River, fishways, fish migration, PIT tagging, fishway trapping, electrofishing.

Summary

The Hawkesbury-Nepean (H-N) river system is a highly regulated coastal system in New South Wales. The construction of at least 81 dams and weirs has obstructed fish passage to approximately half of the H-N system. Migratory species including sea mullet, freshwater mullet and freshwater herring were particularly affected and rarely found upstream of the most downstream barriers in the system. Restricted migration may also impact upon genetic diversity and genetic structure of fish populations along the river.

To address these concerns, the Sydney Catchment Authority (SCA) retrofitted ten new fishways to weirs along the Nepean River from Penrith to Douglas Park, and implemented an environmental flow regime. The fishways are designed to pass fish from 35 mm to 1 m in length and to operate over a wide range of flows. The current project uses a multiple lines of evidence approach to assess the effectiveness of the new fishways at promoting upstream fish passage.

Fish community sampling

Fish community sampling was undertaken at 20 long-term monitoring sites before and after fishway construction to determine changes in fish community structure. Three rounds of sampling were conducted before fishway construction and eight rounds after. Results indicated a dramatic improvement in the upstream distribution of freshwater mullet, sea mullet and freshwater herring. Fishways improved the upstream migration of juvenile Australian bass that

were previously limited by Theresa Park Weir. Several species did not show a marked improvement in distribution, including empire gudgeon and striped gudgeon. It may take more time for these species to expand their distributions.

Fishway trapping

Three of the fishways (Penrith, Theresa Park and Douglas Park) were selected for an intensive assessment to determine if they were operating to design specifications. Fishway trapping was used to evaluate the fish species attempting to migrate through the fishway (entrance trapping) and those that successfully passed (exit trapping). Sampling occurred during spring and summer, beginning in February 2010 and ending in February 2013. Trapping was conducted over a 48 hour period with traps set for 24 hours in each of the entrance and exit. A total of 27 paired entrance and exit samples were collected at Penrith and Theresa Park and 24 paired samples at Douglas Park. Ninteen species were trapped in the fishways and the size of fish in the exit ranged from 20 mm to 1.2 m in length. Data analyses indicated the species composition and sizes of fish in the entrance and exit were similar, suggesting most species and size ranges were successfully passing through the fishways. However, some small-bodied species such as flat-headed gudgeon, dwarf flat-headed gudgeon and firetail gudgeon were not as abundant in the fishway exit.

Fish movement

Passive Integrated Transponder (PIT) technology was used to determine the timing of fish movements and correlate movement with season, time of day and flow. Five fishways were fitted with PIT reader systems; Wallacia, Theresa Park, Cobbitty, Camden and Menangle). A total of 3,798 fish from 14 species were fitted with PIT tags. Unfortunately, due to flooding and power failure, the PIT reader systems were not fully functional throughout the study period. Nevertheless, the data did allow a basic assessment of movement through some of the fishways for Australian bass, the most commonly tagged species. At Camden, 75% of Australian bass entering the fishway successfully progressed to the exit. Recapture of tagged fish during electrofishing and fishway trapping revealed that 94% of Australian bass were recaptured at their original site of tagging, months or years after their first capture. This suggests this species has strong site fidelity and returns to a defined home range following its spawning migration to the estuary.

Genetic analysis

To determine if weirs were fragmenting native fish populations, Australian smelt were collected from sites between each fishway for genetic diversity and population structure assessment. This species was selected because it is common and easy to collect. Results demonstrated that the Australian smelt population below Wallacia Weir was significantly different to populations above the weir. Following fishway installation, genetic analysis suggested upstream gene flow past Wallacia Weir. Pre-fishway data indicated a second barrier existed at Theresa Park Weir, though no improvement to gene flow was detected at this location. A possible explanation for the lack of improvement in gene flow despite trapping data showing that this species successfully uses the Theresa Park fishway is that fragmentation is possibly the result of a natural barrier to gene flow downstream of Theresa Park Weir. There is a large rocky gorge between Theresa Park and Wallacia and it is possible that this may prevent upstream movement and thus restrict upstream gene flow of Australian smelt. Further genetic analysis would assist in answering this question.

Overall, despite the fishways having only been operational for a short time, they have successfully promoted upstream migration of the native fish assemblage in the Nepean River. A wide range of species and size classes are attempting to use the fishways and most are successfully reaching the exit. A few species responded quickly and dramatically. In particular, freshwater mullet, sea mullet and freshwater herring successfully migrated throughout most of the study reach when they were previously restricted to the most downstream sites. While not all species responded to fishway installation as rapidly, the fishways have only been operational for a short time and improvements to the fish community composition are expected to be cumulative over the long-term. The current fish community in the Nepean River is still depauperate compared to the community that was present prior to river regulation. Nevertheless, the substantial response of the fish community in just a few years since the fishways were installed is encouraging and suggests that fish community structure will continue to improve over time.

Introduction

Background

The Hawkesbury-Nepean (H-N) system is the second largest coastal river system in New South Wales (Gehrke & Harris 1996a). Five major dams in its upper reaches collectively store 95% of Sydney's drinking water. The H-N system encompasses a diverse range of fish habitats including rivers, wetlands, lakes and an estuary (Gehrke & Harris 1996a). As a result of this habitat diversity, the H-N system supports a wide range of fish species with a variety of migration strategies.

The H-N system has undergone dramatic changes since the late 19th century when the first of at least 81 dams and weirs were constructed to provide a supply of water for agriculture, industry, urban consumption and flood mitigation (Marsden & Gehrke 1996). There are five storages (Avon, Cordeaux, Nepean, Cataract and Warragamba Dams) operated to regulate the flows within the system (Figure 1; Table 1). In addition, the H-N River, the main river channel within the drainage is further regulated by 15 weirs between Penrith and Pheasants Nest. However, Thurns Weir has been bypassed and Bergins Weir has collapsed and therefore neither structure serves to regulate flows. Broughtons Pass Weir is located on the Cataract River downstream of Cataract Dam and receives additional water from Pheasants Nest Weir via a water transfer tunnel (Figure 1; Table 1).

Migration strategies in the Hawkesbury-Nepean River

The project is focused on reinstating fish migration and fish passage, hence it is important to understand the different migration strategies of fish, as these influence the evaluation of the effectiveness of new fishways.

Migration of fish in rivers is categorised by their movements between freshwater and the sea and the function of that movement. These categories comprise:

***Diadromous* (migrate between freshwater and the sea)**

Anadromous Migrate upstream from the sea to freshwater to spawn.

Catadromous Migrate downstream to the sea or estuary to spawn. After spawning, adult and juvenile fish migrate upstream to return to freshwater.

Amphidromous Spawning occurs in freshwater with larvae drifting to the estuary, juveniles then migrate upstream (McDowall 2007).

***Potamodromous* (migrate wholly within freshwater)**

Potamodromous species migrate within freshwater for spawning, dispersal, feeding and to avoid unfavourable environmental conditions (e.g. droughts). These species often have drifting larvae and migrate upstream to counter downstream displacement in the early life stages.

The spatial scale of potamodromy influences the impact of dams and weirs. If life cycles can be completed within kilometres, then weirs may influence gene flow but can often allow life cycles to be completed. If, however, the spatial scale is over 10s or 100s of kilometres, then weirs can have a major impact on life cycles.

Displacement of adults and juveniles can occur during high flows and upstream migration often occurs to counter this displacement. The extent of connectivity (i.e. fish passage) can structure the fish community following these events.

There are 24 native, three translocated (native Australian fish but not to the H-N system) and four alien freshwater fish species recorded in the lowland freshwater reaches of the H-N system (Table 2). Nineteen of the 24 native species are diadromous. The remaining species are potamodromous, but do not undertake migrations over large spatial scales. For the present study, the diadromous species, which often have annual migrations, are likely to provide a short term response to the installation of new fishways, while the potamodromous species are likely to have a longer-term response as populations redistribute.

The impacts of weirs and dams on the fish of the Hawkesbury-Nepean system

The construction of dams and weirs has obstructed fish passage to approximately half of the H-N system (Marsden & Gehrke 1996). The historical distribution of many fish species in the system is not accurately known given that many of the weirs and dams have been in place for nearly a century. A recent study utilised species distributional modelling to predict the historical distribution of a range of fish species in the Hawkesbury-Nepean system and found that many fish species currently occurring somewhere in the system would have originally occupied all reaches below Maldon Weir (Table 2) (Growns *et al.* 2013). This is in stark contrast to the contemporary distribution (1975-2009; before completion of the new fishways) as determined from NSW Fisheries data (unpublished data). Several studies have confirmed that the weirs and dams in the system have had a major effect on the distribution and abundance of native fish, macro-invertebrate taxa and mussels (Baumgartner & Reynoldson 2007; Brainwood *et al.* 2008; Gehrke *et al.* 1999; Gehrke *et al.* 1996; Growns & Growns 2001; Harris *et al.* 1996). Fish species such as common jollytail (*Galaxias maculatus*), sea mullet (*Mugil cephalus*), freshwater mullet (*Trachystoma petardi*) and freshwater herring (*Potamalosa richmondia*) have greatly reduced abundance and distribution, while the endangered Australian grayling (*Prototroctes maraena*) have rarely been recorded (Baumgartner & Reynoldson 2007; Gehrke *et al.* 1999; Gehrke 1996a; Growns *et al.* 2013).

While reduced fish passage is an obvious consequence of barriers in the H-N system, it is also possible that the barriers are having an impact on the genetic diversity and structure of populations. Recent genetic studies have demonstrated that even relatively low-level barriers can genetically fragment fish populations by disrupting upstream migrations (Blanchet *et al.* 2010; Hänfling & Weetman 2006; McCraney *et al.* 2010; Meldgaard *et al.* 2003). The major dams in the H-N system are an impassable barrier to most fish species, with the possible exceptions of the two freshwater eel species (*Anguilla spp*), Cox's gudgeon (*Gobiomorphus coxii*), climbing galaxias (*Galaxias brevipinnis*) and short headed lamprey (*Mordacia mordax*) given their ability to climb (Bishop & Bell 1978; Gehrke *et al.* 2002; Pusey *et al.* 2004). In contrast, modelling of the weirs from Douglas Park causeway downstream to Theresa Park Weir suggests they are submerged (by high flows) approximately every one or two years (Mallen-Cooper 2009) and the Penrith Weir drowns out regularly, potentially allowing fish passage (Table 1). However, the ecology of migration remains poorly understood – some species may only migrate on the recession of flows or during low flows (David & Closs 2002). Thus, it is possible that the weirs on the Nepean River may be genetically fragmenting populations of some fish species. Genetic fragmentation of fish populations can have a range of consequences including modifying the original genetic structure, reduced genetic diversity and in severe cases, local extinctions (Meldgaard *et al.* 2003; Morita & Yamamoto 2002; Wofford *et al.* 2005).

Figure 1 The location of weirs and dams (crosses) and electrofishing and tagging sites (red dots). Fishway trapping was conducted at Penrith Weir, Theresa Park Weir and Douglas Park Causeway.

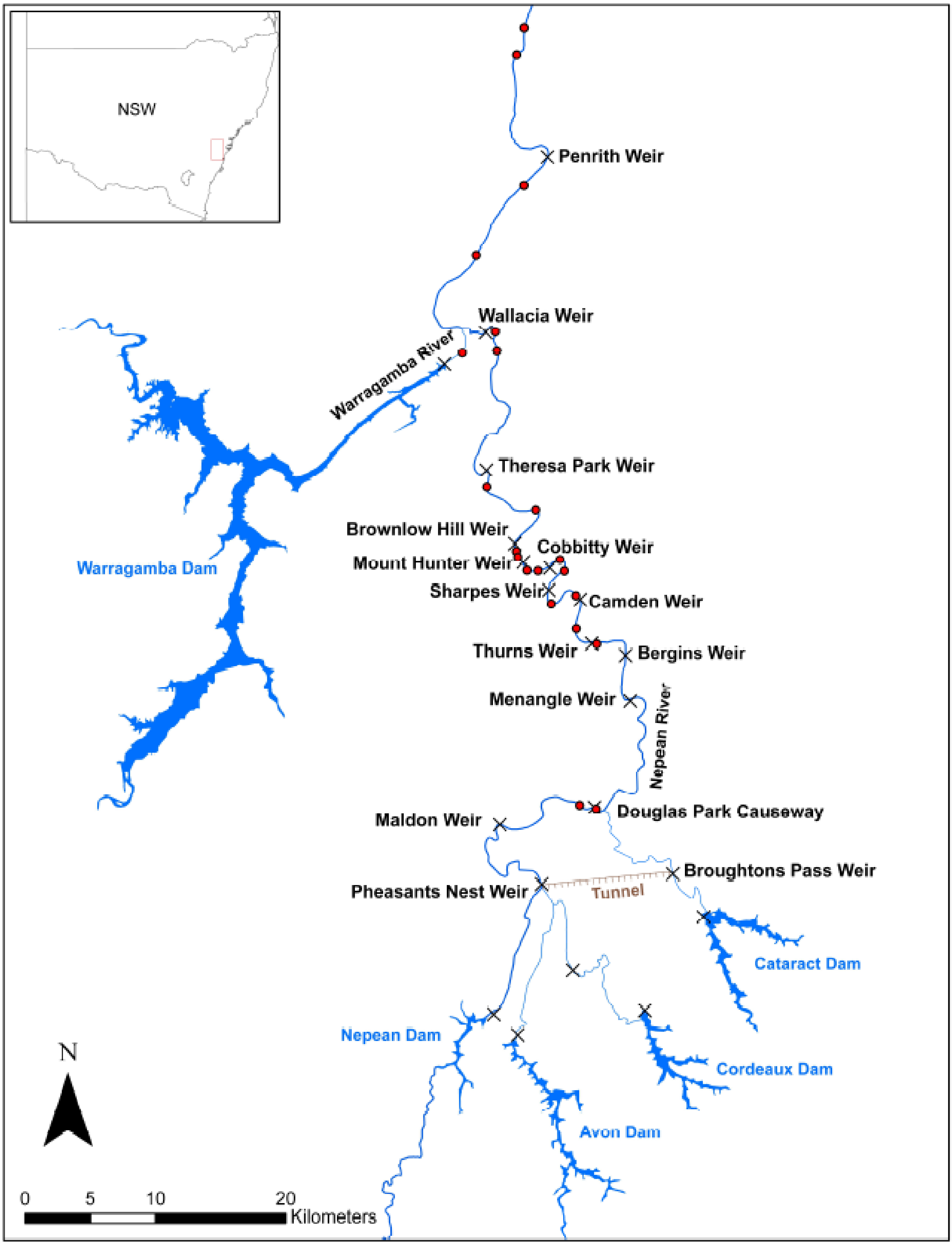


Table 1 Year of construction of weirs, dams and fishways on the Hawkesbury-Nepean system. Refer to Figure 1 for structure locations.

Structure	Year of construction	Relative Level[#] (m)	Height of structure (m)	Drown-out frequency (annual recurrence interval)	Any prior fishway and year of construction	New fishway type and monitoring type
Penrith Weir	1920[1]	15.14[1]	1.3	3.0-5.0[1]	Rock ramp 1920[1] Pool and weir 1973[1], vertical-slot 1988[1]	Vertical-slot, fishway trapping
Wallacia Weir	1907-08[2]	26.7[1]	5.6[2]	Not below 1 in 100 year flood[4]	Pool and weir 1914[1]	Vertical-slot, four reader PIT system
Theresa Park Weir	1975[1]	46.3[1]	3.7	0.8[4]	Steep, narrow baffle 1972[1], rock ramp 1998[1]	Vertical-slot, two reader PIT system, fishway trapping
Brownlow Hill Weir	1907-08[2]	47.05[1]	1.8[2]	0.5[4]	Pool and weir 1928[1]	Vertical-slot
Mount Hunter Weir	1908[2]	49.2[1]	2.2[2]	0.7[4]	Rock ramp 1987[1]	Vertical-slot
Cobbitty Weir	1908/1987[2]*	51.25[1]	2[2]	0.9[4]	Vertical-slot 1987[1]	Vertical-slot, two reader PIT system
Sharpes Weir	1907/1987[2]*	53.65[1]	3.6[2]	1.0[4]	Vertical-slot 1987[1]	Vertical-slot
Camden Weir	1907/1986[2]*	56.33[1]	2.2[2]	1.9[4]	Vertical-slot 1987[1]	Vertical-slot, two reader PIT system
Thurns Weir[†]	1917[2]		2.0[2]	NA	None	NA[†]
Bergins Weir[†]	1913[2]		2.4[2]	NA	None	NA[†]
Menangle Weir	1907-08[2]	60.95[1]	0.7-3.0[2]	1.3[4]	None	Vertical-slot, two reader PIT system
Douglas Park Causeway	1960s[1]	62.1[1]	0.8	0.8[4]	None	Vertical-slot, fishway trapping
Maldon Weir	1968[1]	88.95[1]	16	Not below 1 in 100 year flood[4]	None	None
Pheasants Nest Weir	1888[3]	134.35[1]	4[3]	Uncertain, likely more often than 1 in 100 year flood[1]	None	Vertical-slot, two reader PIT system
Warragamba Dam	1948-1960		142	Does not drown-out	None	NA
Avon Dam	1921-27		72	Does not drown-out	None	NA
Nepean Dam	1925-35		82	Does not drown-out	None	NA
Cordeaux Dam	1918-26		57	Does not drown-out	None	NA

Cataract Dam	1902-1907		56	Does not drown-out	None	NA
Broughtons Pass Weir	1888[3]	130.4[1]	2[3]	Uncertain, likely more often than 1 in 100 year flood[1]	None	NA

*The original weirs failed and were subsequently replaced
[†] These weirs failed and are no longer barriers to fish passage
[#] Height relative to sea level
NA – not applicable
[1] Tony Paull, Sydney Catchment Authority, personal communication
[2] SMEC (2011)
[3] Gehrke *et al.* (1999)
[4] Mallen-Cooper (2009)

Table 2 **Predicted historical distributions (pre-fishways, 1975-2010) of native fish species in the study reaches of the Nepean River based on species distributional modelling (Growns et al. 2013) and expert opinion (Martin Mallen-Cooper pers comm.). The migration category for each species is also given; catadromous (migrate to the sea to breed), amphidromous (spawning occurs in freshwater, larvae drift into the estuary or sea and then migrate upstream), anadromous (migrate from the sea to freshwater to spawn) and potamodromous (move within freshwater only). References for migration category A, according to Gehrke et al. (2002); B, according to Pusey et al. (2004); C, according to Lintermans (2007); D, according to Miles et al. (2009); E, according to McDowall (ed) (1996b); F, according to Walsh et al. (in prep).**

Species (and reference for migration category)	Code	Common name	Downstream of Penrith	Penrith to Wallacia	Wallacia to Theresa Park	Theresa Park to Brownlow Hill	Brownlow Hill to Mt Hunter	Mt Hunter to Cobbity	Cobbity to Sharpes	Sharpes to Camden	Camden to Menangle	Menangle to Douglas Park	Douglas Park to Maldon
Diadromous species (catadromous)													
Anguilla australis (A)	ANGAUS	Short-finned eel	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Anguilla reinhardtii (A)	ANGREI	Long-finned eel	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Galaxias maculatus (A)	GALMAC	Common jollytail	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Mugil cephalus (A)	MUGCEP	Sea mullet	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Notesthes robusta (A,D)	NOTROB	Bullrout	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Percalates novemaculeata (A)	PERNOV	Australian bass	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Potamalosa richmondia (A)	POTRIC	Freshwater herring	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Trachystoma petardi (F)	TRAPET	Freshwater mullet	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Species (and reference for migration category)	**Code**	**Common name**	**Downstream of Penrith**	**Penrith to Wallacia**	**Wallacia to Theresa Park**	**Theresa Park to Brownlow Hill**	**Brownlow Hill to Mt Hunter**	**Mt Hunter to Cobbity**	**Cobbity to Sharpes**	**Sharpes to Camden**	**Camden to Menangle**	**Menangle to Douglas Park**	**Douglas Park to Maldon**
Diadromous species (amphidromous)													
Gobiomorphus australis (A, D)	GOBAUS	Striped gudgeon	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Gobiomorphus coxii (potentially also Potamodromous A,B, D)	GOBCOX	Cox's gudgeon	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Hypseleotris compressa (B)	HYPCOM	Empire gudgeon	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Prototroctes maraena (D)	PROMAR	Australian grayling[#]											
Diadromous species (anadromous)													
Mordacia mordax (C)	MORMOR	Short-headed lamprey[#]											
Potamodromous species													
Ambassis agassizii (B)	AMBAGA	Olive perchlet[†]											
Bidyanus bidyanus (C)	BIDBID	Silver Perch[†]											
Carassius auratus	CARAUR	Goldfish*											
Cyprinus carpio	CYPCAR	Carp*											
Galaxias olidus (A)	GALOLI	Mountain galaxias	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Gambusia holbrooki	GAMHOL	Gambusia*											
Hypseleotris galii (A)	HYPGAL	Firetail gudgeon	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Species (and reference for migration category)	**Code**	**Common name**	**Downstream of Penrith**	**Penrith to Wallacia**	**Wallacia to Theresa Park**	**Theresa Park to Brownlow Hill**	**Brownlow Hill to Mt Hunter**	**Mt Hunter to Cobbity**	**Cobbitty to Sharpes**	**Sharpes to Camden**	**Camden to Menangle**	**Menangle to Douglas Park**	**Douglas Park to Maldon**
Philypnodon grandiceps (C)	PHIGRA	Flat-headed gudgeon	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Philypnodon macrostomus (C)	PHIMAC	Dwarf flathead gudgeon	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pseudomugil signifer (B)	PSESIG	Pacific blue-eye	✓	✓									
Retropinna semoni (potentially also amphidromous B)	RETSEM	Australian smelt	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Salmo trutta	SALTRU	Brown trout*											
Tandanus tandanus (B)	TANTAN	Freshwater catfish†											
Estuarine/marine vagrants													
Acanthopagrus australis	ACAAUS	Yellowfin bream	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Herklotsichthys castelnaui	HERCAS	Southern herring	✓	✓									
Liza argentea	LIZARG	Goldspot mullet	✓	✓	✓	✓							
Percalates colonorum	PERCOL	Estuary Perch	✓										
Platycephalus fuscus	PLAFUS	Dusky flathead	✓										

*alien species
†native species introduced to the Hawkesbury-Nepean catchment
#insufficient data to predict historical distribution

Why are fishways needed in the Nepean River?

It has long been recognised that migration is a critical part of the life cycle of some species of Australian freshwater fish, particularly diadromous species. In recent years it has also become clear that migration and movement is an essential part of the life cycle of most freshwater fish (Pusey *et al.* 2004); for recolonisation (e.g. following droughts) or dispersal and maintenance of meta-populations (spatially separated but genetically linked populations). The Nepean River fish community is comprised of many species that actively migrate as an essential part of their life cycle (Table 2) (Pusey *et al.* 2004). For example, there are many catadromous species that must migrate from fresh to salt water in order to breed (Pusey *et al.* 2004). These include recreationally and commercially important species including Australian bass (*Percalates novemaculeata*), sea mullet and freshwater mullet. The upstream migrations undertaken by adults and juvenile catadromous species are obstructed by multiple weirs and passage is potentially only possible during drown-out flows. Movements by smaller amphidromous species (those that move between fresh and salt water though not for spawning) are also likely to be hampered by weirs.

In NSW, 44 fishways were constructed between 1913 and 1985 to facilitate the upstream migration of fish (Thorncraft & Harris 2000, and references therein). However, the design of these earlier fishways was suited to salmonid species in the northern hemisphere and provided limited fish passage for relatively poor swimming Australian native fish (Mallen-Cooper 1992b, 1996; Mallen-Cooper & Harris 1990). In addition to fundamental design flaws, a lack of maintenance often led to inefficient or no fish passage at all (Harris 1984). For example, an assessment of fishways at Penrith and Brownlow Hill between 1978 and 1980 showed that Penrith had a poorly located entrance and both fishways were blocked and thus inoperable at the time of the survey (Harris 1984). Penrith fishway was upgraded to a vertical slot design in 1987, but still only provided inefficient passage for native fish despite the improved design (Mallen-Cooper 2009). Fishways installed since 1985 have been refined to take native fish swimming ability into consideration and to accommodate a wide range of body sizes of migrating fish. The post-1985 fishways in the Nepean River were the first coastal fishways in Australia to be specifically designed and constructed based on the swimming ability of native fish (Mallen-Cooper & Harris 1990). Ongoing monitoring programs have allowed the adaptation and refinement of modern fishways, to ensure they are successfully passing the target fish community.

The need to provide fish passage in the H-N river system was recognised as far back as 1914 when a pool and weir fishway was retrofitted at Wallacia Weir (Table 1). Since then, a range of different fishways have been retrofitted to weirs from Penrith to Camden. For example, a rock ramp was constructed at Penrith in 1920, followed by a pool and weir fishway in 1973 and then a vertical slot in 1988 (Table 1). However, design and maintenance issues meant that the fishways were largely inefficient or inoperable (Mallen-Cooper 2009). Earlier vertical-slot fishways incorporated design features to reduce water velocity through the vertical-slot baffle, however the turbulence in the pools exceeded the swimming ability of small-bodied fish, while the headwater and tailwater range were poor and so the fishways only operated over a narrow range of flows (Mallen-Cooper 2009). Rock-ramp fishways also had narrow operating ranges, poor entrance conditions and were prone to blockage from debris. Thus the best option to reinstate fish passage along the Nepean River was to de-commission the existing fishways and install state-of-the-art vertical-slot fishways operating over a range of headwater and tailwater conditions and passing the majority of the migrating fish community (Mallen-Cooper 2009).

Reinstating fish passage in the Nepean River

The Sydney Catchment Authority is responsible for supplying water to Sydney and operates under the *Sydney Water Catchment Management Act 1998.* The NSW Government announced new 80/20 environmental flow rules for the Hawkesbury-Nepean River in the Metropolitan Water

Plan. This means that all inflows up to the 80th percentile are released downstream and 20% of the inflows above the 80th percentile are also released. With adoption of the new environmental flows for the river, the Sydney Catchment Authority was given the responsibility to implement measures at the Nepean River weirs that would ensure the passage of environmental flows past the weirs. At the same time, improvements to fish passage at the weirs were to be made. This provided an ideal opportunity to upgrade existing inefficient fishways with a new vertical-slot design tailored to requirements of the native fish community and designed to operate from low flows up to one-in-one year annual recurrence interval flows (97.5% of the flow range). Internal hydraulics of the fishways were designed to allow small-bodied fish to pass during low river flows by keeping the turbulence within pools low and to allow large-bodied fish to locate the fishway entrance during high flows by providing a high attraction flow with higher turbulence within the pools (Mallen-Cooper 2009). Thus, over the broad operating range of these fishways, fish from 35 mm to 1 m in length are expected to be able to successfully pass upstream.

New vertical-slot fishways were installed at all weirs from Penrith Weir to Douglas Park Causeway between 2009 and 2010 (Figure 2). A decision was made not to build a fishway on Maldon Weir largely to protect the endangered ‘eastern’ form of Macquarie perch (*Macquaria australasica*) from coming into contact with the translocated ‘western’ form of Macquarie perch established in the nearby Cataract Dam and Cataract River (Faulks *et al.* 2010; Mallen-Cooper 2009). The low abundance of the ‘eastern’ form in the Cataract River suggests that it may be displaced by the larger ‘western’ form (Mallen-Cooper 2009). Upstream of Maldon Weir a final vertical-slot fishway was installed on Pheasants Nest Weir to reduce population fragmentation of ‘eastern’ Macquarie perch above and below this structure (Mallen-Cooper 2009) and its evaluation was presented in a separate report (Robinson *et al.* 2013a).

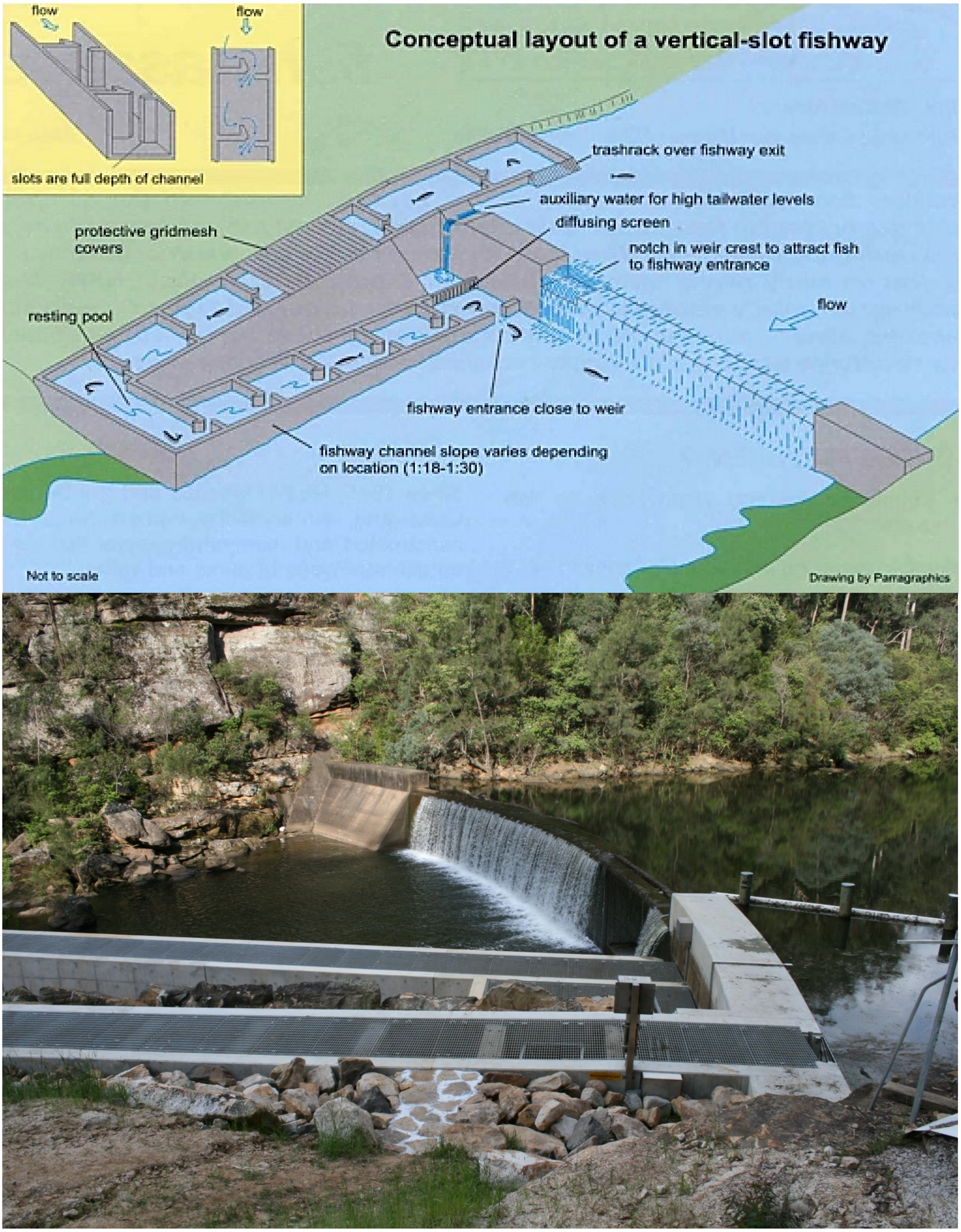
The Nepean fish passage rehabilitation program is the largest ever undertaken on a coastal Australian river system to date, providing a valuable opportunity to evaluate the result of reconnecting approximately 90 km of freshwater habitat to the estuary. All fishways were operational by December 2010 and an intensive monitoring program was undertaken to evaluate the improvements to fish passage. The monitoring program not only serves to assess the effectiveness of the fishways, but also provides valuable data that will assist in refining the fishway design for use in other locations. There are three broad objectives: (i) to determine if fish biodiversity (including species richness, abundance, age structure and genetics) improves as a result of the new fishways, (ii) to assess whether there is increased gene-flow among populations of Australian smelt and (iii) to determine whether environmental flows are stimulating fish movement following fishway construction.

To achieve the above objectives, we divided the monitoring into four components to provide multiple lines of evidence of increased fish passage in the Nepean River following fishway construction:

1. A series of 20 long-term monitoring sites were established from downstream of Penrith Weir to upstream of Douglas Park Causeway (below Maldon Weir). Fish assemblages were assessed using electrofishing and bait-trapping before and after fishway construction. The migratory strategies and the spatial scale of movements present in the H-N River suggest that the diadromous fish community are particularly sensitive to barriers to migration; hence this group as well as the whole fish community were examined.
2. Three fishways were intensively trapped to determine if they were operating to their design specifications, passing fish from 35 mm to more than 1 m long.
3. Passive integrated transponder (PIT) technology was utilised to gain more detailed information on fish movements (i.e. the proportion of fish that enter the fishway that proceeded to the exit, the timing of movements and whether fish also descend the fishway).

4. Genetic analyses were used to determine if the weirs caused population fragmentation in a small-bodied species (Australian smelt), and if so, whether the fragmentation was mitigated following fishway installation.

Figure 2 Conceptual layout of a vertical-slot fishway (top), and the new vertical-slot fishway at Theresa Park (bottom). The exit is at the bottom right of the photo and the entrance is the first baffle adjacent to the attraction flow (photo taken 29th Oct 2010).



Fish community structure of the Nepean River before and after fishway construction

Introduction

The use of fishways to reinstate freshwater fish migration has been used extensively worldwide and fishways are increasingly being constructed in Australia (Barrett & Mallen-Cooper 2006; Gough *et al.* 2012). Many species of freshwater fish in Australia migrate considerable distances to complete critical components of their life cycle (such as spawning migrations) (Pusey *et al.* 2004), and all Australian fish species undertake general movements for feeding, seeking shelter or dispersal (Barrett & Mallen-Cooper 2006; Lucas & Baras 2001).

The Hawkesbury-Nepean river system in south eastern Australia has at least 41 species of freshwater fish (Gehrke 1996b), many of which are migratory. The system is extensively regulated with at least 81 dams and weirs (Marsden & Gehrke 1996). The Nepean River between Maldon and Penrith (approximately 90 river km) has ten major structures that obstruct upstream fish migration during typical flows. Rainfall in the upper catchment can quickly lead to short-term river rises (pulses) throughout lowland reaches of the Nepean River. During these pulses, fish passage barriers can become inundated and upstream passage becomes possible. Hydrologic Engineering Centres River Analysis system (HEC-RAS: www.hec.usace.army.mil) modelling has indicated that while the inundation frequency of some weirs is at least annually, others are not expected to be inundated below the one in 100 year flood level (Mallen-Cooper 2009). Critically, the second most downstream structure – Wallacia Weir – is not expected to be regularly inundated (Table 1). This has serious implications for upstream fish migration, especially for diadromous species. Inundation events alone are insufficient to promote efficient upstream migration, as some species and life stages migrate during low to medium flow periods (Baumgartner & Reynoldson 2007; Gehrke *et al.* 1999; Gehrke *et al.* 1996; Gilligan *et al.* 2003; Harris *et al.* 1996; Mallen-Cooper 2009) . Even for species that are able to migrate past these barriers, such as Australian bass, it is possible that smaller individuals may be less able to do so, resulting in successively larger individuals in upstream sites.

To address the fish passage issues in the Nepean River, the SCA installed ten new vertical-slot fishways from Penrith to Douglas Park in 2009-2010. These fishways were designed to be compatible with the swimming ability of both small and large-bodied native fish (35 mm to 1 m in length) and to operate across a wide range of flow conditions experienced in the Nepean River (Mallen-Cooper 2009). This is the largest scale attempt at reinstating fish passage in a coastal Australian system to date and as such, it is important to assess the changes to the fish community.

This chapter aimed to determine whether the Nepean River fish community between Penrith and Maldon Weir responded to installation of the fishways. We asked three main questions: Firstly, what is the ‘pre-fishway’ state of fish assemblages from downstream of Penrith to Maldon Weir and how do they compare to the predicted historical fish communities? Secondly, does the fish community composition and individual species distributions/relative abundances change following fishway installation? Finally, have the fishways allowed smaller sized fish of catadromous species, such as Australian bass, to migrate further upstream?

Methods

Sites

The current study is focused on the reach of the Nepean River from Maldon in the upper reaches to downstream of Penrith (Figure 1). Twelve weirs were built in this reach between 1907 and 1917 with Theresa Park Weir constructed later in 1975. The weirs serve to store flow and provide pumping pools along the Nepean River for domestic and agricultural purposes. Cobbitty, Sharpes and Camden Weirs failed and were rebuilt in 1986 and 1987 (Table 1). Thurns and Bergins Weirs also failed but were not rebuilt.

Twenty monitoring sites were established to assess the ‘pre-fishways’ status of fish assemblages, with ongoing sampling allowing a comparison to determine the changes to the fish communities ‘post-fishways’ (Figure 1). The river was divided into 11 sampling reaches between the weirs. Two replicate sampling sites were selected downstream of Penrith Weir, and two sites in most river reaches, namely Penrith-Wallacia, Wallacia-Theresa Park, Theresa Park-Brownlow Hill, Brownlow Hill-Mt Hunter, Mt Hunter-Cobbitty, Cobbitty-Sharpes, Sharpes-Camden, Camden-Menangle, Menangle-Douglas Park and Douglas Park-Maldon. A single site was selected between Menangle and Douglas Park given this area was extremely difficult to access. Thurns Weir and Bergins Weir were not considered in this study given they are damaged and are not thought to impede fish passage. An additional site on the Warragamba River was included in the study in March 2011 to benchmark the fish community in this location.

Electrofishing methodology

Sampling was undertaken in accordance with the Sustainable Rivers Audit (SRA) protocols (MBDC 2004), which are consistent with NSW Monitoring, Evaluating and Reporting (MER) protocols for freshwater fish monitoring. Twelve replicate shots with a total power application time of 90 seconds each were undertaken at each site during the day using large electrofishing boats (Smith-Root Model 7.5KVa electrofishing units) (Appendix 1). During each operation, dip nets were used to collect all stunned fish and place them in an aerated live-well. All fish that could not be successfully collected (i.e. they were out of reach of the dip netter or only partially stunned and escaped) but could be positively identified were recorded as ‘observed’. In addition, 10 unbaited concertina type bait-traps (minimum of two hour soak during the day) were set to provide an additional method of sampling the small bodied species in the fish community. At the completion of each electrofishing and bait-trap operation, all fish were identified, counted and a subset measured (50 individuals per species per method). All fish were measured (fork length or total length depending on species) in the electrofishing shot/bait trap that the 50th fish was caught to avoid bias in the size of fish selected for measuring. Fish over 150 mm were fitted with a Passive Integrated Transponder (PIT) tag to allow fish migrations through fishways fitted with PIT readers to be studied. Each site was sampled on three occasions before fishway installation and five occasions post-fishway, with the exception of the Warragamba River site, which was sampled on five occasions from March 2011 onwards (Appendix 2).

Data analysis

What is the pre-fishway state of the fish community from downstream of Penrith Weir to Maldon Weir and how does it compare to the predicted historical fish community?

The pre-fishway state of the fish community throughout the study reach was quantified by: (i) generating the historical distributions of each species pre-fishways and (ii) calculating the mean species richness for each reach pre- and post-fishways and comparing this to the mean historical number of fish species expected in each reach (Table 2). The method described in Growns *et al.* (2013) was used to estimate the historical distributions of species within the study reach. Mean species richness was determined from the pre- and post-fishway data for each reach and displayed on the same plot as that showing historical species richness.

Pre- and post-fishway fish community structure from Penrith Weir to Maldon Weir

Permutational analysis of variance (PERMANOVA) (Anderson *et al.* 2008) was conducted to determine if there was a significant difference in the sampled fish community structure between all reaches and between pre- and post-fishway periods. The analysis used the number of fish caught and observed, standardised (number per 24 hours) and $\log_e$ (X+1) transformed. Bray-Curtis similarities were then calculated. The PERMANOVA model consisted of four factors; season (Se, random, nested within Yr, four levels: winter, spring, summer and autumn), Year (Yr, random, nested in BvA, four levels: 2009, 2011, 2012 and 2013), Before/After (B v A, fixed, two levels: before and after) and Site (S, fixed, twenty levels). Significance values were calculated based on 9,999 unrestricted permutations of the raw data. To visualise the differences in the fish community structure pre- and post-fishways, multi-dimensional scaling (MDS) was used to plot the average fish community data in two dimensions for each site pre- and post-fishways. The average fish community data was calculated by averaging data across the three rounds of the pre-fishway data and five rounds of post-fishway data. Stress values indicate how well the two-dimensional ordination represents the assemblage structure, where a stress value of less than 0.2 is considered a useful representation of data points within the ordination space (Clarke & Warwick 2001). A useful ordination space therefore allows simple interpretation relative to our objectives, as points positioned close within the ordination space have similar fish assemblage composition. Species that contributed the most to the dissimilarity between pre- and post- fishway groups were calculated using SIMPER.

Effect of fishways on spatial structure of the fish community

To complement the overall test for changes in fish assemblage composition, we used a multivariate test to compare spatial seriation in the fish communities through the system before and after fishway installation. Seriation is defined as the gradient of change in the fish community from downstream to upstream reaches. If the weirs are limiting upstream fish migration, it is expected that seriation will be evident, as either a gradual change in the fish community in an upstream direction, or as an abrupt change at one or more fishways. If subsequent installation of the fishways provided sufficient fish passage to alter the fish community composition, it is expected that seriation will decrease during the post-fishway period. To test for seriation, a RELATE analysis was conducted by comparing the Bray-Curtis similarity matrix to a rank order of sites (e.g. Devlin Lane to Penrith downstream = a distance of 1, Devlin Lane to Maldon downstream = a distance of 19; Appendix 3.) for all pre-fishway data pooled and all post-fishway data pooled. Furthermore, given that changes in the fish community are likely to occur at a gradual rate, the three pre- rounds of electrofishing were compared to the first three rounds of post-electrofishing and the last three rounds of post-electrofishing to detect temporal changes. To determine the difference in the fish community after the greatest period of time had passed since the fishways were installed, the first and last rounds of electrofishing were also compared. Finally, RELATE analyses were also used to determine whether the fish community structure before and after fishway installation was in any way related. Significance values were calculated based on 9,999 permutations of the raw data.

Changes in the fish community at individual fishways

Tests for changes in the fish communities before and after fishway installation were carried out on each individual fishway by comparing the sites immediately upstream and downstream using PERMANOVA. Data from all electrofishing samples pre- and post-fishways were pooled. If a significant result was obtained for the pre-and post-fishway interaction with above or below term (BvA x S), follow-up pair-wise tests were carried out to identify site combinations responsible for the significance. When there were fewer than 100 permutations available, the significance values were obtained using Monto Carlo tests. Where results suggested a significant change in the difference between upstream and downstream sites before and after fishway installation, SIMPER analyses were conducted to determine the fish species contributing the most to these changes.

All multivariate analyses, including PERMANOVA, SIMPER, MDS and RELATE were conducted in PRIMER v6 (PRIMER-E Ltd, Ivybridge, UK).

Size distributions

We hypothesised that the weirs may modify fish length-frequency distributions in some species by hampering the upstream movement of smaller individuals with a potentially reduced swimming capability. We selected Australian bass as the study species given its abundance throughout the study reach both pre- and post-fishways, its requirement to undertake upstream migrations at both adult and juvenile life stages (Harris 1983), and its ability to migrate over barriers (at least some of the time) prior to fishway installation. Australian bass adults undertake a downstream migration from May to August to spawn in estuaries before returning upstream (predominantly females, with males remaining in the tidal reaches), while juveniles migrate upstream during spring and summer (Harris & Rowland 1996). We predicted that before fishway installation, the upstream population would be predominantly comprised of large fish, while the downstream populations would have smaller fish, potentially unable to move further upstream because of poorer swimming capability. We expected smaller fish to expand their distribution upstream following fishway installation. To test this, we used two simple approaches. Both approaches selected Australian bass in the 10th (i.e. the smallest 10% of the fish measured at that site) and 90th (i.e. the largest 10% of the fish measured at that site) percentiles of length in any sampling event that had at least 10 bass measured (to moderate the influence of spurious data from small samples and outliers). Firstly, we looked for obvious patterns or changes in fish length pre-and post-fishways by plotting the 10th and 90th percentiles for each sample date against site (ordered from bottom to top). Secondly, we postulated that if the weirs were not a barrier to the migration of any sized fish, there should be no correlation between the lengths of the fish captured and site order. Therefore, we calculated the rank correlation of length versus position along the reach for the 10th and 90th percentiles for the pre- and post-fishway samples. A significant positive correlation with site order would indicate that the size of the smallest and/or largest fish increases in an upstream direction. Furthermore, change in any such correlations post-fishways may indicate change in the length distribution following fishway implementation.

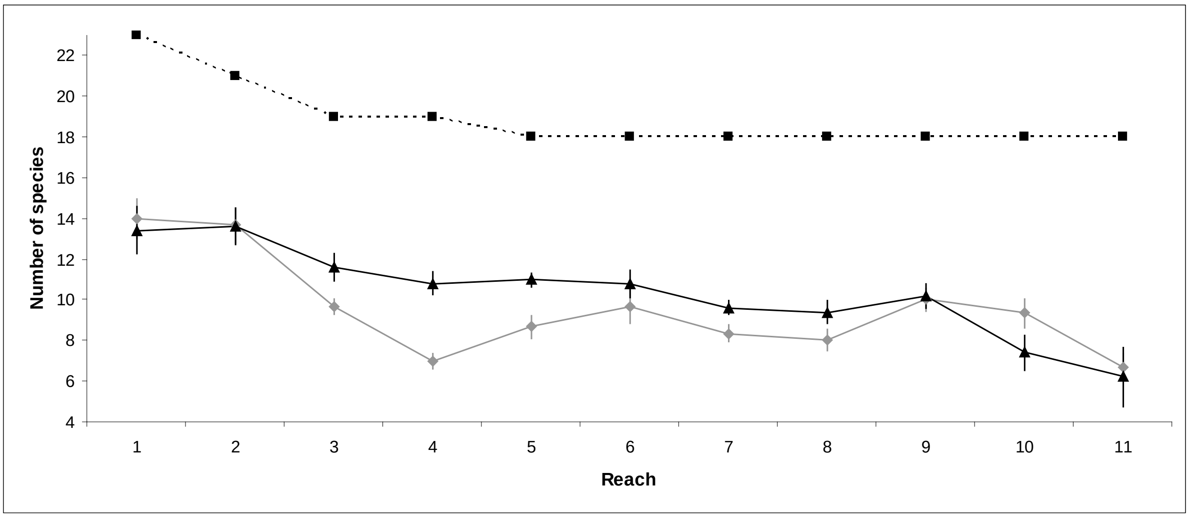
Results

Overall catch summary

Eight rounds of electrofishing were successfully completed; three rounds before and five rounds after commissioning of the fishways. A total of 10,875 fish representing 22 species were caught in the study reach (all methods, including observed fish; Table 3, Appendix 4; Appendix 5). Australian bass were the most commonly caught species (23% of catch) followed by Australian smelt (*Retropinna semoni* 11% of catch). There were four exotic species present in the study area that together contributed 12% of the total catch: common carp (*Cyprinus carpio*; 5% of catch), goldfish (*Carassius auratus*; 5% of catch), eastern gambusia (*Gambusia holbrooki*; 2% of catch) and brown trout (*Salmo trutta*; two fish). Three species native to Australian rivers outside the Hawkesbury-Nepean catchment were sampled including freshwater catfish (*Tandanus tandanus*), olive perchlet (*Ambassis agassizii*) and silver perch (*Bidyanus bidyanus*). Freshwater catfish are well established in the study reach (4% of catch) while olive perchlet were only sampled in the two most downstream sites (four individuals, all in bait traps) and only a single silver perch was sampled.

The mean species richness before fishway commissioning was lowest at the most upstream reach and remained relatively low throughout the middle reaches, especially at reach four (between Theresa Park and Brownlow Hill Weirs; Figure 3). Following fishway installation, mean species richness increased throughout the middle reaches and remained relatively unchanged at the most downstream and upstream reaches (Figure 3). Mean species richness was considerably lower than the predicted species richness for both time periods (Figure 3).

Figure 3 **Predicted number of species (dotted line) and mean observed species richness (± SE) before (grey line) and after (solid line) fishway installation for each river reach. Sites are ordered from the most downstream reach on the left to the most upstream reach on the right. The reaches are 1-downstream of Penrith, 2-Penrith-Wallacia, 3-Wallacia-Theresa Park, 4-Theresa Park-Brownlow Hill, 5-Brownlow Hill-Mt Hunter, 6-Mt Hunter-Cobbitty, 7-Cobbitty-Sharpes, 8-Sharpes-Camden, 9-Camden-Menangle, 10-Menangle-Douglas Park and 11-Douglas Park-Maldon.**



Pre- and post-fishway fish community structure from Penrith to Maldon

All native species collected would historically have been found throughout the study reach (Table 2). Three species predicted or known to occur in the reach were not collected: common jollytail (*Galaxias maculatus*), mountain galaxias (*Galaxias olidus*) and Pacific blue-eye (*Pseudomugil signifier*).

Pre-fishways sampling showed that many species were restricted to the lower portion of the study reach; in particular, of the ten diadromous species sampled, only Australian bass and long-finned eel were present throughout the study area (Table 3). Post-fishways, four of these species – freshwater herring, sea mullet, freshwater mullet and Cox's gudgeon – extended their ranges substantially upstream (Table 3). Estuary perch (*Percalates colonorum*) (a largely estuarine species) and bullrout (*Notesthes robusta*), were in low abundances and showed no significant change in distribution. Pre-fishways, the two amphidromous gudgeons (empire gudgeon (*Hypseleotris compressa*) and striped gudgeon (*Gobiomorphus australis*) were restricted to sites downstream of Wallacia Weir. Post-fishways, empire gudgeon did not expand their distribution upstream and only three striped gudgeon were sampled above Wallacia Weir. However, striped gudgeon were trapped in reasonable numbers in the Theresa Park Weir fishway based on fishway data (next chapter), demonstrating their distribution extended further upstream of Wallacia than the electrofishing data suggests. Fishway trapping also showed that empire gudgeon could negotiate the internal hydraulics of the fishways, but unlike striped gudgeon they were not recorded upstream of Wallacia, corroborating the electrofishing and bait-trapping data.

Potamodromous species were largely found throughout the study reach both before and after fishway commissioning, consistent with expectations.

Table 3 **Total catch (includes caught and observed fish combined) for each site before (Year 1, 2009) and after (Years 2-4, 2011-2013) fishway commissioning. Not all species were sampled in all years. A dash represents no caught and observed fish.**

	Australian bass				**Australian smelt**				**Freshwater herring**				**Long-finned eel**			
Year	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Devlin Lane	37	131	75	15	11	18	26		15	111	152	19	23	14	10	3
Penrith - downstream	99	111	60	15	12	21	1	1	2	76	85	54	10	4	8	-
Penrith - upstream	56	82	46	25	43	88	1	2	14	5	7	25	13	12	6	2
Wallacia - downstream	67	113	36	13	35	15	35	1	14	7	8	26	14	8	2	-
Wallacia - upstream	42	68	41	3	7	32	6	14	6	36	31	5	14	7	3	-
Theresa Park - downstream	72	50	44	1	6	5	-	-	-	43	19	10	17	6	8	-
Theresa Park - upstream	23	36	28	17	-	-	10	2	-	7	15	7	18	13	9	6
Brownlow Hill downstream	28	28	28	10	2	4	10	-	-	7	9	9	25	8	7	2
Brownlow Hill upstream	29	17	19	2	1	-	3	-	-	10	2	3	17	4	7	4
Mt Hunter - downstream	25	16	20	3	11	8	44	20	-	3	1	1	36	6	10	4
Mt Hunter - upstream	18	10	36	3	29	5	16	3	-	-	4	5	25	5	6	4
Cobbitty downstream	24	35	16	4	91	9	17	3	4	2	12	12	10	13	13	2
Cobbitty upstream	33	36	38	3	4	-	4	3	-	2	2	19	29	18	11	3
Sharpes - downstream	44	25	22	3	12	1	7	-	1	-	11	3	14	8	9	2
Sharpes - upstream	35	31	20	9	24	-	10	4	-	-	13	1	11	5	11	1
Camden downstream	49	26	31	8	22	17	11	39	-	-	7	1	25	13	11	3
Camden upstream	25	14	22	5	5	-	12	27	-	-	-	1	13	5	10	-
Menangle - downstream	30	19	23	8	3	42	56	10	-	-	-	-	15	7	7	1
Douglas Park downstream	42	40	33	17	81	-	-	3	-	-	-	-	45	19	13	18
Maldon - downstream	6	13	17	11	50	41	5	10	-	-	-	-	28	5	18	4
Grand Total	**784**	**901**	**655**	**175**	**449**	**306**	**274**	**142**	**56**	**309**	**378**	**201**	**402**	**180**	**179**	**59**

	Sea mullet				Flat-headed gudgeon				Freshwater mullet				Firetail gudgeon			
Year	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Devlin Lane	61	12	101	38	23	8	1	2	20	15	53	17	-	6	-	-
Penrith - downstream	34	60	83	24	37	16	6	1	12	73	12	23	3	-	-	-
Penrith - upstream	26	30	26	4	65	23	7	4	8	32	3	29	-	5	3	9
Wallacia - downstream	36	9	17	17	52	19	11	9	18	103	49	30	1	1	2	-
Wallacia - upstream	3	20	28	10	16	5	6	9	13	12	42	10	4	13	1	1
Theresa Park - downstream	-	19	10	5	21	1	2	6	-	18	21	14	10	25	3	2
Theresa Park - upstream	-	1	1	2	30	5	2	2	-	-	2	5	15	24	1	-
Brownlow Hill downstream	-	4	5	1	19	3	-	1	-	-	2	6	16	4	5	2
Brownlow Hill upstream	-	-	3	-	9	4	-	4	-	-	4	1	10	5	-	-
Mt Hunter - downstream	-	6	5	1	24	7	2	2	-	-	-	-	54	42	3	
Mt Hunter - upstream	-	-		-	16	12	1	7	-	-	6	-	25	33	1	2
Cobbitty downstream	-	-	6	-	8	4	2	-	-	-	1	2	22	2	-	-
Cobbitty upstream	1	1	4	-	14	5	-	5	-	2	1	1	27	60	1	3
Sharpes - downstream	-	3	5	-	12	1	-	2	-	-	4	8	10	13	7	1
Sharpes - upstream	-	4	5	-	16	2	3	3	-	-	5	2	7	13	3	2
Camden downstream	-	7	1	-	11	3	3	1	-	-	7	-	1	5	13	4
Camden upstream	-	-	1	-	24	7	4	8	-	-	3	2	32	19	1	5
Menangle - downstream	-	-		-	11	8	6	10	-	1	5	8	30	30	9	1
Douglas Park downstream	-	3	1	1	19	-	1	2	-	1	-	9	26	2	1	2
Maldon - downstream	-	-		1	16	1	1	5	-	-	-	-	5	-	-	7
Grand Total	**161**	**179**	**302**	**104**	**443**	**134**	**58**	**83**	**71**	**257**	**220**	**167**	**298**	**302**	**54**	**41**

	Common carp				Goldfish				Freshwater catfish				Empire gudgeon			
Year	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Devlin Lane	7	11	2	4	38	7	1	-	17	8	13	5	162	33	11	-
Penrith - downstream	4	3	7	5	4	5	-	-	3	3	4	1	45	55	8	-
Penrith - upstream	11	8	15	5	36	5	12	-	2	13	6	2	3	2	58	1
Wallacia - downstream	4	1	16	3	14	18	-	-	10	14	4	-	10	7	16	12
Wallacia - upstream	7	13	13	6	9	8	3	3	-	4	1	-	-	-	-	-
Theresa Park - downstream	8	19	21	4	48	7	3	7	1	2	3	-	-	-	-	-
Theresa Park - upstream	12	13	19	4	-	5	1	-	5	5	1	1	-	-	-	-
Brownlow Hill downstream	10	5	25	-	-	3	-	-	3	2	2	1	-	-	-	-
Brownlow Hill upstream	8	28	3	5	10	3	9	2	4	3	5	2	-	-	-	-
Mt Hunter - downstream	8	13	2	3	8	2	1	-	9	5	7	2	-	-	-	-
Mt Hunter - upstream	6	4	8	8	10	5	1	-	6	4	6	1	-	-	-	-
Cobbitty downstream	13	10	8	6	3	5	4	1	8	6	5	-	-	-	-	-
Cobbitty upstream	20	8	9	9	30	9	1	-	13	7	5	1	-	-	-	-
Sharpes - downstream	13	13	13	2	12	4	7	5	7	5	2	3	-	-	-	-
Sharpes - upstream	13	11	6	-	6	3	-	-	9	3	6	3	-	-	-	-
Camden downstream	10	19	1	-	20	2	-	-	21	8	11	2	-	-	-	-
Camden upstream	-	1	-	-	23	8	8	2	18	10	13	2	-	-	-	-
Menangle - downstream	4	1	1	-	23	5	2	-	24	10	11	3	-	-	-	-
Douglas Park downstream	3	9		-	11	15	13	-	4	7	2	3	-	-	-	-
Maldon - downstream	3	-	-	-	1	2	9	-	-	-	3	1	-	-	-	-
Grand Total	**164**	**190**	**169**	**64**	**306**	**121**	**75**	**20**	**164**	**119**	**110**	**33**	**220**	**97**	**93**	**13**

	Eastern gambusia				**Cox's gudgeon**				**Carp gudgeon**		**Striped gudgeon**				**Dwarf flat-headed gudgeon**			
Year	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**3**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Devlin Lane	67	-	-	-	-	2	2	-	-	-	23	4	2	-	1	-	2	-
Penrith - downstream	7	-	-	-	-	12	-	-	-	2	12	6	1	-	2	-	-	-
Penrith - upstream	2	1	-	-	-	15	4	-	7	-	2	1	3	-	2	1	-	-
Wallacia - downstream	3	2	-	-	-	10	1	-	-	-	1	1	2	-	-	-	-	-
Wallacia - upstream	5	-	-	-	-	2	1	-	12	-	-	-	-	1	3	-	-	3
Theresa Park - downstream	2	-	-	-	-	3	-	-	20	-	-	1	-	1	3	-	-	1
Theresa Park - upstream	-	-	-	-	-	2	2	-	1	-	-	-	-	-	-	-	-	-
Brownlow Hill downstream	-	17	-	-	-	-	2	-	-	-	-	-	-	-	2	-	-	-
Brownlow Hill upstream	5	-	-	-	-	-	4	-	3	-	-	-	-	-	-	-	-	-
Mt Hunter - downstream	-	1	-	-	-	2	5	-	2	-	-	-	-	-	2	1	-	-
Mt Hunter - upstream	3	8	-	-	-	4	1	1	-	-	-	-	-	-	1	-	1	-
Cobbitty downstream	-	-	2	-	-	3	2	2	2	-	-	-	-	-	1	-	-	1
Cobbitty upstream	-	-	-	-	-	2	-	1	7	-	-	-	-	-	-	-	-	-
Sharpes - downstream	-	-	-	-	-	4	-	5	1	-	-	-	-	-	-	-	-	-
Sharpes - upstream	-	-	-	1	-	-	5	-	6	-	-	-	-	-	1	1	-	-
Camden downstream	-	-	-	-	-	3	2	-	1	-	-	-	-	-	-	-	-	-
Camden upstream	17	1	-	-	-	-	-	-	50	-	-	-	-	-	1	-	-	-
Menangle - downstream	16	23	1	-	1	3	5	8	-	-	-	-	-	-	7	-	2	-
Douglas Park downstream	4	-	-	-	2	2	1	4	-	-	-	-	-	-	9	1	1	-
Maldon - downstream	-	-	-	-	3	8	4	4	-	-	-	-	-	-	2	-	1	-
Grand Total	**131**	**53**	**3**	**1**	**6**	**77**	**41**	**25**	**112**	**2**	**38**	**13**	**8**	**2**	**37**	**4**	**7**	**5**

	Bullrout			Olive perchlet	Brown trout	Silver perch	Estuary perch	Grand Total
Year	**1**	**2**	**3**	**2**	**4**	**3**	**4**	
Devlin Lane	11	1	2	2	-	-	1	**1,456**
Penrith - downstream	-	-	1	2	-	-	-	**1,135**
Penrith - upstream	-	-	1	-	-	-	-	**919**
Wallacia - downstream	1	3	-	-	-	-	-	**921**
Wallacia - upstream	-	-	-	-	2	-	-	**604**
Theresa Park - downstream	-	-	-	-	-	-	-	**592**
Theresa Park - upstream	-	-	-	-	-	-	-	**352**
Brownlow Hill downstream	-	-	-	-	-	-	-	**317**
Brownlow Hill upstream	-	-	-	-	-	-	-	**252**
Mt Hunter - downstream	-	-	-	-	-	-	-	**427**
Mt Hunter - upstream	-	-	-	-	-	-	-	**350**
Cobbitty downstream	-	-	-	-	-	-	-	**396**
Cobbitty upstream	-	-	-	-	-	-	-	**452**
Sharpes - downstream	-	-	-	-	-	-	-	**324**
Sharpes - upstream	-	-	-	-	-	-	-	**314**
Camden downstream	-	-	-	-	-	-	-	**419**
Camden upstream	-	-	-	-	-	-	-	**399**
Menangle - downstream	-	-	-	-	-	1	-	**491**
Douglas Park downstream	-	-	-	-	-	-	-	**470**
Maldon - downstream	-	-	-	-	-	-	-	**285**
Grand Total	**12**	**4**	**4**	**4**	**2**	**1**	**1**	**10,875**

Differences in the fish assemblages in the reach before and after fishway installation were not consistent across all sites (BvA x S, Pseudo-F = 1.30, df = 27, 38, p = 0.033; Table 4). The ordination clearly indicates that before fishway installation sites 1 - 4 were distinct from the upstream sites, while after fishway installation sites 1 - 6 were distinct from the upstream population, suggesting the fishways have allowed upstream migration resulting in the fish communities at sites 5 and 6 more closely resembling the most downstream sites (Figure 4). Species that were primarily responsible for the overall differences in the pre- and post- fishway groups were freshwater herring, freshwater mullet, sea mullet, Australian smelt and goldfish (SIMPER; Table 5). There were significant community differences among seasons within years and among years before and/or years after the fishways were installed (Se(Yr(BvA)), Pseudo-F = 3.43, df = 6,6, p < 0.001; Yr(BvA) x S, Pseudo-F = 1.31, df = 38, 71, p = 0.011; Table 4).

Table 4 Summary of PERMANOVA results across all fishways. The comparison between sites above and below the fishway pre- and post-fishways are indicated by a star (*) and significant (α=0.05) P values are in bold. (B*v*A=Before *vs* After, S=Site, Yr=Year, Se=Season, df=degrees of freedom, SS=sum of squares, MS=mean squares. P values were based on 9999 unrestricted permutations of the raw data).

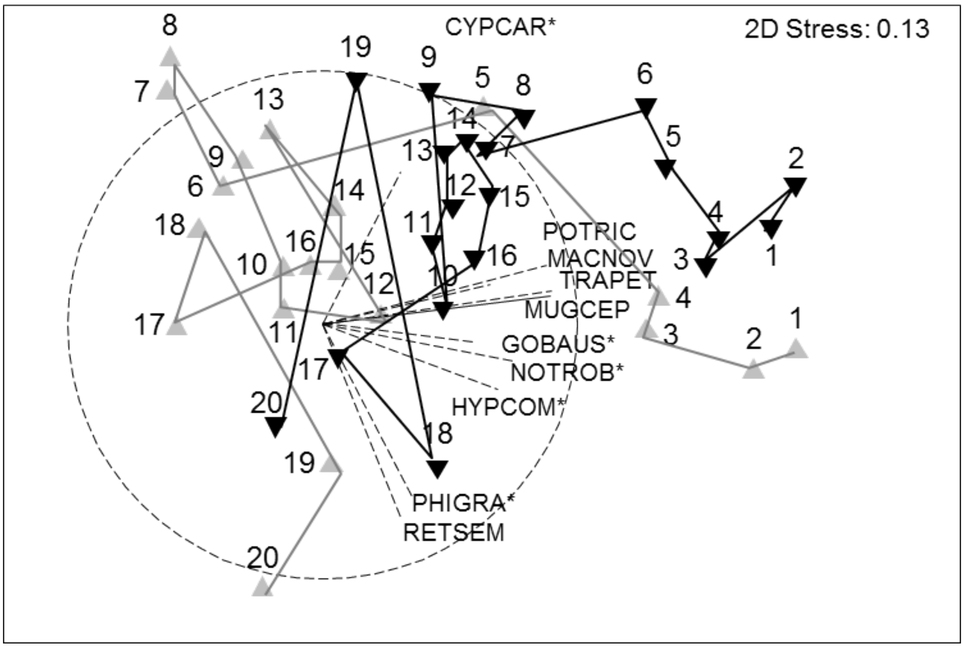
Factor	*df*	SS	MS	Pseudo-F	*P*	Components of variation (%)
B*v*A	1	9596	9596	3.11	0.052	0.13
S	19	60511	3185	3.72	**0.000**	0.28
Yr(B*v*A)	2	4515	2257	1.38	0.276	0.02
BvA x S	19	18388	968	1.30	**0.033**	0.06
Se (Yr(B*v*A))	6	9792	1632	3.43	**0.001**	0.07
Yr (B*v*A) x S*	38	23865	628	1.31	**0.011**	0.07
Se (Yr(BvA)) x S	68	32637	480	1.01	0.503	0.00
Residual	6	2859	476			0.37

Effect of fishways on spatial structure of the fish community

There was an identifiable pattern of change in the fish communities along the length of the river both pre- and post-fishways. The fish community had a strong gradient of change from downstream to upstream before (Spearman’s r = 0.395, p = 0.0001) and after the fishways were installed (Spearman’s r = 0.469, p = 0.0001; Figure 4). The gradient of change was still evident in the final round of electrofishing (Spearman’s r = 0.548, p = 0.0001; Figure 4).

Spatial patterns in the fish community structure from downstream to upstream pre-fishway and the first three rounds of post-fishway were similar (rounds 1, 2 and 3, with rounds 4, 5 and 6; Spearman’s r= 0.357, p = 0.0001). When only the final three sampling rounds were compared to the three rounds before fishways, correlation was still significant but had decreased by almost half (rounds 1, 2 and 3, with rounds 6, 7 and 8; Spearman’s r = 0.221, p= 0.0001). Furthermore, a comparison of just the first and last rounds of electrofishing indicated there was no longer a significant match in the spatial pattern of the fish community (Spearman’s r = 0.097, p = 0.163). Thus these data suggest that the gradient in fish community structure in a downstream to upstream direction is changing post-fishways, but at a gradual rate. This is consistent with expectations.

Figure 4 **Multi-dimensional scaling (MDS) ordination showing the dissimilarity of the fish community before (average of three rounds, grey triangles) and after (average of five rounds, black triangles) fishway installation.**



The trajectories illustrated in Figure 4 show the order of sites from the most downstream site, Devlin Lane (1), to upstream of Douglas Park (20) and also indicate the seriation of the fish community. The plot is overlaid with vectors showing the direction of the relationship of the fish species most strongly associated with the sites using Spearman’s rank correlation co-efficient (Rs > 0.6). The length of the vector indicates the strength of the correlation and the dashed circle indicates the maximum achievable correlation of 1. Species marked with an asterisk have strong correlations with the space but still do not explain more variation than expected by chance (see Table 5). Species codes are given in Table 2.

Table 5 Fish species contributing to the mean dissimilarity between fish assemblages before and after fishway installation across the entire study area. (Av. Abund = average abundance (log abundance +1) of each fish species, Av. Diss=the contribution to pre- and post-fishway dissimilarity, Contrib.%=indicates the proportion of dissimilarity that a specie contributes to the overall dissimilarity between pre- and post-fishway groups) *Species contributing more than a random proportion are in bold (note, only species that account for more than 7.5% of the dissimilarity (as 91.7% ÷12 species-7.5%) explain greater than a random amount).

	Before vs. after average dissimilarity = 36.98%					
Species	Av. Abund Before	Av. Aund After	Av. Diss	Diss/SD	*Contrib. %	Cum.%
Freshwater herring	0.4	1.6	4.6	1.4	**12.5**	12.5
Freshwater mullet	0.4	1.4	4.2	1.6	**11.3**	23.9
Sea mullet	0.6	1.3	4.0	1.5	**10.9**	34.8
Australian smelt	1.7	1.8	3.8	1.3	**10.2**	45.0
Goldfish	1.5	1.1	3.0	1.4	**8.1**	53.0
Common carp	1.2	1.5	2.4	1.2	6.5	59.6
Flat-headed gudgeon	1.0	0.7	2.2	1.4	5.9	65.4
Freshwater catfish	1.1	1.2	2.2	1.3	5.8	71.2
Cox's gudgeon	0.1	0.6	2.1	1.4	5.6	76.8
Australian bass	2.5	2.8	1.9	1.2	5.2	82.0
Empire gudgeon	0.4	0.4	1.8	0.6	4.9	86.9
Long-finned eel	2.0	1.6	1.7	1.4	4.7	91.6

Changes in the fish community by fishway

Significant changes in the difference between downstream vs. upstream fish communities before and after fishway installation were only observed at Penrith, Wallacia and Menangle (BvA x S, Pseudo-F = 3.02, df = 1, p = 0.020, Pseudo-F = 3.11, df = 1, p = 0.029 and Pseudo-F = 5.68, df = 1, p = 0.034 respectively; Table 6).

Follow-up pair-wise PERMANOVA for Penrith revealed that the upstream site was not different before and after (p = 0.249) but the downstream site had a significantly different fish community pre- and post-fishway (similarity B vs. A similarity = 58%, p < 0.046). The upstream and downstream sites were an average of 7.5% more similar after the fishway (Table 7).

In contrast, the downstream and upstream site fish communities at Wallacia were not significantly different pre-fishway (similarity site 4 vs site 5 before = 49%, p = 0.56; Table 7) The fish community in the upstream site post-fishway was not significantly different to the downstream site post-fishway (A4 vs. A5 similarity = 64%, p = 0.077) or itself pre-fishway (B5 vs. A5 similarity = 51%, p = 0.055), but was significantly different to the downstream site pre-fishway (B4 vs. A5 similarity = 58%, p = 0.05; Table 7). This suggests a change in the upstream site due to fish moving through the fishway. The species primarily responsible for the differences pre- and post-fishways were freshwater mullet, sea mullet, freshwater herring, Australian smelt and common carp (Table 8).

At Menangle, the downstream and upstream sites were significantly different pre- and post-fishway (B18 vs. B19 and A18 vs. A19 $p < 0.05$), but more similar pre- (63 %) than post-fishway (54%), suggesting the fishway had started to alter the fish community at the upstream site.

Size distributions of Australian bass

The number of Australian bass required to obtain a reasonable estimate of size distributions was set to a minimum of 10 per reach and this only occurred consistently along most reaches in the October to December sampling periods in 2009, 2011 and 2012, and the April to June period in 2012 (Figure 5). In October-December 2009 (pre-fishways) there was a significant increase in the size of small fish (10th percentile) and the size of large fish (90th percentile) in an upstream direction (Figure 5; Table 9). These differences were from smaller fish being found downstream of reach 7 (U/S Theresa Park). Similarly, in 2011 (post-fishways) there was also a significant increase in the size of the smallest and largest fish, due to smaller fish downstream of reach 7 (Figure 5; Table 9). In October-December 2012 (post-fishways), fish were still significantly smaller at downstream sites, but there was no corresponding significant increase in the size of large fish. The pattern in April-June 2012 is less clear, but there does appear to be a mixture of small and large fish in the upper sites (Figure 5). The magnitude of the correlation coefficients of the smaller fish with upstream location was smaller in post-fishway samples (Table 9) suggesting the relationship may be weaker and smaller fish may be tending to migrate up the system.

Table 6 **Summary of PERMANOVA results for individual fishways. Significant P values (α = 0.05) are in bold.**

	df	SS	MS	Pseudo-F	*P*	*df*	SS	*MS*	Pseudo-F	*P*
Penrith						**Wallacia**				
B*v*A	1	2502	2502	2.72	0.051	1	2598	2598	3.54	**0.029**
S	1	749	749	3.33	**0.016**	1	2233	2233	3.71	**0.012**
Yr(B*v*A)	2	1544	772	0.87	0.568	2	1234	617	0.52	0.825
B*v*A x S*	1	644	644	3.02	0.020	1	1806	1806	3.11	**0.029**
Se (Yr(B*v*A))	4	3375	844	1.52	0.199	4	4716	1179	2.07	0.118
Yr (B*v*A) x S	2	473	236	1.87	0.127	2	980	490	1.84	0.140
Se (Yr(BvA)) x S	2	253	126	0.23	0.996	2	532	266	0.47	0.891
Residual	2	1110	555			2	1142	571		
Theresa Park						**Brownlow Hill**				
B*v*A	1	5038	5038	6.66	**0.006**	1	3043	3043	2.26	0.096
S	1	1475	1475	2.91	0.060	1	1579	1579	1.19	0.356
Yr(B*v*A)	2	1131	565	1.20	0.369	2	2030	1015	1.29	0.314
B*v*A x S*	1	905	905	1.82	0.175	1	492	492	0.46	0.890
Se (Yr(B*v*A))	5	1924	385	2.16	0.233	4	3152	788	2.02	**0.047**
Yr (B*v*A) x S	2	895	448	1.30	0.359	2	1987	993	2.54	**0.022**
Se (Yr(B*v*A)) x S	2	691	345	1.94	0.265	4	NA	NA		
Residual	1	178	178			15	1563	-391		
Mt Hunter						**Cobbitty**				
B*v*A	1	1871	1871	2.10	0.090	1	849	849	0.70	0.708
S	1	447	447	1.36	0.305	1	638	638	1.42	0.347
Yr(B*v*A)	2	1537	769	0.70	0.770	2	2328	1164	1.55	0.248
B*v*A x S*	1	989	989	2.37	0.085	1	300	300	0.91	0.517
Se (Yr(B*v*A))	4	4365	1091	1.95	0.068	4	3010	752	1.23	0.328
Yr (B*v*A) x S	2	713	357	0.64	0.766	2	888	444	0.72	0.695
Se (Yr(B*v*A)) x S	4	NA	NA			4	NA	NA		
Residual		2237	559				2452	613		

	df	**SS**	**MS**	**Pseudo-F**	***P***	*df*	**SS**	***MS***	**Pseudo-F**	***P***
Sharpes						**Camden**				
B*v*A	1	937	937	0.57	0.878	1	2240	2240	0.98	0.527
S	1	298	298	0.38	0.824	1	932	932	1.97	0.185
Yr(B*v*A)	2	3320	1660	2.44	**0.017**	2	3395	1697	3.83	**0.011**
B*v*A x S*	1	808	808	0.95	0.502	1	132	132	0.43	0.810
Se (Yr(B*v*A))	5	2839	568	2.02	0.080	5	1930	386	0.65	0.819
Yr (B*v*A) x S	2	1553	777	2.76	**0.032**	2	902	451	0.76	0.664
Se (Yr(BvA)) x S	3	NA	NA			3	NA	NA		
Residual		845	282				1782	594		
Menangle						**Douglas Park**				
B*v*A	1	1437	1437	1.19	0.447	1	3158	3158	2.02	0.140
S	1	1376	1376	3.09	0.105	1	2959	2959	2.64	0.081
Yr(B*v*A)	2	1827	914	2.41	0.123	2	2318	1159	1.21	0.348
B*v*A x S*	1	2673	2673	5.68	**0.034**	1	773	773	0.76	0.611
Se (Yr(B*v*A))	4	1516	379	1.11	0.409	5	4422	884	5.89	**0.002**
Yr (B*v*A) x S	2	668	334	0.98	0.491	2	1331	665	4.43	**0.011**
Se (Yr(BvA)) x S	4	NA	NA			3	NA	NA		
Residual		1364	341				451	150		

Table 7 **Summary of pair-wise PERMANOVA results for the differences in the fish community between sites above and below Penrith, Wallacia and Menangle fishways. Signficant Monte Carlo P-values (α = 0.05) are in bold.**

Groups	t	Unique perms	*P*(MC)	Average similarity (%)
Penrith				
B2, A2	2.099	315	0.046	58
B2, B3	1.303	3	0.248	59
B2, A3	1.579	315	0.129	60
A2, B3	1.903	315	0.054	59
A2, A3	1.090	8408	0.337	67
B3, A3	1.245	315	0.249	64
Wallacia				
B4, A4	1.244	314	0.285	61
B4, B5	0.882	3	0.560	49
B4, A5	2.179	315	0.047	58
A4, B5	1.671	315	0.112	46
A4, A5	1.558	8405	0.078	64
B5, A5	2.073	314	0.055	51
Menangle				
B18, A18	2.319	840	0.056	60
B18, B19	1.971	10	0.041	63
B18, A19	0.929	839	0.550	66
A18, B19	1.979	840	0.077	64
A18, A19	2.218	8409	0.031	54
B19, A19	1.126	838	0.365	60

Table 8 Fish species contributing to the mean dissimilarity between the fish assemblages above and below Wallacia Weir before and after fishway installation. Av. Abund is the average abundance (log10(x + 1) of each fish species. Av. Diss is the contribution to between pre- and post-fishway dissimilarity. The contribution to dissimilarity (Contrib%) indicates the proportion of dissimilarity that a species contributes to the overall dissimilarity between pre- and post-fishway groups. Species contributing more than a random proportion are in bold. Note that by random chance each of these species should contribution about 7.5% of the dissimilarity (i.e. 92.5% / 12 species = 7.5%0.

Species	Av. Abund Before	Av. Abund After	Av.Diss	Diss/SD	*Contrib %	Cum.%
Freshwater mullet	1.2	2.8	6.4	1.4	**13.9**	13.9
Sea mullet	1.2	2.3	5.1	1.5	**11.1**	25.0
Freshwater herring	1.0	2.2	5.1	1.3	**11.0**	36.0
Australian smelt	1.3	1.9	4.8	1.3	**10.3**	46.4
Common carp	0.8	1.6	3.6	1.3	**7.8**	54.1
Goldfish	1.2	1.1	3.0	1.4	6.6	60.7
Long-finned eel	1.6	0.9	2.7	1.2	6.0	66.6
Australian bass	2.8	3.0	2.7	1.5	5.9	72.5
Flat-headed gudgeon	1.2	0.8	2.6	1.3	5.8	78.3
Freshwater catfish	0.7	0.9	2.6	1.2	5.7	84.0
Empire gudgeon	0.5	0.7	2.5	1.0	5.5	89.5
Cox's gudgeon	0.0	0.5	1.4	0.7	3.0	92.5

Figure 5 10th and 90th percentile of lengths of Australian Bass collected in each electrofishing trip between March 2009 and June 2013. Only instances where 10 or more fish were collected are presented.

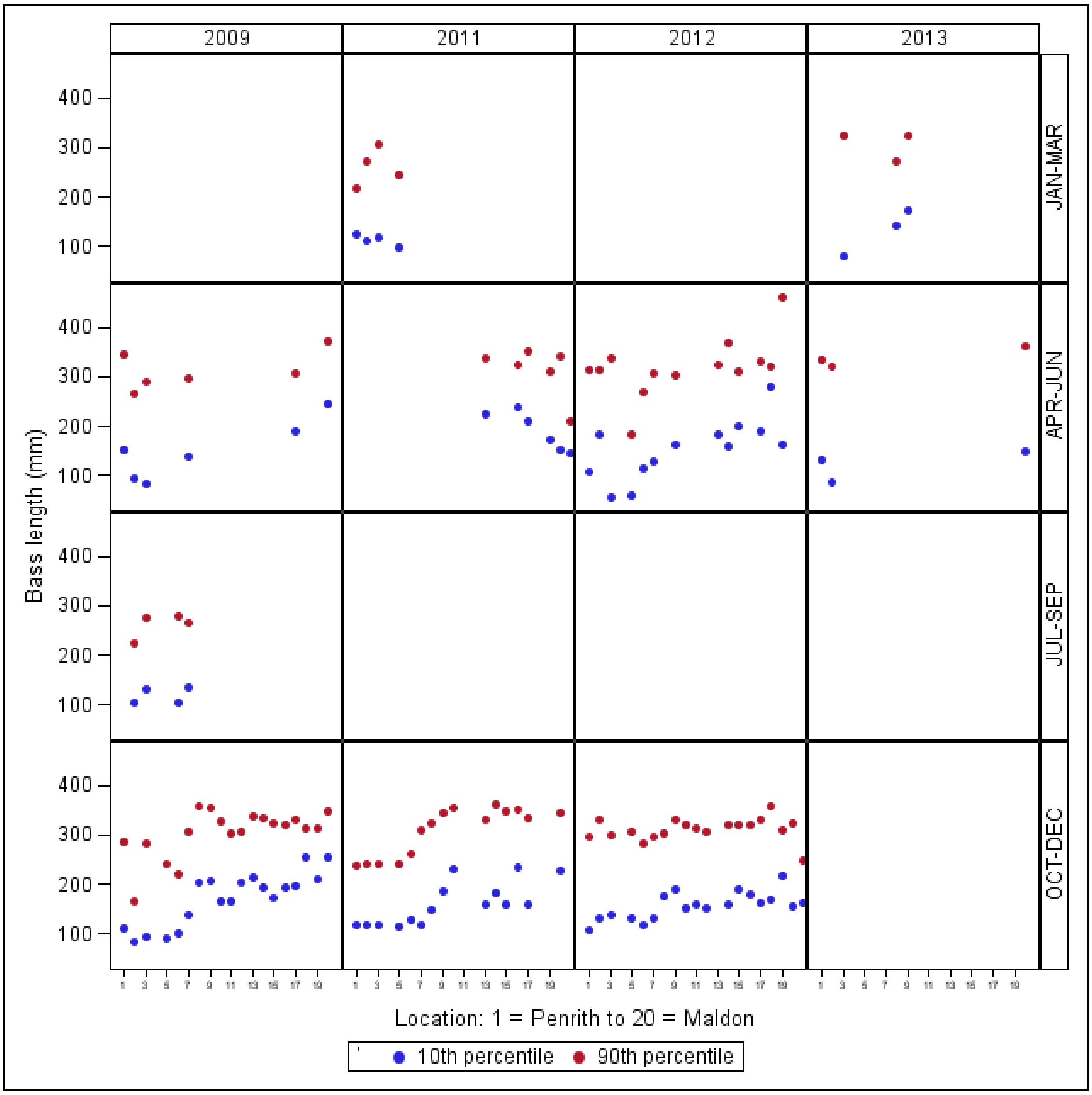


Table 9 Spearman's rank correlation for size of smallest Australian bass (10th percentile) and largest Australian bass (90th percentile) collected at each site and rank order of location of the site in the reach (Penrith to Maldon). Only dates where at least 10 sites are included. Positive correlation value indicates that the length of fish in each percentile increases in an upstream direction. (ns = not significant).

Year	Quarter	Number of sites with ≥ 10 bass	10th percentile		90th percentile	
			R_s	p-value	R_s	p-value
2009	Oct-Dec	19	0.79	0.0001	0.57	0.0109
2011	Oct-Dec	15	0.82	0.0002	0.84	0.0001
2012	Apr-Jun	13	0.69	0.0086	0.44	ns
2012	Oct-Dec	19	0.68	0.0015	0.30	ns

Discussion

Comparison of fish assemblages before and after fishway installation

This study presents evidence that the ten Nepean River weirs were a substantial barrier to native fish migration before the series of new fishways were installed in 2009 and 2010. The lack of adequate fish passage had resulted in significant spatial structuring of the fish community in a downstream to upstream direction. Following fishway installation (and associated provision of environmental flows), this spatial structure was still evident, though not to the same extent, indicating that the fishways have had a positive influence on fish community structure. While analysis of fish communities upstream and downstream of individual fishways did not always detect a statistically significant change pre- and post-fishways, the results of the study taken in their entirety clearly demonstrate that the fish community is undergoing change following fishway installation. Further sampling is recommended to assess ongoing and long term improvements in fish community structure in response to improved fish passage.

Of the 14 native species collected prior to fishway installation, only five species were collected throughout the study reach (i.e. in 90% or more of sites; Australian bass, long-finned eel, Australian smelt, freshwater catfish and flat-headed gudgeon), despite predictions that most would have occurred throughout the reach historically. Further, only two of the ten diadromous species sampled during the study were found throughout the system pre-fishways (Australian bass and long-finned eel), highlighting the sensitivity of this group of fish to the fishways. Sampling inefficiency may account for some species being overlooked in some sampling reaches, particularly the less common species. However, this bias would have effected sampling during both before and after fishway commissioning and the pre-fishway distribution of species is consistent with the findings of a previous study in this area (Baumgartner & Reynoldson 2007). Thus, data presented here is likely to be an accurate reflection of the state of the fish community at the time of sampling. The mean species richness declined in an upstream direction, consistent with two earlier studies that found higher species richness at the most downstream sites (Baumgartner & Reynoldson 2007; Gehrke *et al.* 1996).

After the fishways were installed, three additional species were collected in 90% or more sites (sea mullet, freshwater mullet, Cox's gudgeon), while freshwater herring were collected in 85% of sites. While the overall species richness improved throughout the study reach, there was still a pattern of reduced richness in an upstream direction. Electrofishing and bait-trapping combined with fishway trap data (next chapter) shows that biodiversity and distribution have improved, though not to the full extent of species' historical distributions(Growns *et al*. 2013). The provision of fishways alone may not necessarily encourage all species to return to former habitats. While the Nepean River has received environmental flows concomitantly with provision of fish passage, it is still essentially a series of lentic water bodies interspersed between lotic reaches that do not closely resemble mesohabitats within the original river. The improvements expected from installing fishways, whilst extremely important, are unlikely to match those that result from complete weir removal (Bednarek 2001). Nevertheless, for a river system integral to the water supply and agricultural needs of the Sydney area, the rapid improvements achieved through installation of the Nepean fishways are unprecedented for a coastal river in Australia or worldwide.

The first major fish passage barrier on the river is Wallacia Weir, the second weir in the system. This is not surprising given the first weir, Penrith Weir, was the only weir in the study already fitted with a partially operational fishway (Mallen-Cooper 2009). Wallacia Weir clearly hampered upstream migration of sea mullet, freshwater mullet and freshwater herring, illustrated by the fact that each species dramatically expanded its distributions upstream following installation of the fishway at this weir (Table 3). However, bullrout did not expand its distribution past Wallacia Weir despite being a catadromous species that migrates upstream to maintain populations in freshwater habitats (Miles *et al.* 2009; Pusey *et al.* 2004). Two amphidromous species, empire gudgeon and striped gudgeon, also appeared to be affected by Wallacia Weir. These species are known or presumed to spawn in freshwater. Like other amphidromous fishes, there is evidence that early life stages utilise the marine or estuarine environment (Miles *et al.* 2009; Pusey *et al.* 2004) and upstream migration is necessary for maintenance of populations in freshwater habitats. The new fishways have allowed striped gudgeon to expand their distribution a short distance upstream to Theresa Park Weir (next chapter). In contrast, the distribution of empire gudgeon and bullrout remains unchanged. This is despite fishway trap data showing that both species successfully pass upstream through the Penrith fishway (next chapter). There are three possible explanations: i) these species are present in low numbers upstream of Wallacia and recovering populations are yet to be detected, ii) the habitat upstream of Wallacia may be unsuitable for these species given they are sensitive to degraded banks and flow regulation (Gehrke *et al.* 1999; Growns *et al.* 1998), or iii) they are naturally lowland species that do not penetrate upstream to the same degree as other diadromous species(Baumgartner & Reynoldson 2007; Gehrke *et al.* 1999; Gehrke *et al.* 2002; Growns *et al.* 2003; Growns *et al.* 1998; Rolls 2011; Pusey *et al.* 2004; Miles *et al.* 2009).

Cox's gudgeon were initially found only at the three most upstream sites within the reach, where they were only present in very low numbers, however they are known to be present in higher numbers at sites further upstream than our study reach (Robinson *et al.* 2013b). Following fishway construction, the species was detected at nearly every site and was also recorded in large numbers moving through Penrith, Theresa Park and Douglas Park fishways (next chapter). Given the species' well known ability to climb (Bishop & Bell 1978), its absence at the downstream sites before fishway installation requires further explanation. Cox's gudgeon are capable exploiting both lowland and slope reaches in the systems that they occupy. Previous studies have found that the species is more abundant in unregulated lowland rivers (Gehrke 1997a) and unregulated slope reaches (Gehrke 1997b; Gehrke & Harris 2001; Rolls 2011). In south-east Queensland, Cox's gudgeon are more commonly associated with rapids, riffles and runs (Pusey *et al.* 2004). It is reasonable to suggest that river regulation in the lowlands of the Nepean River created unsuitable conditions for this species given lotic habitat was greatly reduced. The sudden appearance of this species in high numbers throughout the lowland reaches suggests that reinstatement of environmental flows have restored habitat conditions more conducive to this species given that it was rarely reported from lowland reaches prior to fishway installation (Baumgartner & Reynoldson 2007; Gehrke *et al.* 1996; Gehrke & Harris 2001; Growns *et al.* 2003; Growns *et al.* 1998).

While there has been considerable confusion over the diadromous status of Cox's gudgeon, recent otolith chemistry research suggests it is likely to be 'marginally' amphidromous, i.e. spawning occurs in freshwater and larvae are washed downstream into areas of low salinity within the tidal freshwater-estuary interface (Miles *et al.* 2009). Consequently, fishways appear to be facilitating upstream movement of smaller Cox's gudgeon less capable of climbing over large structures to access upstream habitats (Baumgartner & Reynoldson 2007; Gehrke *et al.* 2002; Robinson *et al.* 2013a; Rolls 2011).

We have not examined recruitment patterns in this study, which are likely to vary from year to year. Favourable recruitment may also be a factor in the observed increased abundance of Cox's gudgeon. Most Cox's gudgeon in the fishways were juveniles, reinforcing the notion that improved recruitment may be related to improved environmental flows. More detailed studies on age, growth and larval ecology would be needed to clarify these hypotheses.

Historical fish community vs. present day fish community

Of the 22 species that distributional modelling predicted should occur somewhere in the study area, seven were not detected. Fisheries records from 1992-2007 have recorded five of these species at sites downstream of the study reach (Appendix 6). Four of these are estuarine (yellowfin bream (*Acanthopagrus australis*), dusky flathead (*Platycephalus fuscus*), goldspot mullet (*Liza argentea*) and Castelnau's herring (*Herklotsicthys castelnaui*)), and are only expected downstream of Penrith Weir and the tidal limit, while one species (common jollytail) should have been present further upstream. It is possible that the latter species may make its way further upstream in the future. The remaining two species predicted to occur but not detected by this study or any other Fisheries records dating back to 1992 are Australian grayling (*Prototroctes maraena*) and Pacific blue-eye (*Pseudomugil signifer*).

Size distributions (Australian bass)

Australian bass are one of only two catadromous species that were regularly found throughout the study reach prior to fishway installation, suggesting they were able to negotiate the older pre-existing fishways and/or were able to migrate past the weirs during high flows. Nevertheless, the data presented here demonstrate that the upstream migrations of both the smallest 10% and largest 10% of fish had been hampered by the weirs. Theresa Park Weir appeared to be the point at which there was a shift in the size of Australian bass downstream and upstream. Consequently, while Wallacia Weir was responsible for the major shift in fish community structure before fishway installation, it didn't appear to have the same effect on Australian bass with respect to the size of fish. It is likely that their relatively strong swimming ability (Bishop & Bell 1978; Mallen-Cooper 1992a) allowed this species to either pass over the weir in high flows (assuming the modelling of 1:100 year drown-out is an overestimate) or through the pre-existing pool-and-weir fishway, which was operational whenever the weir spilled. However, it appears Australian bass could not use the pre-existing pool-and-orifice fishway at Theresa Park, which was blocked and inoperable, or the later rock-ramp fishway installed in 2000.

Following the installation of new fishways in 2009 and 2010, the smallest 10% of fish were still significantly larger at upstream sites, but the largest 10% were no longer significantly different in length throughout the study reach. These data may suggest that the smallest fish are still hampered in their upstream migration. However, this assumption may be incorrect given juvenile fish migrate upstream during the spring and summer months after spending their first few months of life in the estuaries and reaching 100 mm by the end of their first year. Thus will be growing rapidly as they migrate upstream (Harris & Rowland 1996; Mallen-Cooper 1992a). Therefore, the smallest fish upstream are expected to be larger than at the most downstream sites, as reflected in the post-fishway results showing a more gradual increase in size of the smallest fish in an upstream direction. The results of this component of the study are important given that it may have been erroneously assumed that the species was unaffected by the weirs if total catch alone had been analysed. Similarly skewed size distributions have been presented for species occurring upstream and downstream of barriers elsewhere, including Cox's gudgeon (Gehrke *et al.* 2002; Robinson *et al.* 2013a), long-finned eels (Gehrke *et al.* 2002) and Australian smelt (Baumgartner & Reynoldson 2007), reiterating the importance of not relying on abundance alone when assessing the impact of barriers on a fish species.

Despite the fact that this study only collected three years of post-fishway data, early results demonstrate several positive changes in the fish community. The distribution of several diadromous species was dramatically increased and the distributions of other potentially less mobile diadromous fishes have also begun to expand. In addition, the fishways have allowed smaller size classes of at least one species (Australian bass) to migrate upstream. While the Nepean River fishways do not negate all of the problems created by weirs such as sedimentation, bank erosion and predominance of lentic habitats, they do allow connectivity between otherwise fragmented populations, assisting in restoration of degraded fish communities. Further sampling over the coming years would provide additional data on the ongoing response of the fish community to the new fishways.

Assessment of the performance of three vertical-slot fishways in the Nepean River

Introduction

Worldwide, barriers to fish migration adversely affect fish populations by preventing upstream and downstream migration, causing injury when fish pass over spillways into the downstream pool, and modifying the hydrological regime (Gough *et al.* 2012). Fish passage at these structures is typically provided by installing a fishway, of which there are many different designs. The type of fishway selected will depend on the hydrological conditions at the installation site and the biological characteristics of the target species, such as swimming ability (Larinier 2002). Fishways constructed in Australia up to the mid-1980s were based on designs for salmonids and as such tended to be inefficient at passing the comparatively poorly swimming native species (Harris 1984; Kowarsky & Ross 1981; Mallen-Cooper & Brand 2007; Russell 1991). Since the mid-1980s, the design of fishways used in Australia has been refined to better suit the swimming ability of the native fish community (Mallen-Cooper 1992a; Mallen-Cooper 1994).

The Nepean River of coastal eastern Australia supports a diverse fish community including many diadromous species requiring access to estuaries/sea to complete their life cycle (Table 2). The length of species in the Nepean River rangesfrom approximately 30 mm (e.g. Australian smelt, dwarf flat-headed gudgeons (*Philypnodon macrostomus*) and juvenile Australian bass) to greater than one metre (long-finned eel). The Nepean River is highly modified, several major dams within its catchment (the largest being Warragamba, Cataract, Nepean, Avon and Cordeaux Dams). There are twelve weirs along the length of the Nepean River from Penrith upstream to Pheasants Nest (Figure 1). While there were existing fishways on some of these weirs, they were not operating effectively, obstructing upstream migration of many species(Baumgartner & Reynoldson 2007; Gehrke *et al.* 1999; Gehrke *et al.* 1996; Harris *et al*. 1996; Mallen-Cooper 2009). Consequently, ten new low–turbulence vertical-slot fishways were designed that utilised a variable baffle shape and provided a low maximum water velocity of 1.4 m/s (Mallen-Cooper 2009). These criteria resulted in a floor slope of 1:20 and an average head loss per baffle of 100 mm. The new fishways were designed to pass fish within the full length range of species in assemblage, with passage for both small and large-bodied fish during low flows and large-bodied fish during high flows. Fishways were retrofitted to all ten weirs from Penrith to Douglas Park, with construction commencing in 2009 and all fishways fully operational by December 2010.

Given the very broad size range of fish the fishways were designed to pass, it was important to carry out monitoring to ensure the fishways are operating to design specifications. This can be achieved by comparing the fish community in the entrance of the fishway to that in the exit; the assumption being that all fish can locate the entrance and continue through to the exit. If the species and size ranges caught in the entrance and exit are similar, it can be concluded that the fishways are performing as expected.

The current study uses direct fishway trapping to address two main questions: (i) Are the fishways passing the fish community that is present in the Nepean River ranging in length from 35 mm to >1m in length?, and (ii) Do some size classes within species have greater success at navigating the fishway than others?

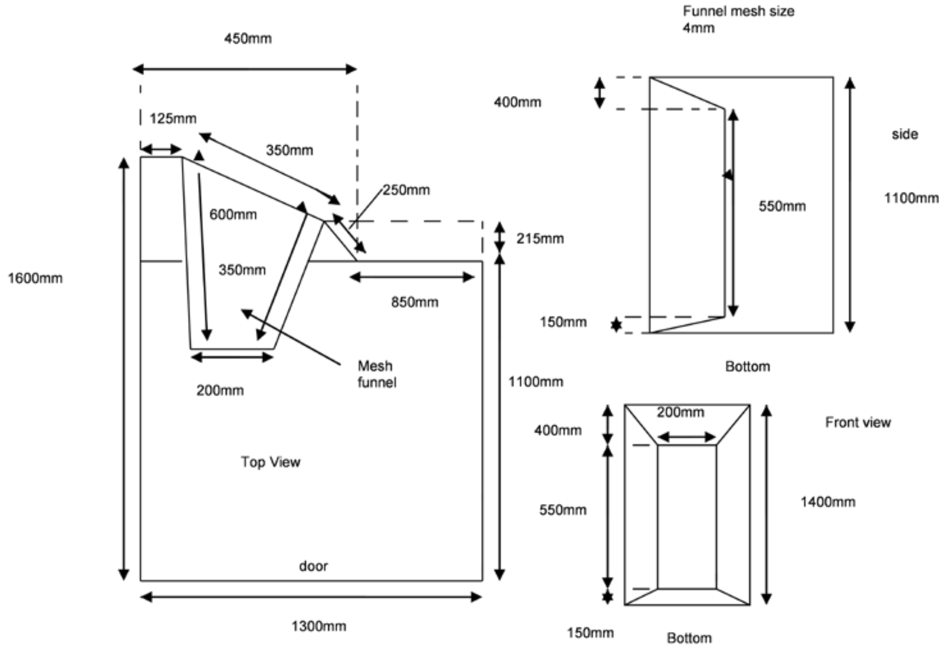
Methods

Fishway Trapping

To supplement the information obtained from fish community sampling, direct trapping was performed within three fishways: Penrith, Theresa Park and Douglas Park. Penrith was selected because it is the most downstream weir in the system. Theresa Park was selected for its location above both Penrith and Wallacia Weirs (the latter weir does not drown-out below the one in one hundred-year flood), thus it is important to determine if migratory species can routinely migrate past these initial barriers. Trapping was performed at Douglas Park Causeway as it is the final barrier in the study area to be fitted with a fishway and the presence of diadromous species here would indicate that the downstream fishways are operating effectively.

Fishway traps were 1.6 m long by 1.3 m wide, with the Douglas Park fish trap 1.1 m high whilst both Penrith and Theresa Park traps were 1.4 m high (Figure 6). The entrance to the trap was aligned to the slot of the fishway and fitted with a mesh and brush funnel to reduce fish escape after entering. The funnels on all traps were 350 mm wide at the entrance and extended back into the trap 600 mm on one side and 350 mm on the other. The exit of the funnel (where fish entered the main body of the fish trap) was 200mm wide and 550 mm high. Fish traps were covered in 4 mm square mesh to ensure small fish were retained. The funnel was covered in 4 mm square mesh and was also fitted with 80 mm long nylon brushes to enable the cage to cover the entire vertical-slot. Traps were deployed and retrieved from the fishway using a gantry crane (Graham Handling Equipment) and Hitachi electric hoist (Model 1S1, capacity 1000 kg).

Figure 6 **Design of fishway trap used to sample Douglas Park fishway.**



Trapping was conducted within the peak fish migration period of spring and summer (September to March) from 2010 to 2013. Monitoring involved a point-quantification of the number of fish i) attempting to ascend the fishway (entrance trapping), and ii) successfully ascending the fishway (exit trapping). Trapping was stratified so that a paired ‘entrance’ and ‘exit’ sample were performed in a 48-hour period, with traps set for 24 hours at each location. The order of entrance and exit samples was randomised during each sampling week to avoid sampling bias. Trapping typically commenced on a Tuesday morning at 08:30 and was completed by 08:30 on a

Saturday. Thus a total of two paired samples at each trapping location were collected in each trapping period (Appendix 7).

Entrance trapping provides a sample of migrating fish in the river and exit trapping is a sample of those fish that are migrating and successfully ascend the fishway. The entrance sample assumes that migrating fish can locate the fishway entrance and that there is no behavioural inhibition to enter the fishway. To ensure swimming ability is not a limiting factor in this sample and that the weakest swimming fish, which are usually the smallest, can enter the fishway, the velocity at the fishway entrance was reduced by inserting stop logs and reducing discharge at the exit of the fishway to approximately 1.0 m/s (50 mm head loss) (Mallen-Cooper 1999; Mallen-Cooper & Brand 2007). The entrance head loss was measured at the beginning and end of each sample to ensure it had not changed substantially. Exit trapping was conducted with the fishway under normal operation and without flow-control stop logs.

Following a 24-hour trapping period, the trap was retrieved and all fish were transferred to an aerated tub of river water, identified, counted and up to a maximum of 50 fish per species were measured for fork length (FL - fork tailed species) or total length (TL - all other species).

This experimental design enables a comparison of fish species and sizes attempting to migrate upstream (based on the entrance samples) and whether they are successful in ascending when the fishway is operating under normal conditions (based on the exit samples). If fish communities at the fishway exit are statistically similar to those at the entrance, or have a broader range of species or sizes then the fishway is regarded as operating successfully.

Data Analysis

Fish community structure

Data analyses were conducted using S-PLUS (TIBCO Software Inc., Palo Alto, CA) and PRIMER v6 (PRIMER-E Ltd, Ivybridge, UK). The number of fish of each species caught was standardised by time (number per 24 hours) and $\log_e$ (X+1) transformed. Bray-Curtis similarities were calculated from the transformed data and used in all subsequent analyses. Each fishway was analysed separately. To determine if there were significant differences in the fish community structure in the entrance and exit of the fishways, permutational analysis of variance (PERMANOVA) (Anderson *et al.* 2008) was conducted. To assess whether any differences between entrance and exit were independent of the timing of sampling, the model consisted of four factors: entrance/exit (fixed factor), season (fixed factor), month (nested within season; random factor) and year (random factor). Significance values were based on 9,999 permutations of the data. Multidimensional scaling plots (MDS) were used to visually compare the fish community at the entrance and exit of each fishway. Ordination stress values are included where a stress value of less than 0.2 indicates a useful representation of data points within the ordination space (Clarke & Warwick 2001).

Size of species ascending fishways

Two-tailed Kolmogorov-Smirnov tests (KS) (Sokal & Rohlf 1996) were performed to assess differences in length-frequency distributions between the entrance and exit of each fishway of individual species where at least 20 fish were caught in the entrance and exit. If the fishway is successfully passing the full size distribution of each species, the KS test will find a non-significant difference in the length-frequency distributions between entrance and exit, or significant difference with a wider size range at the exit.

Results

Prior to entrance sampling, the head loss through the fishway entrance slot was reduced to as close to 50 mm (normally 100 mm) as possible but varied widely depending on river flow. Head loss at Penrith ranged from 30-240 mm pre-trapping and 20-200 mm post-trapping, Theresa

Park ranged from 30-150 mm pre-trapping and 15-160 mm post-trapping and Douglas Park ranged from 40-120 mm pre-trapping and 20-120 mm post-trapping.

Fish species sampled

Twenty-seven paired entrance and exit samples were collected at Penrith and Theresa Park, while 24 paired samples were collected at Douglas Park. A total of 26,139 fish representing 19 species were caught across all three fishways and there was a higher abundance sampled in the exit than the entrance of fishways (Table 10). Empire gudgeon were the most commonly sampled species at Penrith fishway (43% of catch), while Cox's gudgeon was the most commonly sampled species at Theresa Park and Douglas Park (35% and 80% of catch respectively). Australian bass were also frequently sampled at all three fishways, while alien species (carp, goldfish and gambusia) were rarely sampled. Two species of fish native to the Murray-Darling Basin were sampled; silver perch and freshwater catfish, the latter species at all three fishways (Table 10).

Many species were sampled at both the entrance and the exit of the fishways, though some species were sampled primarily at either the entrance or the exit. For example, 2,368 freshwater herring were sampled from the exit of the Penrith fishway, but only three were sampled from the entrance (Table 10). In contrast, flat-headed gudgeon (*Philypnodon grandiceps*) were largely collected at the entrance of the fishways with 41 and 20 fish sampled from the entrance of Penrith and Douglas Park fishways respectively, but none in the exit, and 79 in the entrance of Theresa Park fishway and only three in the exit (Table 10). Firetail gudgeon (*Hypseleotris galii*), dwarf flat-headed gudgeon, freshwater catfish, long-finned eel, short-finned eel, carp, gambusia and goldfish were all sampled, but in low numbers throughout the study.

Table 10 **Total number of fish caught in the entrance and exit of Penrith, Theresa Park and Douglas Park fishways. Data from each of the paired sample days (27 paired sample days for Penrith and Theresa Park and 24 paired sample days for Douglas Park) are pooled.**

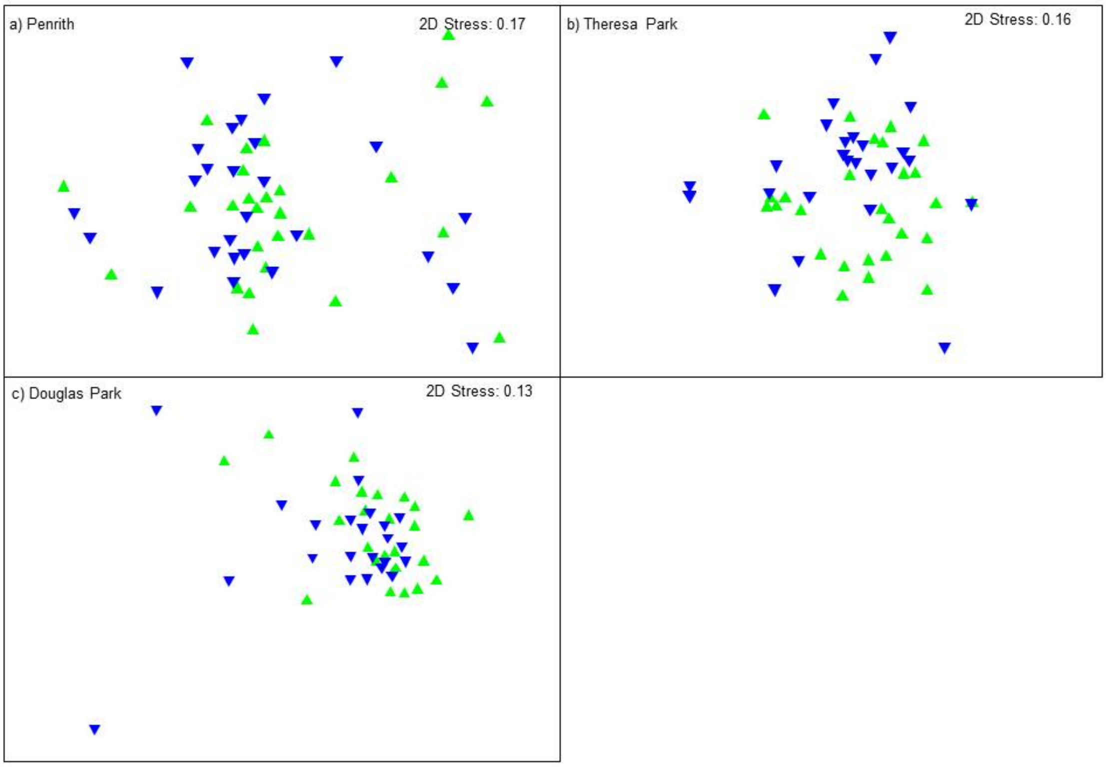
	Penrith		Theresa Park		Douglas Park		Total
Common name	**Entrance**	**Exit**	**Entrance**	**Exit**	**Entrance**	**Exit**	
Cox's gudgeon	801	1,120	188	207	3,989	3,572	9,877
Empire gudgeon	3,472	3,213					6,685
Australian smelt	513	1,336	91	149	794	948	3,831
Freshwater herring	3	2,368		1			2,372
Australian bass	1,045	418	68	172	9	47	1,759
Striped gudgeon	469	386	47	36			938
Freshwater mullet	49	48	2	68		8	175
Flat-headed gudgeon	41		79	3	20		143
Sea mullet	23	92		4			119
Firetail gudgeon	2		8	1	65	12	87
Bullrout	24	58					82
Dwarf flat-headed gudgeon		1	8		12		21
Long-finned eel	1	9	1	2	4	1	18
Common carp		9		2		3	14
Freshwater catfish$^{\pm}$		4		1		5	10
Short-finned eel	1	2					3
Eastern gambusia					2		2
Goldfish			1				1
Silver perch$^{\pm}$		1					1
Total	6,444	9,065	493	646	4,895	4,596	26,139

$^{\pm}$ Native fish introduced to the Hawkesbury-Nepean river system.

Fish community structure

Overall, the fish communities were not significantly different at the entrance and exit of any of the fishways over the course of the study (all $p > 0.05$; Table 11). The only significant effects found in the PERMANOVA analyses of Penrith and Theresa Park fishway communities was a significant interaction between months within season and years ($p = 0.0002$ and $p = 0.0038$, respectively; Table 11). That is, the fish communities varied between months within seasons, and the variation was not uniform across years. Multidimensional scaling ordination of the fish community displays the lack of a significant difference between the entrance and exit of each fishway as evidenced by the entrance and exit samples overlapping within the ordination space (Figure 7).

Figure 7 Multi-dimensional scaling (MDS) ordination of the fish communities in spring and summer sampling between the entrance (green triangles) and exit (blue triangles) at (a) Penrith Fishway, (b) Theresa Park Fishway and (c) Douglas Park Fishway between 2010 and 2013.



Size of species ascending fishways

The three fishways successfully passed a wide size range of fish. Fish in the exit measured 21-1,200 mm (Penrith; Table 12), 23-1,100 mm (Theresa Park; Table 13) and 20-700 mm (Douglas Park; Table 14). Significantly larger Australian smelt were caught at the exit of Penrith and Theresa Park fishways compared to the entrance, but there was no significant difference at Douglas Park. In contrast, many more small sea mullet (≈40 mm) were caught in the exit of Penrith fishway and the largest fish (>220 mm) were also only sampled in the exit. Cox's gudgeon were sampled at all three fishways and had a significantly different size distribution at the entrance and exit of each. These differences for Cox's gudgeon were not consistent, with larger fish at the exit at Penrith and Douglas Park and larger fish at the entrance at Theresa Park.

For most of the species with statistically significant length differences between entrance and exit, the average differences in length were small - only a few millimetres - and not biologically significant (Figure 8; Figure 9; Figure 10). The exception to this was freshwater mullet at Penrith (average of 291 mm and max of 460 mm at the exit; average 144 mm and max 250 mm at the entrance).

Table 11 **Summary of PERMANOVA results evaluating the differences in the fish community between entrance and exit, month, season and year for Penrith, Theresa Park and Douglas Park fishways. df, degrees of freedom; SS, sum of squares. Significant P values are in bold.**

	Penrith				Theresa Park				Douglas Park			
Factors	**df**	**SS**	**Pseudo-F**	**P**	**df**	**SS**	**Pseudo-F**	**P**	**df**	**SS**	**Pseudo-F**	**P**
Main effects												
Entrance/exit	1	4453.3	2.5878	0.0828	1	2137.1	0.8522	0.5614	1	2679.0	2.8239	0.1122
Year	2	12140.0	2.1580	0.1296	2	12337.0	2.0430	0.2132	2	2837.7	1.2729	0.3930
Season	1	10632.0	1.5258	0.2972	1	3971.8	0.5804	0.7530	1	3542.0	2.8851	0.1532
Month (Season)	5	27522.0	2.0559	0.1581	5	24622.0	1.8999	0.1786	4	6179.4	1.5418	0.3190
Interactions												
Entrance/exit x Year	2	1386.8	0.5570	0.7727	2	3291.1	1.5993	0.3016	2	1287.1	1.6962	0.2641
Entrance/exit x Season	1	1150.7	1.0631	0.4725	1	615.9	0.6091	0.5978	1	648.0	1.9231	0.2879
Season x Year	1	3950.0	1.4344	0.2951	1	6524.6	2.0699	0.1904	1	344.9	0.3647	0.7227
Entrance/exit x Month (Season)	5	7898.2	1.2219	0.3537	5	11994.0	2.5010	0.0953	4	1481.4	0.9695	0.5462
Month (Season) x Year	3	7615.0	3.5045	**0.0002**	3	7580.2	2.7802	**0.0038**	2	2050.7	1.8372	0.1090
Entrance/exit x Year x Season	1	815.7	0.6451	0.5844	1	435.9	0.5793	0.4995	1	189.3	0.6429	0.5622
Entrance/exit x Month (Season) x Year	3	3752.8	1.7271	0.0596	3	2822.2	1.0351	0.4374	2	745.5	0.6679	0.6585
Residual	28	20280.0			28	25448.0			25	13953.0		

Table 12 The number of fish measured and average length of fish in the entrance and exit of Penrith fishway during 27 paired sample days. Kolmogorov-Smirnov (KS) tests are given for species where more than 20 individuals were sampled at the entrance and exit. Significant results (p<0.05) are in bold).

Common Name	Number of fish		Average Length (mm)		Size range (mm)	KS Test statistic	P-value	Conclusion
	Entrance	Exit	Entrance	Exit				
Australian smelt	417	675	45.1	46.7	21-72	0.169	**0.000**	Smaller fish in entrance
Cox's gudgeon	527	509	39.0	39.7	21-156	0.143	**0.000**	Smaller fish in entrance
Empire gudgeon	375	379	39.2	39.2	22-60	0.061	0.455	No difference
Australian bass	319	311	61.4	69.1	30-337	0.152	**0.001**	Smaller fish in entrance
Striped gudgeon	179	146	43.3	43.2	26-65	0.037	0.999	No difference
Freshwater herring	3	153	134.0	149.2	92-233			Mid-sized fish not in entrance
Sea mullet	23	92	101.0	114.8	32-410	0.446	**0.001**	Smaller fish in entrance
Freshwater mullet	49	48	144.1	291.0	123-460	0.709	**0.000**	Smaller fish in entrance
Bullrout	24	58	82.9	85.2	52-230	0.185	0.497	No difference
Flat-headed gudgeon	41				42-77			
Long-finned eel	1	9			95-1,200			
Common carp*	0	9			600-740			
Freshwater catfish[†]		4			420-462			
Short-finned eel	1	2			105-770			
Firetail gudgeon	2				34-37			

Silver perch[†]		1	199
Dwarf flat-headed gudgeon		1	54
Site Total	**1,961**	**2,397**	

*Alien species
† Native species introduced to the H-N catchment

Table 13 The number of fish measured and average length of fish in the entrance and exit of Theresa Park fishway during 27 paired sample days. Kolmogorov-Smirnov (KS) tests are given for species where more than 20 individuals were sampled at the entrance and exit. Significant results ($p<0.05$) are in bold.

Common Name	Number of fish		Average Length (mm)		Size range (mm)	KS Test statistic	P-value	Conclusion
	Entrance	Exit	Entrance	Exit				
Cox's gudgeon	185	207	48.2	43.7	23-119	0.232	**0.000**	Smaller fish in exit
Australian bass	67	170	144.8	179.2	95-402	0.313	**0.000**	Smaller fish in entrance
Australian smelt	91	130	44.2	48.4	31-60	0.341	**0.000**	Smaller fish in entrance
Flat-headed gudgeon	79	3			29-72			
Striped gudgeon	47	36	47.0	44.4	37-60	0.242	0.149	No difference
Freshwater mullet	2	68			192-376			
Firetail gudgeon	8	1			28-36			
Dwarf flat-headed gudgeon	8				41-49			
Sea mullet		4			161-218			
Long-finned eel	1	2			490-1,100			
Common carp*		2			720-760			
Goldfish*	1				162			
Freshwater herring		1			190			
Freshwater catfish†		1			425			
Site total	**489**	**625**						

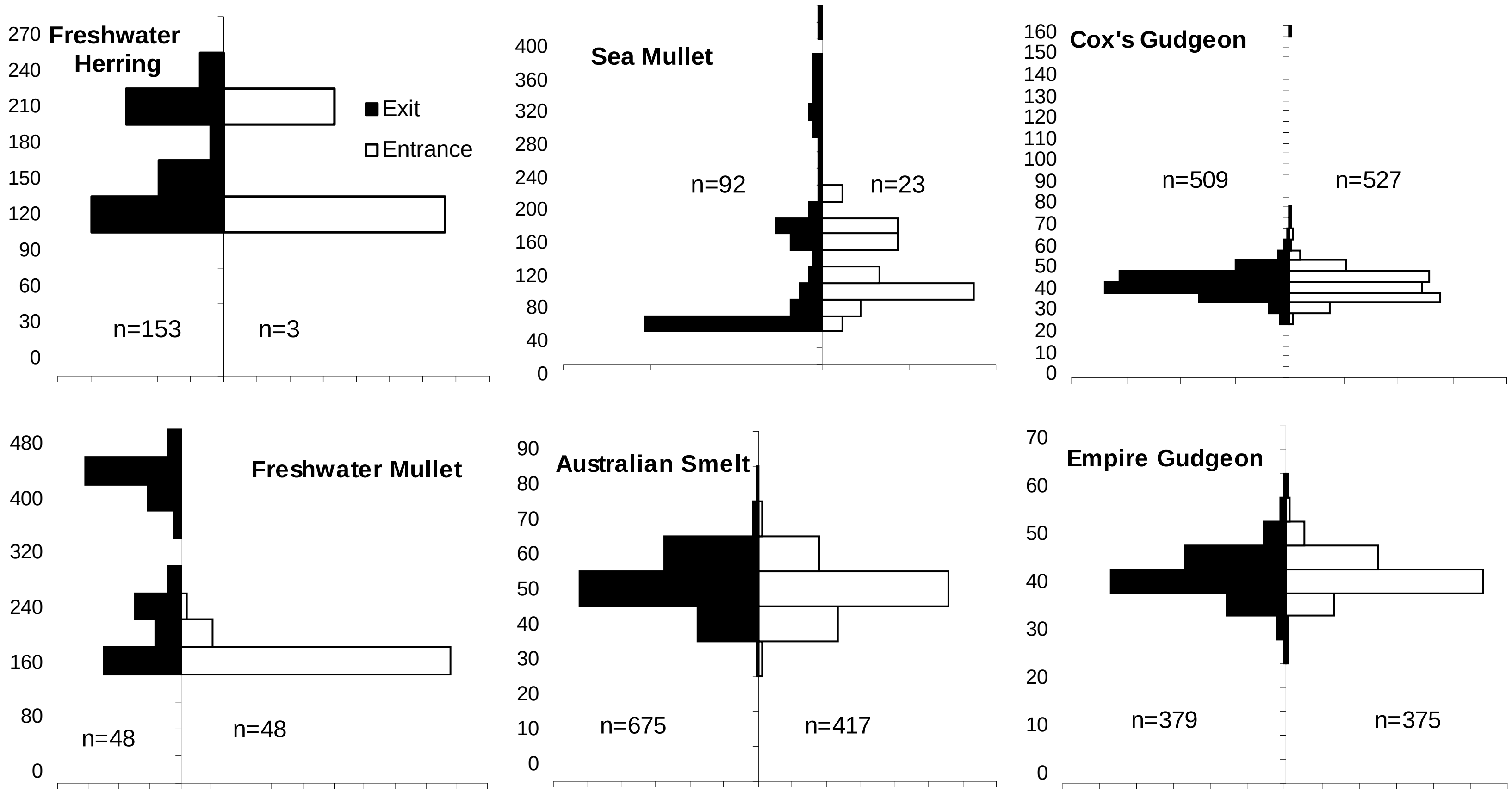
*Alien species
† Native species introduced to the H-N catchment

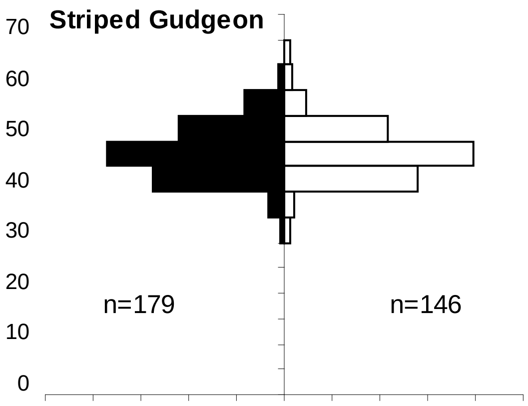
Table 14 The number of fish measured and average length of fish in the entrance and exit of Douglas Park fishway during 24 paired sample days. Kolmogorov-Smirnov (KS) tests are given for species where more than 20 individuals were sampled at the entrance and exit. Significant results ($p<0.05$) are in bold.

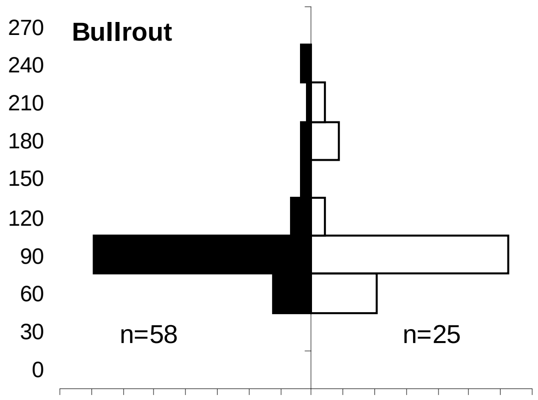
Common Name	Number of fish		Average Length (mm)		Size range (mm)	KS Test statistic	P-value	Conclusion
	Entrance	Exit	Entrance	Exit				
Cox's gudgeon	884	777	43.2	42.3	20-119	0.179	**0.000**	Larger fish in exit
Australian smelt	525	600	44.6	44.8	21-69	0.032	0.923	No difference
Firetail gudgeon	65	12	37.9	36.3	31-49			
Australian bass	9	47	173.1	207.5	90-375			
Flat-headed gudgeon	20				31-65			
Dwarf flat-headed gudgeon	12				30-47			
Freshwater mullet		8			230-265			
Long-finned eel	4	1			380-900			
Freshwater catfish[†]		5			470-530			
Common carp*		3			481-700			
Eastern gambusia*	2				35-41			
Site total	**1,521**	**1,453**						

*Alien species
† Native species introduced to the H-N catchment

Figure 8 Relative length frequency distributions for the species most commonly sampled from the entrance and exit of Penrith fishway (each horizontal axis represents 10%). Note the vertical scale is length (mm) and is different for each species.




Striped Gudgeon
70
60
50
40
30
20
10
0
n=179
n=146


Bullrout
270
240
210
180
150
120
90
60
30
0
n=58
n=25

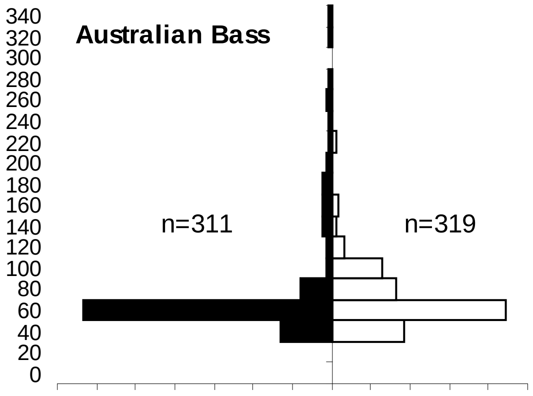

Australian Bass
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
n=311
n=319

Figure 9 Relative length frequency distributions for the species most commonly sampled from the entrance and exit of Theresa Park fishway (each horizontal axis represents 10%). Note the vertical scale is length (mm) and is different for each species.

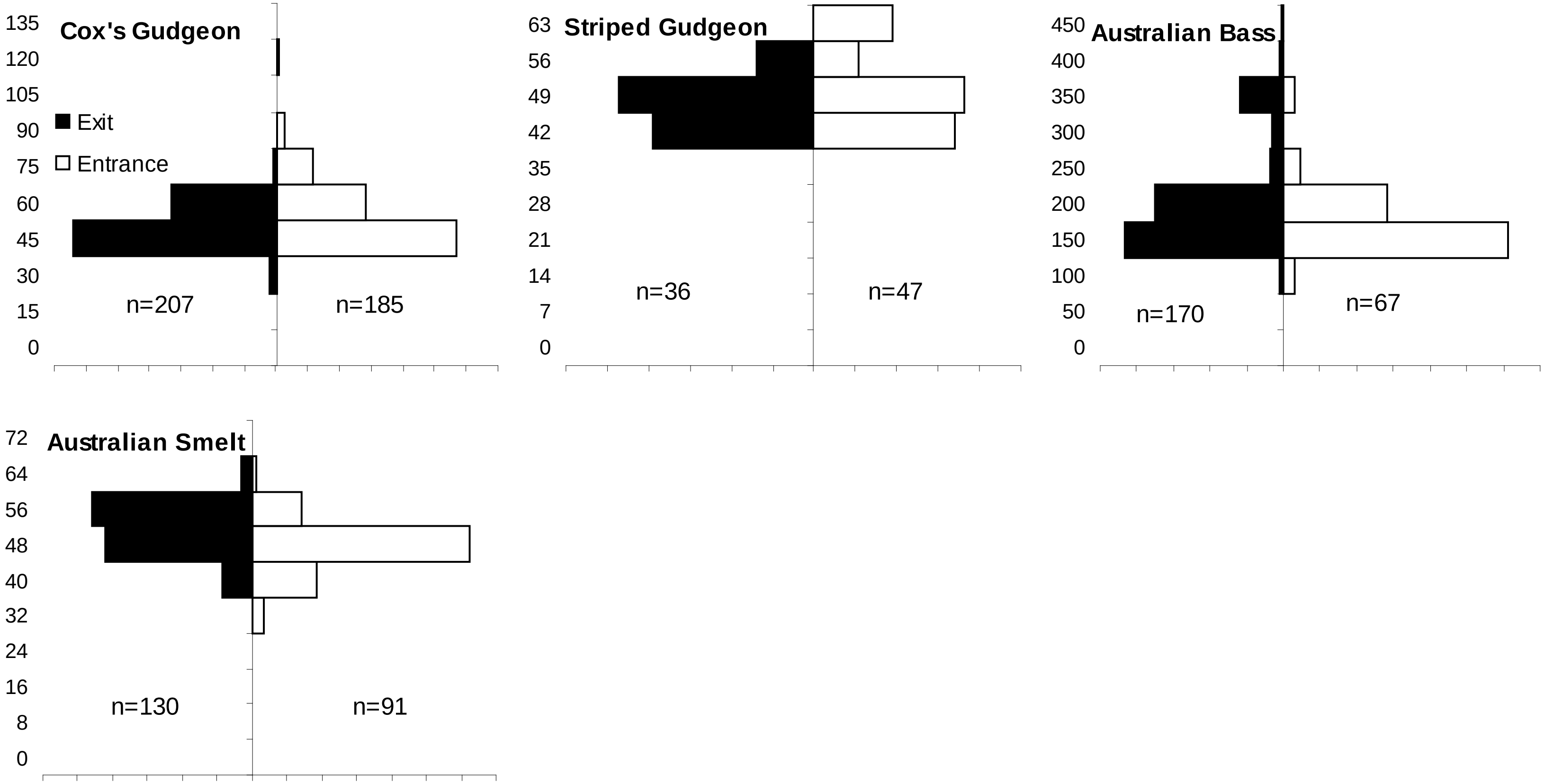
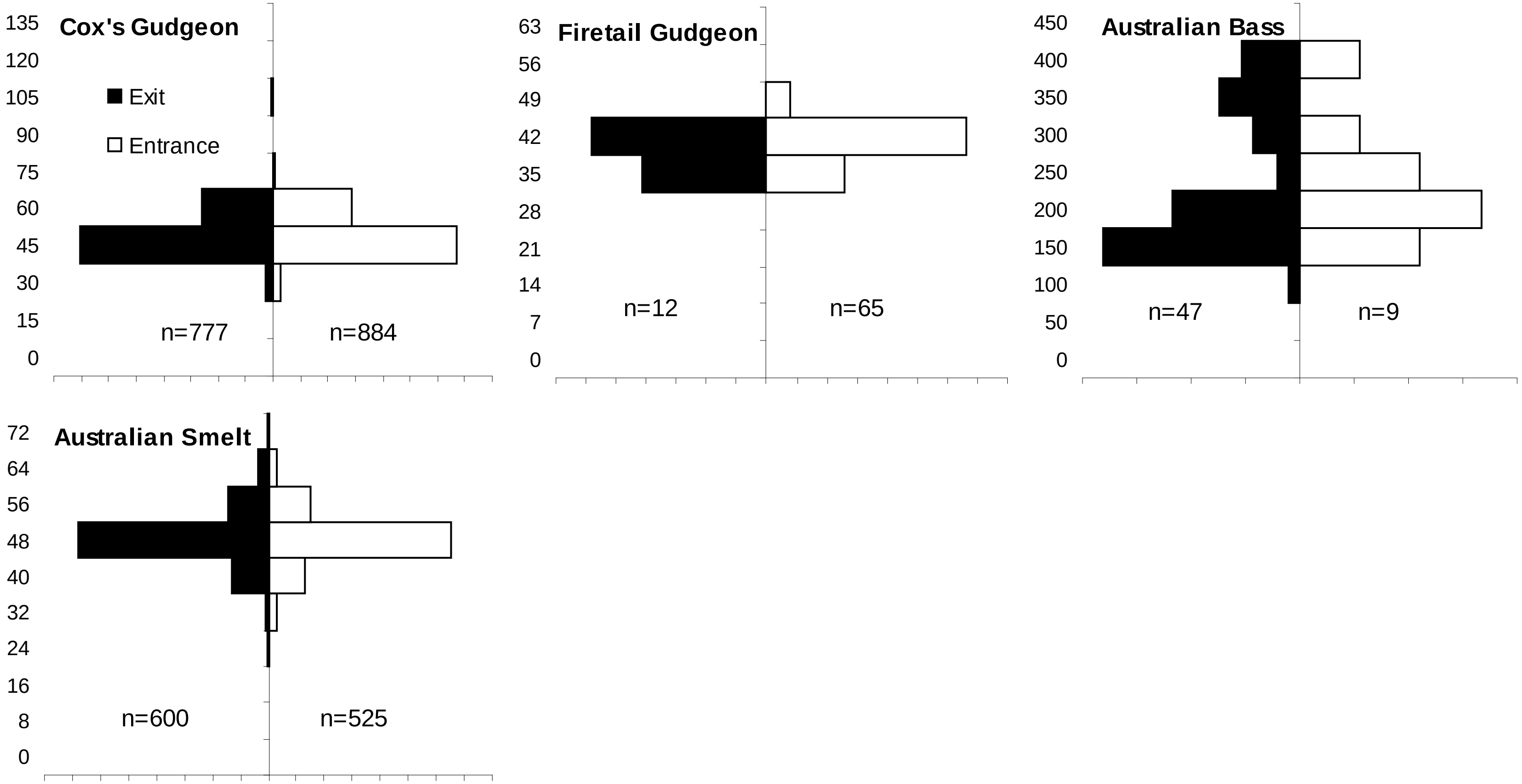


Figure 10 **Relative length frequency distributions for the species most commonly sampled from the entrance and exit of Douglas Park fishway (each horizontal axis represents 10%). Note the vertical scale is length (mm) and is different for each species.**



Discussion

Fish species using the fishway

Fishway trapping at Penrith, Theresa Park and Douglas Park demonstrated that all three fishways are passing the size range of fish according to their design specifications. Each fishway was successfully passing fish between 35 mm and 1000 mm as well as many fish that were considerably smaller than the minimum of 35 mm.

The fish assemblage as a whole was found to be the same at the entrance and the exit of the fishways, and individual species comparisons show the fishways were successfully passing most species. There were, however, exceptions with three small-bodied potamodromous species. Flat-headed gudgeon were more abundant in the entrance samples while dwarf flat-headed gudgeon and firetail gudgeon were rarely or never captured in the exit of the fishways. These results are despite many small-bodied species (<100 mm) successfully reaching the fishway exit, including Australian smelt, empire gudgeon and striped gudgeon.

There are three possible explanations for these data, that these species:

i) were not migrating but entering the entrance chamber to feed or seek shelter,

ii) had high trapping efficiency in the entrance samples but not the exit samples,

iii) have a very poor swimming ability and are unable to ascend the fishways.

It is possible these species may not have been attempting to migrate upstream and their presence in the entrance of the fishway could be related to the fish seeking shelter or food. Larger predatory fish can utilise the entrance or lower pool of a fishway to feed, especially when the velocity is reduced as this enables very small aquatic fauna such as juvenile shrimp to enter the fishway and provide a ready food source (Mallen-Cooper pers. comm.). However, this behaviour has not been observed for small-bodied fishes.

The movement biology of these species is also not comprehensively known, though there is some evidence that flat-headed gudgeon (and possibly some misidentified dwarf flat-headed gudgeon) undertake both downstream and upstream migrations (Jennings et al. 2008; Pusey et al*et al*. 2004, and references therein; Stuart *et al.* 2008). Firetail gudgeon may also undertake upstream migrations following increases in discharge (Pusey *et al*. 2004). Thus it is possible that flat-headed gudgeon (and possibly also dwarf flat-headed gudgeon) and firetail gudgeon sampled in the Nepean fishways are genuinely attempting to migrate upstream.

The failure of many individuals to be recorded at the exit of the fishway could also be due to trapping efficiency. Cone-traps are a common design in artisanal fisheries and they are usually more efficient with more discharge rather than less discharge, as fish find it harder to locate the cone and escape. Entrance trapping was conducted by reducing flows through the fishway while exit trapping was carried out under normal hydraulic conditions with more discharge; thus the hydraulic conditions within the traps were different. Hence, it seems unlikely that flat-headed gudgeon and the other two species had higher escapement rates at the exit.

Swimming ability is very closely related to body form (Videler & Wardle 1991) which gives an indication of the swimming ability of flat-headed gudgeon, dwarf flat-headed gudgeon and firetail gudgeon. A percoid-shaped fish such as Australian bass has a generalist shape that will have good acceleration and burst speed, with average prolonged swimming ability. Freshwater herring are more streamlined with a forked tailed; they have good burst speed, reasonable acceleration and above average prolonged swimming ability. Bullrout are less streamlined and have large pectoral fins with a round tail which provides short distance acceleration and burst speed – suitable for an ambush predator - but very poor prolonged swimming ability. Flat-headed gudgeon are reasonably streamlined with a large tailfin for their body size (Figure 11), suggesting they have very good burst swimming ability. Flat-headed gudgeon have a similar body shape compared with striped gudgeon (Figure 12) which successfully ascends the Nepean fishways.

Figure 11 Flat-headed gudgeon. (Source: Gunther Schmida)

Figure 12 Striped gudgeon (Source: Australian Museum, copyright Robert McCormack).

Laboratory experiments on the swimming ability of flat-headed gudgeon confirm high burst swimming ability (Bice 2004), but poorer prolonged swimming ability compared to Australian smelt (Kilsby & Walker 2010), which has a very streamlined body shape. In a vertical-slot fishway, fish need to use burst swimming ability to negotiate the slot in the fishway baffle and then either rest in the pools or use prolonged swimming ability to reach the exit. Flat-headed gudgeon actively seek velocity refuges (Kilsby & Walker 2012) and so could be expected to find these in the fishway pools.

Flat-headed gudgeon have been recorded negotiating vertical-slot fishways in other systems. At the Murray River barrages in South Australia, flat-headed gudgeon 21-58 mm in length pass through a vertical-slot fishway with higher velocities and turbulence than the Nepean fishways, but it is a short two-pool fishway (Stuart *et al.* 2005). The Tinana Barrage fishway (Mary River, Queensland) has a vertical-slot fishway that is 13 to 26 pools long (depending on the tide) and has the same maximum water velocity as the Nepean fishways and a slightly higher turbulence. Monitoring of this fishway reported similar results as the Nepean fishways for empire gudgeon (Figure 13), passing fish 25-60 mm in length, but also passes flat-headed gudgeon 20-60 mm in length.

Figure 13 Empire gudgeon (Source: Australian Museum, copyright Robert McCormack).

Of the three possible explanations for the lack of flat-headed gudgeon at the exit of the fishways it seems unlikely that, given the species body shape and its successful passage in other fishways. It is possible these fish were using the entrance of the fishways as shelter or for feeding. Only further investigation would explain their behaviour.

Empire gudgeons may provide an effective indicator of the ability of small-bodied fish to ascend vertical-slot fishways. Passage of this species is inhibited in vertical-slot fishways with turbulence > 40 Watts/m^3 similar to other small-bodied fish species of the same size (Stuart & Mallen-Cooper 1999; Stuart & Berghuis 2002). The design specifications of the Nepean fishways results in turbulence of 35 Watts/m^3 at low river flows (i.e. the weir just spilling). Data in the present study shows that empire gudgeons were ascending the fishways successfully under these conditions. The passage of this species provides some of the most compelling evidence that the Nepean fishways are passing the smallest fish attempting to migrate and that the fishways represent one of the most effective designs in coastal rivers of Australia at present.

Freshwater herring presented an unexpected result as they were rarely caught in the entrance of Penrith fishway (three fish) but 2,368 were caught in the exit. In addition, only a single individual was caught at Theresa Park fishway (in the exit) despite electrofishing sampling showing the species was caught upstream of this weir. The inconsistent capture of this species in the fishways is likely to be due to a combination of factors. Reducing the flows through the fishway to conduct entrance trapping may have diminished the attraction flow below that necessary to trigger this species to seek out the fishway. It is also possible that, as mentioned above, the entrance and exit traps had differing efficiencies and the exit trap was more efficient for this species. For example, this species may be naturally wary and thus hesitant to enter the first fishway slot when there is a funnel and cage in place. But if they have exhibited the courage to enter the first slot and travel through to the end of the fishway during an exit trapping phase, they may be more confident to then enter a trap. In either case, the presence of this species post-fishways at the majority of sites upstream of Wallacia Weir, and their successful passage through Penrith Weir fishway, is strongly suggestive that the species is successfully ascending the fishways.

Australian bass used the fishways in high numbers, particularly Penrith Fishway. The average size of the fish moving through the fishways increased substantially from Penrith (61.4 mm to

69.1 mm) to Douglas Park (173.1 mm to 207.5 mm). Female Australian bass are much larger than males (Harris & Rowland 1996) and are known to travel upstream following spawning, while males remain in the upper estuaries or lower freshwater reaches (Harris & Rowland 1996; Reinfelds *et al.* 2013). Consequently, the clear increase in size of Australian bass from Penrith to Douglas Park is likely due to the sexual segregation of this species, with young-of-year fish and juveniles tending to remain at the more downstream sites and large, mature females progressing further upstream. The lack of very small Australian bass in the Douglas Park fishway may also be a reflection of its distance from the estuary. By the time fish reach Douglas Park they have likely grown substantially bigger than when they passed through Penrith Fishway.

Size of fish ascending the fishways

The ecological objective of a fishway is to pass 90% of each migratory life stage (whether juvenile, sub-adult or adult) of each species (Mallen-Cooper 2000). The intent of this objective is to restore ecological processes of juvenile and sub-adult dispersal, and adult migrations. Size distributions of fish within fishways are used to measure this objective and the Kolmogorov-Smirnov (KS) test is one of a few tests that compares frequency distributions.

While the fishways in the present study were successfully passing the required size range of fish to meet design specifications, KS tests indicated that the size distributions of many species were different in the entrance and exit of the fishways. However, the utility of this test is limited because it only determines that there is a statistical difference in the distributions, without quantifying the extent of the difference or where it lies, which is important when making biological interpretations. For example, the distributions may have different average lengths, different range of lengths or a different shaped demographic even though the minimum and maximum sizes are the same. Consequently, when interpreting the results of the KS tests, it is important to consider the size distributions, the species ecology and the trapping methods to evaluate whether significant differences detected are ecologically meaningful.

In the present study there were two types of comparative (entrance-exit) distributions that were statistically significant: either larger fish were present at the exit or the distribution within the same size range (minimum-maximum length) was different. Examples of the first group are larger sea mullet and freshwater mullet that were not caught in the entrance of the Penrith fishway, but were caught in the exit. The lack of large fish in the entrance is probably a result of the sampling methodology, as discussed above. Reducing the flow through the fishway when trapping the entrance greatly reduces the flow through the fishway, and thus the attraction flow that is essential to large-bodied individuals is also reduced.

The full size range of sea mullet was detected at the fishway exit, particularly the relatively weaker-swimming juvenile size classes, so the statistically significant difference in size distribution is not ecologically significant and Penrith Fishway is clearly working well for this species. Freshwater mullet would appear to have a significant bias to smaller fish at the entrance, which may suggest that weaker-swimming juveniles are restricted from ascending the fishway. However, these fish are 160 mm, while sea mullet as small as 40 mm were able to ascend the fishway. The two mullet species have the same body shape and are very likely to have similar swimming ability. Hence, again the result appears statistically significant but not ecologically significant.

Examples of size distributions where the range was similar but the distribution within the range was significantly different include Cox's gudgeon, Australian smelt and Australian bass. For each of these species an examination of the length-frequency distributions shows that juveniles, sub-adults and adults are well represented at the exit of the fishways. Hence, for these species the fishways are meeting the ecological objective.

Conclusions

The only species where there appears to be evidence of a failure to successfully negotiate the fishways are the small-bodied species mentioned above: flat-headed gudgeon, firetail gudgeon and dwarf-flat-headed gudgeon. However, as mentioned above, the swimming ability of flat-headed gudgeon and the presence of the weaker-swimming empire gudgeons using the fishways suggest that these fish could ascend the fishway if they were migrating.

This study has provided additional evidence that a range of small bodied potamodromous species may attempt to undertake movements or migrations as an important part of their life-cycle (Baumgartner & Harris 2007;Jennings *et al.* 2008; Stuart *et al.* 2008).

The monitoring has demonstrated that the fishways on the Nepean river system are operating to design specifications and meeting their ecological objective. This was confirmed by comparing the fish assemblage in the entrance and exit of the fishway and the size distribution. The size range collected was as expected and we conclude that the fishways are performing as designed.

Using PIT tags to assess fish movement in the Nepean River

Introduction

Rivers worldwide have been extensively modified, contributing to the decline of many species. Of particular concern is the obstruction of crucial fish migration pathways through the installation of dams and weirs. To mitigate the effects of these barriers, fishways are typically constructed to allow upstream and downstream passage. It is important when undertaking any form of habitat rehabilitation that there is associated monitoring to evaluate the success of the intervention. The design of a fishway is crucial because the swimming ability of the species of fish or fish community of interest will influence the specifications of the fishway. For example, fishways developed for salmonids are generally unsuitable for native Australian fish (Mallen-Cooper & Brand 2007; Mallen-Cooper & Harris 1990). Monitoring of the performance of fishways is important to enable designs to be continually refined to maximise their effectiveness for passing the fish species or fish community of interest.

Fishway performance can be assessed using a range of methods including monitoring the fish community above and below the fishway using electrofishing, or directly monitoring fish using the fishway via fishway trapping. These methods are used to evaluate changes in the fish community and to assess whether the fishway is performing to design specifications by passing fish of the intended size range. However, these methods can only be carried out intermittently and do not give detailed information on the behaviour of fish during passage through the fishway, nor provide information on potential migration cues such as flow, time of year/day and water temperature. This level of information can be obtained by implanting fish with Passive Integrated Transponder (PIT) tags to track movements of individual fish as they pass through fishways fitted with PIT readers. PIT tags are relatively inexpensive and many fish can be tagged cheaply. Information collected each time a fish passes through a fishway for the rest of its life can therefore result in the collection of large amounts of fish movement data in an inexpensive manner. This can provide valuable information on the migration patterns of a range of species given that fish as small as 150 mm in length can be tagged.

The aim of this chapter is to summarise the fish species tagged throughout the project and to provide preliminary information on movements of these tagged individuals through fishways. Fish are tagged for life and the data will continue to be collected over coming years.

Methods

Fish Tagging

With the exception of two species, all fish over 150 mm in size collected during routine electrofishing and fishway trapping were anaesthetised in a solution of 25mg/L of AQUI-S (Ridley Agriproducts) and injected with a passive integrated transponder (PIT) tag (either a Hallprint food-safe 22 mm half duplex, Hallprint food-safe 11 mm half duplex, or Karltek glass 23 mm half-duplex). Those species not tagged were bullrout (poisonous spines) and freshwater herring (unacceptably high handling mortality). Tags were initially injected into the dorsal musculature, but recaptures of two PIT tagged Australian bass in the process of expelling their PIT tags in October 2009 indicated that PIT retention was questionable (

Figure 14). Subsequently, PIT tag retention trials in golden perch (*Macquaria ambigua*) and silver perch demonstrated that PIT retention rates in both species were 100% when tagged intraperitoneally compared to just 68% and 22% respectively when tagged in the dorsal musculature (Lee Baumgartner, unpublished data). Consequently, a decision was made to tag fish intraperitoneally from the 25th of October 2010. All PIT tagged fish were also fitted with external dart tags (Hallprint) to enable anglers to report their catch to researchers.

Figure 14 PIT tag rejection in an Australian bass as identified from its swollen nape, anterior to where the PIT tag was injected (left), and, near complete PIT tag rejection in a second Australian bass with the PIT tag protruding through the nape, anterior to where the PIT tag was injected (right). Both photos captured in 2009.

PIT reader sites and configuration

Logistical constraints restricted the installation of PIT reader systems to five of the ten fishways: Wallacia, Theresa Park, Cobbitty, Camden and Menangle. Wallacia Weir has previously been demonstrated as a major barrier to fish migration and is important given the abundance of diadromous species in the Nepean River (Anon 2006; Baumgartner & Reynoldson 2007; Gehrke & Harris 1996b; Mallen-Cooper & Smit 2005). The river reach between Wallacia and Theresa Park Weirs includes a steep rocky section (Bents Basin) where fish passage can be limited for extended periods during low flows. Thus a PIT reader system at Theresa Park fishway is important to assess fish movement through this section of river. Cobbitty and Camden fishways are in the middle reach of the system. Menangle is the second last of the new fishways.

Twin-antennae PIT reader systems (Karltek) were installed from Theresa Park Weir to Menangle Weir (entrance and exit), while Wallacia Weir was fitted with a four antennae system (entrance, first vertical-slot, the middle vertical-slot and the exit). Twin-antennae systems allow a determination of whether a fish passed through the fishway and the direction it was travelling. Systems with four antennae also provide these directional data but provide additional information on components of the fishway design. For instance, new entrance configurations have been developed for the Nepean River fishways, whereby keyed slots have been installed to maintain an acceptable attraction flow during times of high river flow. A multi-reader system allows researchers to investigate fish responses to the new design that operates over a limited range of flows but targets a specific aspect of fish migration behaviour. PIT information can then be used to determine the success of the entrance by analysing passage data and can also relate movements to flow.

When a PIT tagged fish passed through a fishway, the antenna number, PIT tag number, date and time were recorded. These data were downloaded from the control boxes at each fishway and imported into the PIT Information System (Karltek).

Operation of PIT readers

Unfortunately, the PIT reader systems were not fully functional throughout the life of the project. The systems at Cobbitty, Wallacia and Theresa Park fishways were badly damaged by flooding in March 2012 and again in March 2013.

Data Analysis

PIT systems were not fully functional during the study and the results contain only basic movement data rather than the in-depth analyses. More detailed analyses will be conducted once the repair and waterproofing of the PIT systems is complete and more data are collected.

The summary data presented here includes;

- number of each species tagged by each method
- which species have been detected in fishways
- recorded numbers of successful and unsuccessful attempts at migration through fishways
- fish passage efficiency

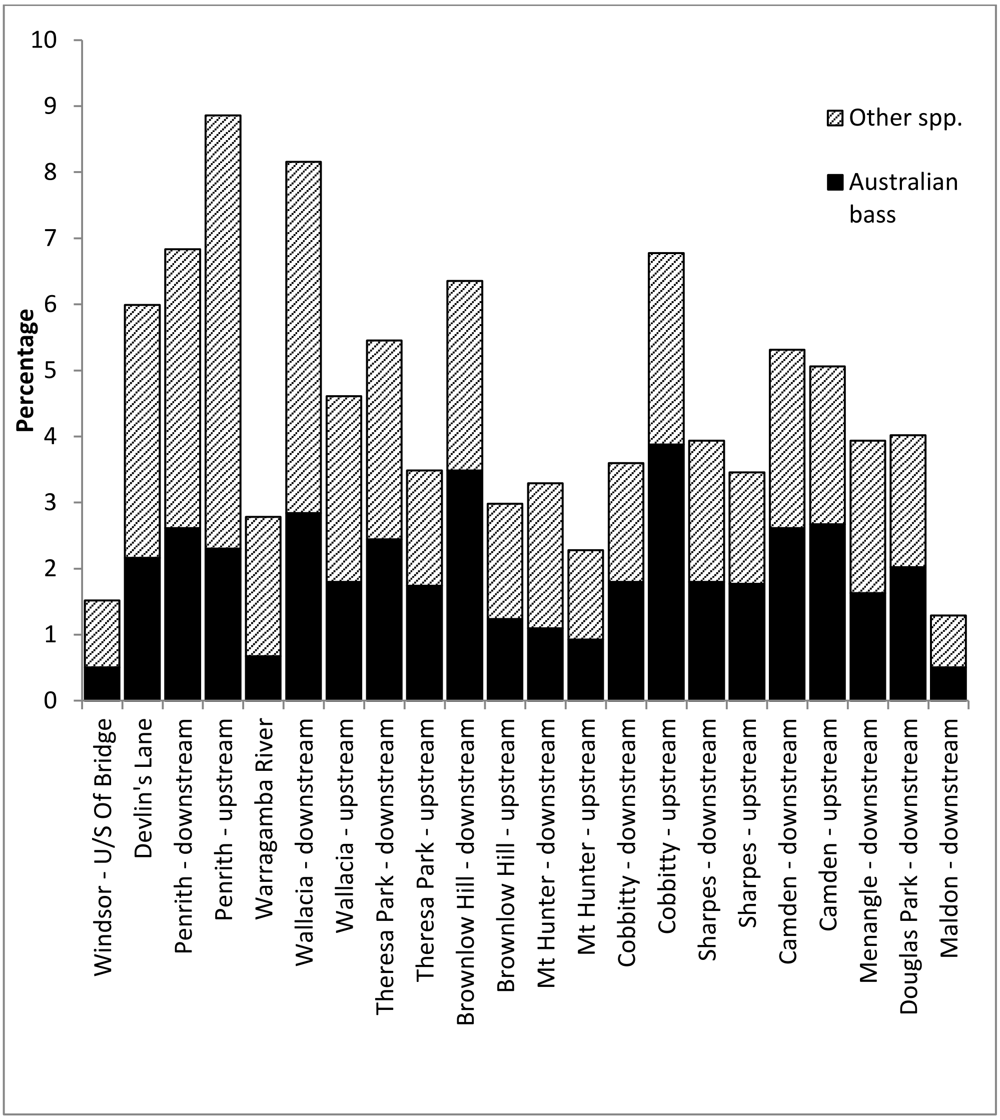
Results

Up until March 2013, a total of 3,798 fish from 14 species were tagged (Table 15). Forty percent of tags were deployed in Australian bass, with freshwater mullet, common carp, short-finned eels, freshwater catfish and sea mullet also having more than 300 individuals tagged (Table 15). Fish were tagged throughout the system, including more than 300 individuals tagged above and below the first and last fishways in the system with PIT antennae: Wallacia and Menangle respectively (Figure 15).

Table 15 **Number of fish PIT tagged in the Nepean River during this study. Fish were collected during electrofishing surveys, targeted electrofishing tagout trips (E) or during fishway trapping (T). Detections are from antennae in any of the fishways fitted with PIT systems.**

Species/Year	2009	2010	2011		2012		2013		Cumulative detections
	E	T	T	E	T	E	T	E	
Australian bass	439	107	45	602	7	411	1	61	72
Brown trout						7		2	
Bullrout	1								
Common carp	137	5	3	152	1	60	1	4	2
Estuary perch						4			
Freshwater catfish	138	6		125	1	111	1	23	1
Freshwater herring	2								
Freshwater mullet	52	2	32	164	2	129		48	
Goldfish	137			1					4
Long-finned eel	228	5	15	93		17		2	7
Rainbow trout						1			
Sea mullet	111	1	1	173	2	95	3	24	3
Short-finned eel			1			1			
Silver perch						1			

Figure 15 **Location of fish tagged during electrofishing trips in the Nepean River between 2009 and 2013. Sites are ordered from downstream (left) to upstream (right).**



During the limited time where both entrance and exit antennae were functioning, there were 43 recorded ascents and 5 recorded descents (Table 16). The largest number of fish passages was recorded at Camden Fishway, with 24 Australian bass ascending the fishway. These fish took between 19 and 145 minutes to pass (Table 16). A further eight Australian bass were recorded entering the fishway but not passing through to the exit (Table 17) giving a preliminary estimate of 75% efficiency for this species at the Camden Fishway.

Eighty-one PIT tagged fish were recaptured in subsequent electrofishing trips and 86 fish were reported as captured by anglers (Table 18). Fifty-one of 54 (94%) Australian bass recaptured in electrofishing trips were collected at the same locations they were tagged, while only 40% of the 60 Australian bass tags returned by anglers were collected at their tagging location (Table 18). One Australian bass was reported and released by an angler 1,218 days after it was tagged, and one short-finned eel tag was harvested by an angler 1,278 days after tagging (Table 18). All of the electrofished Australian bass recaptures and 59 of the 60 reported by anglers were released alive.

Table 16 **Summary of recorded ascents by fish in 5 fishways in the Nepean River. Data are cumulative over the 4 years of the study, but are only included below when both entrance and exit PIT receivers were operational.**

Fishway	Species	Simple Ascents	Minimum ascent time (mins)	Maximum ascent time (mins)	Descents
Camden	Australian bass	24	19	145	1
	Common carp	1	31	31	0
	Freshwater mullet	1	7	7	0
	Long-finned eel	1	103	103	0
	Total	**35**			
Cobbitty	Australian bass	2	28	152	2
	Long-finned eel	1	98	98	0
	Total	**4**			
Menangle	***Total***	**0**			
Theresa Park	Australian bass	1	25	25	0
	Total	**4**			
Wallacia	Australian bass	0	N/A	N/A	1
	Total	**0**			
Overall Total		**86**			**4**

Table 17 Summary of fishway passage efficiency in the Nepean River at four sites.

	Camden				Cobbitty		Menangle	Wallacia	Total
Data	**Australian bass**	**Common carp**	**Freshwater mullet**	**Long-finned eel**	**Australian bass**	**Long-finned eel**	**Australian bass**	**Australian bass**	
Sum of Entrance Only	8	0	0	1	1	0	0	0	10
Sum of Simple Ascents	24	1	1	1	2	1	1	0	32
Passage efficiency (%)	75	100	100	50	67	100	100		
Sum of No Ascents	1	0	0	0	2	0	0	1	4

Table 18 Summary of tagged fish that were recaptured either by electrofishing in scheduled sampling trips or by anglers. Duration is the number of days between tag and recapture.

Species	Number of recaptures (electrofishing)	New location	Duration (days)	Size at recapture (mm)	Number recaptures (anglers)	New location	Duration (days)	Size at recapture (mm)
Long-finned eel	4		117-168	520-630	1	1	1278	800
Goldfish	2		57	276	1	1	172	270
Carp	10		136-596	520-680	4	2	59-643	530-700
Australian bass	54	3	49-518	170-375	60	24	1-1,218	150-430
Sea mullet					16	16	150-840	360-450
Freshwater catfish	13		56-358	445-550	1		338	440
Freshwater mullet	8	2	139-758	239-418	3	2	12-1,064	340-620

Discussion

Over 3,500 fish representing 14 species were PIT tagged over the course of this study and initial data from the PIT tag readers indicates four of these species have successfully ascended fishways. Australian bass were the most commonly tagged species and also the most common species recorded using the fishways. Electrofishing and angler recapture data indicates that Australian bass exhibit substantial site fidelity. A homing migration after spawning was first suggested by Harris (1983). Here we have shown that 94% of the 54 Australian bass recaptured through electrofishing were caught at the original site of tagging. In contrast, only 40% of Australian bass angler recaptures were caught at their original tagging site. While these data initially appear conflicting, when viewed with this species' ecology in mind they are fully concordant. Australian bass undertake downstream migrations to the estuary to breed from May to August and return in spring and summer (Harris 1986; Harris & Rowland 1996; Reinfelds *et al.* 2013). Electrofishing was typically carried out during late spring to early autumn, when most fish were expected to have returned to the river following spawning in the estuary. In contrast, anglers could potentially target bass during the cooler months and are therefore much more likely to encounter a tagged fish when it is actively migrating. Thus the data presented here are highly suggestive of a return to a home range following spawning. An acoustic tagging study of Australian bass in the nearby Shoalhaven River also indicated reasonably high levels of site fidelity (Walsh *et al.* 2012), thus there is now increasing evidence that this species, like other percichthyids, exhibits a high degree of homing behaviour after large-scale movements. Further information on the movement of tagged individuals in the Nepean River from the PIT systems will be important to verify that most fish did in fact leave the tagging site and travel downstream given a recent study has demonstrated that not all Australian bass in the nearby Shoalhaven River migrate to the estuary during the winter/spring months (Walsh *et al.* 2012).

Effect of weirs on the genetic structure of Australian smelt pre- and post-fishways

Introduction

Dams and weirs play an important role in providing water for irrigation, hydroelectricity and consumptive purposes, but the associated changes in river hydrology, hydraulics and physical barriers to migration have had a major impact on fish communities. These barriers can fragment populations of freshwater fish, potentially isolating them from critical spawning or feeding habitat. This can ultimately result in local extinction of populations above the barrier, particularly of diadromous species (Cadwallader 1978; Gehrke *et al.* 2002; Jellyman & Harding 2012). While some potamodromous species can survive in isolated dams, they do not necessarily escape unaffected. For example, they are more susceptible to inbreeding, which could result in a loss of genetic diversity, increased prevalence of deformities and ultimately an increased risk of extinction (Morita & Yamamoto 2000, 2002; Neraas & Spruell 2001; Yamamoto *et al.* 2004). Furthermore, it has been demonstrated that even low-level structures are capable of significantly affecting genetic structure of fish populations by disrupting upstream migrations (Blanchet *et al.* 2010; Hänfling & Weetman 2006; McCraney *et al.* 2010; Meldgaard *et al.* 2003; Raeymaekers *et al.* 2009). One possible solution to promote upstream migration and consequently gene flow is to install fishways at impassable barriers (Blanchet *et al.* 2010; Raeymaekers *et al.* 2009). Many fishways have been installed on rivers worldwide, but assessment of their effectiveness has largely been limited to improvements in fish distribution and community structure, while improvements to gene flow and population structure have rarely been addressed (But see Meldgaard *et al.* 2003; Raeymaekers *et al.* 2009; Reid *et al.* 2008).

The Nepean River of south-eastern Australia is one of the most highly regulated coastal rivers and its upper catchments provide the vast majority of water to the city of Sydney. Five major dams (Warragamba, Avon, Cordeaux, Nepean and Cataract) are located in the headwaters of the system and 13 smaller weirs (between 1.8 and 16 m high) further regulate the flows between Pheasants Nest and Penrith (Figure 1). While some of these weirs had fishways installed (Table 1), they were designed for salmonid species and poorly maintained, providing inefficient or no fish passage for the comparatively weaker swimming native fish in the Nepean River (Harris 1984; Mallen-Cooper 2009). A number of studies have demonstrated that weirs have affected the upstream distribution of several species within this system, particularly catadromous species that require access to the estuary to spawn (Baumgartner & Reynoldson 2007; Gehrke *et al.* 1999; Gehrke 1996a; Growns *et al.* 2013). The second weir upstream of the estuarine limit in particular, Wallacia Weir, has had a substantial effect on the upstream fish community structure, probably due to its infrequent drown-out occurrence of once in 100 years. Consequently, fish were unlikely to be able to pass Wallacia Weir, thus limiting their ability to colonise sites further upstream irrespective of the drown-out frequency of those weirs further upstream. In contrast, all weirs between Theresa Park and Douglas Park inclusive drown-out approximately once per year (Mallen-Cooper 2009), thus it is possible that fish may be able to migrate past these weirs during high flows. However, the ability of a fish to migrate upstream past a submerged weir will be dependent on whether it migrates under high flows, and if so, its swimming ability given drown-out occurs during very high flows that generate greater water velocity. There is evidence that larger diadromous species such as Australian bass are capable of upstream migrations during high flows given their presence upstream of the weirs (Baumgartner & Reynoldson 2007). Smaller potamodromous species such as Australian smelt are also found above these barriers, though it is not known if their presence is a result of local spawning at each site or a combination of local spawning and upstream migration. In an effort to improve fish passage along the Nepean River for fish ranging in size from 35 mm to more than 1 m in length, ten of the weirs between Penrith and Douglas Park were retrofitted with vertical-slot fishways. This provided a rare opportunity to assess whether a series of low-level weirs, some of which regularly drown-out, fragmented the population genetic structure of fish populations, and if so, whether the fragmentation was subsequently ameliorated by the installation of the fishways. To our knowledge, there are no other studies on a similar scale that have been conducted anywhere in the world to date.

We selected the potamodromous Australian smelt as our target species based on its small size and comparatively weaker swimming ability that may limit its capability to migrate effectively past weirs during high flows, and because it was ubiquitous throughout the study reach. In addition, it is also present in three of the headwater dams (Nepean, Avon and Cordeaux Dams) which were included in this study to allow a direct comparison to be made between low-level barriers that may be passable during high flow events and high-level barriers that are never passable. Nepean, Avon and Cordeaux Dams (Figure 1) have been in place since 1925, 1921 and 1918 respectively. Using an estimated life-span of approximately two years (Pusey *et al.* 2004), Australian smelt have been isolated in these dams for approximately 44 to 47 generations. Therefore it is likely that these impounded populations have undergone substantial genetic drift and possibly inbreeding, which has likely led to considerable population genetic differentiation to populations below the dams. It is more difficult to predict the degree of population structuring in populations downstream of the dams between each weir, but it could feasibly range from complete genetic panmixia (no genetic structure), to pronounced genetic structure between weirs, depending on the ability of the species to pass barriers during high flows and the effective population size of each population unit.

Microsatellite data was used to address three main questions: (i) is there evidence of genetic structure consistent with one or more weirs and is there an additive effect of weirs in an upstream direction, (ii) if genetic structure is present, do the fishways improve genetic connectivity between populations; and (iii) have isolated populations (in the dams) undergone a greater amount of genetic differentiation than those in the river?

Methods

Sample collection

Australian smelt were collected from 11 river reaches from downstream of Penrith Weir to Maldon Weir and from Nepean, Avon and Cordeaux Dams using boat electrofishing during fish community sampling (Figure 1). Sampling was conducted between May and December 2009 (before the fishways were installed) and again between April 2012 and March 2013 (after the fishways were commissioned). We attempted to collect 30 Australian smelt per reach from each time period. Whole fish were euthanised in a lethal concentration of AQUI-S and preserved in 100% ethanol.

DNA extraction and microsatellite genotyping

DNA was extracted from all samples using the Jet Quick DNA (Genomed) extraction kit following the manufacturer's instructions. Nine microsatellite loci were selected for analysis (dye labels in parentheses): SM-18 (D4), SM-26 (D3), SM-49 (D2), SM-77 (D3) and SM-80 (D2) (Hillyer *et al.* 2006), C2 (D4), C7 (D2), E7 (D4) (Woods 2008) and E8 (D3 - F: CGGAAGTGGAGGTTCAGCA R: GACGAGTTCATCACGGGAAA) (Woods, unpublished data). The samples collected prior to the fishways being commissioned were PCRed in 96 well plates with two positive and two negative controls on each plate. Reactions were carried out in a 12 µL volume containing 0.05 µL of forward primer at 30 µM, 0.1 µL of reverse primer at 30 µM and 0.15 µL of M13 labelled primer either D4, D3 or D2 (CACGACGTTGTAAAACGAC) at 30 µM, 1.25 µL dNTP mix at 5 mM each, 1.25 µL of $MgCl2$ at 25 mM, 1.25 µL of 5X reaction buffer, 0.5 units Taq DNA polymerase (Promega) and 5 – 20 ng DNA. PCR cycling conditions consisted of an initial denaturation step at 95ºC for 2 min, 30 cycles of 95ºC for 30 secs, 55ºC for 45 sec, 72ºC for 60 secs, and a final extension at 72ºC for 10 mins. PCR products were pooled into three bins: bin 1 (SM-49, C2 and E8), bin2 (SM-77, C7 and E7) and bin 3 (SM-18, SM-26 and SM-80), and mixed with SLS buffer containing the internal size standard 400 (Beckman Coulter) and run on a CEQ 8000 Genetic Analysis System (Beckman Coulter). Peaks were scored using CEQ software (Beckman Coulter), and carefully scrutinised by an experienced operator. To ensure alleles were consistently sized, raw allele lengths were assigned into bins using FLEXIBIN (Amos 2007). Extracted DNA from samples collected after the fishways were commissioned was sent for PCR, genotyping and allele calling at the Australian Genome Research Facility (AGRF – Melbourne, Australia).

Genetic diversity

All of the following analyses were conducted separately for all pre- and post-fishway data. Conformance of each locus to Hardy-Weinberg expectations (HWE) was calculated in GENEPOP 3.3 (Raymond & Rousset 1995) using exact probability tests and 10,000 permutations. Tests for linkage disequilibrium of each locus pair within each site was also carried out in GENEPOP 3.3 (Raymond & Rousset 1995) with 10,000 permutations to assess significance. Observed (HO) and expected heterozygosity (HE) were calculated in GENALEX 6 (Peakall & Smouse 2006), and allelic richness (AR - allelic diversity corrected for sample size) and the inbreeding coefficient (FIS) were calculated in FSTAT 2.9.3 (Goudet 1995). Permutation tests (15,000 permutations) were also carried out in FSTAT 2.9.3 to test if the impounded populations had lost a significant amount of genetic diversity (AR and HO) compared to the river populations, and to test if genetic diversity in the riverine populations decreased in an upstream direction. AR was plotted against sample reach for the pre- and post-fishway data to explore whether genetic diversity had changed post-fishways.

Population structure

Population genetic differentiation F_{ST} (Weir & Cockerham 1984) was calculated in ARLEQUIN (Excoffier *et al.* 2005) using 10,000 permutations of the data to assess significance. F_{ST} ranges from 0 (no genetic differentiation) to 1 (fixation of different alleles in different populations). To test whether genetic differentiation was consistent with an isolation by distance pattern (IBD, where populations are more genetically similar to those closest to them than those further away), tests for seriation were conducted using the RELATE function in PRIMER v6. In addition, genetic distance matrices (F_{ST}) were compared to geographic distance matrices (river km) to determine if there was a significant correlation between genetic and geographic distance. The genetic distance matrices for the pre-and post-fishway samples were also compared using RELATE to determine if there were significant changes in genetic structure since the fishways were installed. The F_{ST} matrix was used to construct multi-dimensional scaling plots (MDS) using PRIMER v6 (Clarke & Gorley 2006) to aid in visualisation and interpretation of the data.

The analyses above require populations to be pre-defined, which can be problematic with a potentially interconnected population of fish in a river. Therefore, Structure 2.2 (Pritchard *et al.* 2000) was used to 'assign' individual fish to one or more genetic clusters (K). Analyses were conducted using the 'admixture model' and correlated allele frequencies (Falush *et al.* 2003) with 100,000 burn-ins, 100,000 Markov Chain Monte-Carlo repetitions, and 5 replicate runs per K (K = 1 – 8 for the pre-fishway data and k =1 – 10 for the post-fishway data) to ensure the results were consistent across runs. To estimate the most likely number of populations (K), the average likelihood [Pr(X/K)] of each K was plotted to determine the value with the highest likelihood. In addition, the method of Evanno *et al.* (2005) was used to estimate the value of ∆K, which is based on the second order rate of change of the log probability [P(X/K)]. These two methods were used in conjunction with knowledge of the river system and species to determine the most likely number of K.

Population bottleneck

It is possible that populations of Australian smelt above dams and weirs might experience a loss of genetic diversity (heterozygosity) due to a reduction in population size and/or isolation, which is known as a population bottleneck. Therefore, we tested for recent bottlenecks using the program BOTTLENECK 1.2.02 (Piry *et al.* 1999). This program is very sensitive as it exploits the tendency of bottlenecked populations to lose allelic diversity before heterozygosity. Thus, recently bottlenecked populations will temporarily display a heterozygosity excess compared to that of a population that is presumed to be at mutation-drift equilibrium (Cornuet & Luikart 1996). We used the two-phase mutation model (TPM) given that it performs best with microsatellite data (Di Rienzo *et al.* 1994) and the Wilcoxon signed-rank test to assess the significance of the results. The program should ideally be run with a minimum 12 loci and 30 individuals (Piry *et al.* 1999). Therefore, it is acknowledged that there may be a reduction in statistical power to detect recent bottlenecks given that we have used only eight loci and fewer than 30 individuals for some populations.

Results

Genetic diversity and population structure

Eight loci amplified successfully and were polymorphic (multiple alleles per locus) across most populations. Three loci were monomorphic (only one allele detected) in Cordeaux Dam (C2, E7 and SM-77), while one was monomorphic at Downstream Sharpes (post-fishways, E7). In the pre-fishway samples, 14 locus/population combinations departed from HWE. Following sequential Bonferroni correction (Rice 1989), this reduced to five locus/population combinations (Table 19). In the post-fishway samples, 18 locus/population combinations departed from HWE, reducing to seven following Bonferroni correction (Table 19). All departures were due to a heterozygote deficiency. This may imply the presence of alleles that were not detected (null alleles) due to mutations in the primer binding site (Jarne & Lagoda 1996). Alternatively, this may indicate that there is inbreeding or unresolved population structure (Frankham *et al.* 2002). Across all pre-fishway populations, no locus pairs exhibited linkage disequilibrium, while in the post-fishway population only one of the 28 locus pairs (E7 and SM-80) exhibited linkage disequilibrium (based on Bonferroni corrected results, table adjusted to 1 population x 28 locus pairs). Prior to Bonferroni correction, no locus pair was consistently linked across populations.

Genetic diversity ranged from HE = 0.30 (Cordeaux Dam) to HE = 0.76 downstream Wallacia (post-fishways) (Table 19). The mean signal of inbreeding (FIS) was low in all populations, ranging from -0.12 to 0.21 (Table 19). Allelic richness ranged from AR = 1.72 (Cordeaux Dam) to AR = 2.94 (downstream Penrith pre-fishways) (Table 19). Cordeaux Dam had a much lower level of genetic diversity due to the fixation of a single allele at three loci (C2, E7 and SM-77) while the other two populations isolated by dams had a comparable level of genetic diversity to the riverine populations. Permutation tests indicated HO and AR were not significantly different in the dams compared to the river. However, AR was significantly lower in upstream river sites compared to downstream river sites pre-fishways, but was not significantly different post-fishways, suggesting an increase in AR upstream post-fishways (Table 20; Figure 16). Pre-fishways, HO was significantly lower in the middle reaches (3 to 9) compared to the downstream reaches (1-2) ($P = 0.015$), but was not significantly different post-fishways (Table 20).

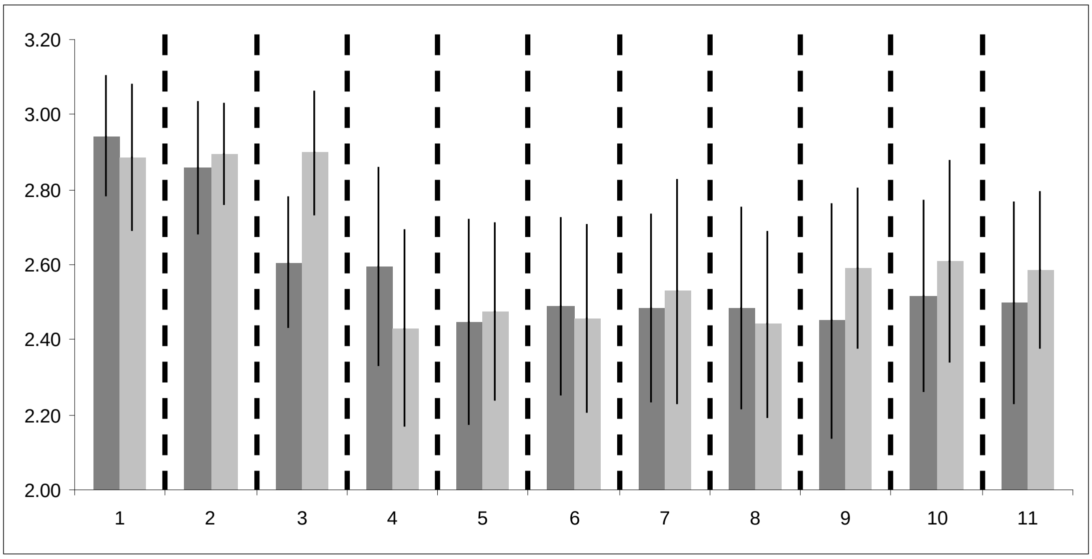
Table 19 Sample size (N), mean number of alleles (Na), allelic richness (allelic diversity corrected for sample size, AR), observed heterozygosity (HO), expected heterozygosity (HE) and inbreeding coefficient (FIS) for all pre- and post-fishway populations based on eight microsatellite loci. D/S = downstream.

	N		Mean Na		AR		HO		HE		FIS		HW disequilibrium	
Population	**pre-**	**post-**	**pre-**	**post-**	**pre-**	**post-**	**pre-**	**post-**	**pre-**	**post-**	**pre-**	**post-**	**pre-**	**post-**
D/S Penrith	22	29	8.25	8.75	2.94	2.89	0.67	0.75	0.76	0.75	0.15	0.02		SM49
D/S Wallacia	54	30	10.13	8.75	2.86	2.89	0.65	0.66	0.75	0.76	0.14	0.14	SM49, SM80	E7
D/S Theresa Park	5	21	4.00	8.13	2.61	2.90	0.66	0.67	0.62	0.75	0.05	0.13		
D/S Brownlow Hill	10	21	5.13	6.88	2.59	2.43	0.61	0.52	0.62	0.59	0.09	0.14		
D/S Mt Hunter	10	30	4.88	7.38	2.45	2.47	0.54	0.61	0.57	0.62	0.11	0.02	E8	
D/S Cobbitty	40	28	8.25	7.50	2.49	2.46	0.56	0.60	0.62	0.60	0.10	0.01	SM49, SM80	
D/S Sharpes	11	7	5.63	4.75	2.48	2.53	0.59	0.55	0.59	0.58	0.06	0.12		
D/S Camden	40	30	9.00	7.50	2.48	2.44	0.52	0.60	0.61	0.60	0.16	0.01	E8	
D/S Menangle	4	30	3.25	9.25	2.45	2.59	0.65	0.60	0.50	0.65	-0.12	0.09		E8
D/S Douglas Park	68	3	9.75	3.13	2.52	2.61	0.60	0.60	0.62	0.56	0.05	0.16		
D/S Maldon	2	12	2.50	5.63	2.50	2.59	0.69	0.64	0.52	0.64	0.00	0.05		
Cordeaux Dam		30		4.00		1.72		0.28		0.30		0.09		
Avon Dam		30		7.00		2.68		0.62		0.70		0.14		SM49, E7
Nepean Dam		30		7.50		2.79		0.59		0.73		0.21		SM49, E7

Table 20 Results of permutation tests for significant differences in observed heterozygosity (HO) and allelic richness (AR) between groupings of the data. The p-value indicates whether there were significant differences between the groups tested. Significant values (p<0.05) are in bold. NA = not applicable.

Group	Pre-fishways		Post-fishways	
	P-value AR	P-value HO	P-value AR	P-value HO
Reaches 1 & 2 vs. reaches 3-9 vs. reaches 10-11	**0.020**	0.180	0.340	0.475
Reaches 1& 2 vs. reaches 3 – 9	**0.007**	**0.015**	0.152	0.232
Reaches 1 & 2 vs. reaches 10 - 11	**0.021**	0.438	0.309	0.458
Dam (Nepean, Avon and Cordeaux) vs. River (11 reaches)	NA	NA	0.26	0.15

Figure 16 Mean allelic richness (± SE) pre-fishways (dark grey bars) and post-fishways (light-grey bars) for each river reach from the most downstream reach on the left to the most upstream reach on the right. The reaches are: 1- downstream of Penrith, 2- Penrith-Wallacia, 33 - Wallacia-Theresa Park, 4 - Theresa Park-Brownlow Hill, 5- Brownlow Hill-Mt Hunter, 6 - Mt Hunter-Cobbitty, 7 - Cobbitty-Sharpes, 8- Sharpes-Camden, 9 - Camden-Menangle, 10 - Menangle-Douglas Park and 11-Douglas Park-Maldon. Weirs are represented by dashed vertical lines.



Population structure

The highest F_{ST} values were obtained for population comparisons involving Cordeaux Dam (Table 22). Populations downstream of Penrith and Wallacia were different to most other populations both pre- and post-fishways (Table 21 and Table 22). Populations in the middle and upper reaches of the river (downstream Brownlow Hill to downstream Maldon) were not significantly different to each other in most cases both pre- and post fishways. However, where there were significant differences between populations in the middle and upper reaches pre-fishways, all became non-significant or reduced in magnitude post-fishways. For example, Australian smelt downstream of Brownlow Hill were significantly different to those downstream of Mt Hunter pre-fishways, but there was no significant difference post-fishways (Table 21 and Table 22). A longitudinal gradient in genetic diversity consistent with isolation by distance was found in both the pre- and post-fishway samples (Rho = 0.502, p = 0.0009 and Rho = 0.492, p =

0.0008 respectively), which is evident in the MDS plots (Figure 17). The longitudinal gradient was primarily due to the influence of the four most divergent populations (those downstream of Wallacia, Penrith, Theresa Park and Maldon) with the populations in the middle reaches displaying little difference from each other pre- and post-fishways. The pre-fishway and post-fishway samples were significantly correlated with geographic distance (Rho = 0.578, p = 0.004 and Rho = 0.612, p = 0.0025 respectively), suggesting populations closer together were more genetically similar than those further apart. The pattern of genetic diversity pre-and post-fishways was significantly correlated (p = 0.0016) indicating the genetic structuring had changed little temporally.

Results from *STRUCTURE* for the pre-fishway group indicated that the posterior probability P(X|*K*) was maximal at *K* = 5, while Δ*K* was maximal at *K* = 3. Regardless of the *K* value, the data was split into two main groups; above and below Wallacia Weir, thus it was decided to use Δ*K* to interpret the pre-fishway data. At *K* = 3 (Δ*K*) the populations downstream of Wallacia Weir were clearly distinct from upstream populations with most fish belonging to cluster 2 (Figure 18; Table 23). The upstream populations were split between cluster 2 and 3 with the exception of the population downstream of Theresa Park that also had nearly 30% membership in cluster 2 (Figure 18; Table 23). For the post-fishway group (which included the dams), P(X|*K*) was maximal at K = 8, while Δ*K* was maximal at *K* = 2. The Δ*K* method of Evanno *et al.* (2005) detects the uppermost hierarchical structure in the data, thus in the post-fishway data it is possible that the highly divergent Cordeaux Dam population is overwhelming finer scale structure present in the other populations. To avoid any confusion from the impounded populations, the Nepean, Cordeaux and Avon Dams were removed from the post-fishway data set and *STRUCTURE* was run again for the river reaches and dam populations separately. Following this second analysis for the river reaches, P(X|*K*) was maximal at K = 5, while Δ*K* was maximal at *K* = 2. Similarly to the pre-fishway data, the populations downstream of Wallacia were consistently separated from the upstream sites (Figure 19; Table 23), and therefore the estimate of Δ*K* was considered to be the most appropriate *K* to evaluate results. At *K* = 2, populations downstream of Wallacia were predominantly classified into cluster 2 while those upstream of Wallacia were classified strongly into cluster 1, with the exception of the population downstream of Theresa Park that was equally split between cluster 1 and 2 (Figure 19; Table 23). Therefore, the pre- and post-fishway data indicate there is a genetic break around Wallacia Weir. It is clear that there is also a genetic break around Theresa Park Weir as evidenced by the mixed cluster membership of both the pre- and post-fishway populations. The STRUCTURE run of the three dams was easily interpreted without having to calculate Δ*K.* There were clearly two genetic clusters, one containing all the fish from Cordeaux Dam and the other containing all the fish from Nepean and Avon Dams (Figure 20). The results remained unchanged from *K* = 2 to *K* = 5.

Population bottleneck

There was no evidence of population bottlenecks in any of the populations. In the pre-fishway populations, there was a significant heterozygosity deficiency in three reaches; downstream Cobbitty, downstream Camden and downstream Maldon (p = 0.004, p = 0.004 and p = 0.006 respectively). Post-fishways, heterozygosity deficiencies were exhibited by the downstream Brownlow Hill, downstream Cobbitty, downstream Camden and downstream Menangle reaches (p = 0.027, p = 0.02, p = 0.014 and p = 0.004 respectively), as well as Cordeaux Dam (p = 0.004). A heterozygosity deficiency is potentially attributed to an influx of rare alleles from a divergent population, or it could be due to recent population expansion (Cornuet & Luikart 1996; Maruyama & Fuerst 1985).

Table 21 **Pairwise FST estimates (below diagonal) and river km between sites (above diagonal) for samples collected pre-fishways (with river kilometres). FST values range from 0 (no differentiation between populations) to 1 (fixation of different alleles in populations). FST ranges from 0 (populations are genetically identical) to 1 (populations are fixed for different alleles). Values greater than 0.15 indicate populations are considered as substantially differentiated due to restricted gene flow (bold).**

	Reach	Sample size	D/S Penrith	D/S Wallacia	D/S Theresa Park	D/S Brownlow Hill	D/S Mt Hunter	D/S Cobbitty	D/S Sharpes	D/S Camden	D/S Menangle	D/S Douglas Park	D/S Maldon
D/S Penrith	1	22		20	34	53	57	59	63	68	74	94	96
D/S Wallacia	2	54	0.000		14	33	37	39	43	48	54	75	76
D/S Theresa Park	3	5*	**0.065**	**0.037**		19	23	25	29	34	40	61	62
D/S Brownlow Hill	4	10*	**0.062**	**0.039**	**0.070**		5	7	10	16	21	42	43
D/S Mt Hunter	5	10*	**0.112**	**0.086**	**0.084**	**0.043**		2	5	11	16	37	39
D/S Cobbitty	6	40	**0.106**	**0.076**	**0.066**	0.003	-0.003		3	9	14	35	37
D/S Sharpes	7	11*	**0.095**	**0.057**	**0.040**	0.022	0.000	-0.006		6	11	32	33
D/S Camden	8	40	**0.108**	**0.076**	**0.060**	-0.002	0.005	-0.003	-0.004		5	26	28
D/S Menangle	9	4*	**0.080**	0.034	0.036	0.023	-0.038	-0.050	-0.060	-0.041		21	22
D/S Douglas Park	10	68	**0.103**	**0.065**	**0.073**	0.001	**0.016**	-0.004	0.000	-0.009	-0.004		2
D/S Maldon	11	2*	0.067	0.072	**0.126**	**0.132**	0.124	**0.098**	0.076	**0.099**	0.034	**0.122**	

*Note that F_{ST} values for site comparisons where fewer than 15 samples were collected should be interpreted with caution.

Table 22 Pairwise FST estimates (below diagonal) and river km between sites (above diagonal) for samples collected post-fishways (including the dams). FST values range from 0 (no differentiation between populations) to 1 (fixation of different alleles in populations). FST ranges from 0 (populations are genetically identical) to 1 (populations are fixed for different alleles). Values greater than 0.15 indicate populations are considered as substantially differentiated due to restricted gene flow (bold).

	Reach	Sample size	D/S Penrith	D/S Wallacia	D/S Theresa Park	D/S Brownlow Hill	D/S Mt Hunter	D/S Cobbitty	D/S Sharpes	D/S Camden	D/S Menangle	D/S Douglas Park	D/S Maldon	Cordeaux Dam	Avon Dam
D/S Penrith	1	29													
D/S Wallacia	2	30	**0.014**												
D/S Theresa Park	3	21	**0.031**	**0.014**											
D/S Brownlow Hill	4	21	**0.119**	**0.090**	**0.030**										
D/S Mt Hunter	5	30	**0.110**	**0.085**	**0.023**	-0.007									
D/S Cobbitty	6	28	**0.118**	**0.084**	**0.030**	-0.002	-0.005								
D/S Sharpes	7	7	**0.115**	**0.086**	0.025	-0.001	-0.006	0.003							
D/S Camden	8	30	**0.122**	**0.099**	**0.033**	0.004	-0.001	-0.002	-0.009						
D/S Menangle	9	30	**0.092**	**0.062**	**0.014**	0.001	-0.001	-0.003	-0.002	-0.002					
D/S Douglas Park	10	3	**0.063**	0.027	-0.013	-0.033	-0.026	-0.026	-0.004	-0.014	-0.030				
D/S Maldon	11	12	**0.110**	**0.070**	**0.030**	**0.034**	**0.033**	**0.029**	0.015	0.020	0.010	-0.021			
Cordeaux Dam		30	**0.288**	**0.269**	**0.225**	**0.174**	**0.165**	**0.201**	**0.204**	**0.151**	**0.148**	**0.312**	**0.220**		
Avon Dam		30	**0.046**	**0.035**	**0.029**	**0.074**	**0.064**	**0.073**	**0.066**	**0.071**	**0.053**	0.031	**0.059**	**0.295**	
Nepean Dam		30	**0.047**	**0.043**	**0.035**	**0.098**	**0.090**	**0.104**	**0.109**	**0.107**	**0.075**	0.050	**0.083**	**0.302**	**0.017**

Figure 17 Multi-dimensional scaling ordination (MDS) of pairwise F_{ST} values for Australian smelt for each site (a) pre-fishways and (b) post-fishways. The trajectory shows the order of sites from the most downstream (D/S; D/S Penrith) to the most upstream (D/S Maldon).

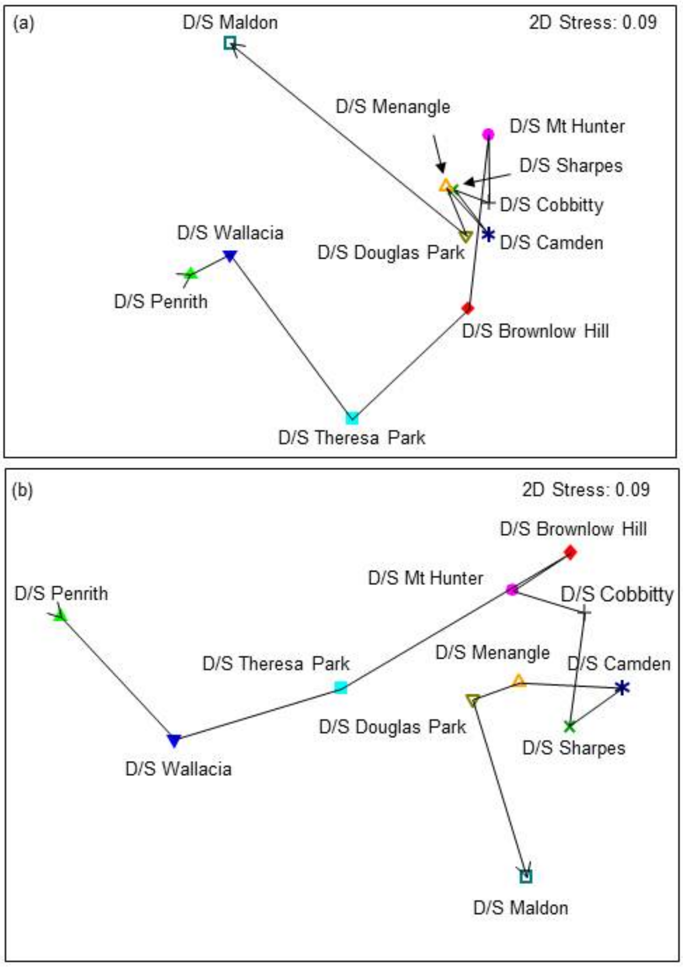


Figure 18 Pre-fishway proportional membership coefficient (Q) plots of Australian smelt from 11 river reaches (excluding the dams) for K = 3 (∆K). Individual fish are represented by vertical bars and population boundaries are defined by thin black vertical lines. D/S – downstream. Colours indicate the genetic population ‘cluster’ that each individual belongs to.

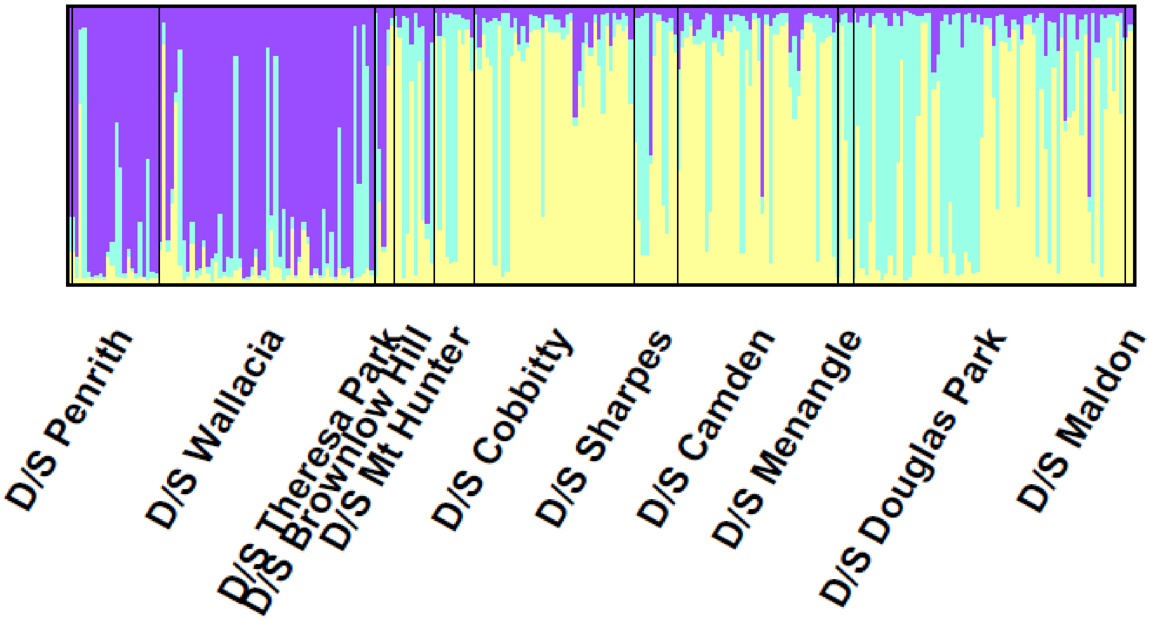


Figure 19 Post-fishway proportional membership coefficient (Q) plots of Australian smelt from 11 river reaches (excluding the dams) for K = 2 (∆K). Individual fish are represented by vertical bars and population boundaries are defined by thin vertical lines. D/S = downstream. Colours indicate the genetic population ‘cluster’ that each individual belongs to.

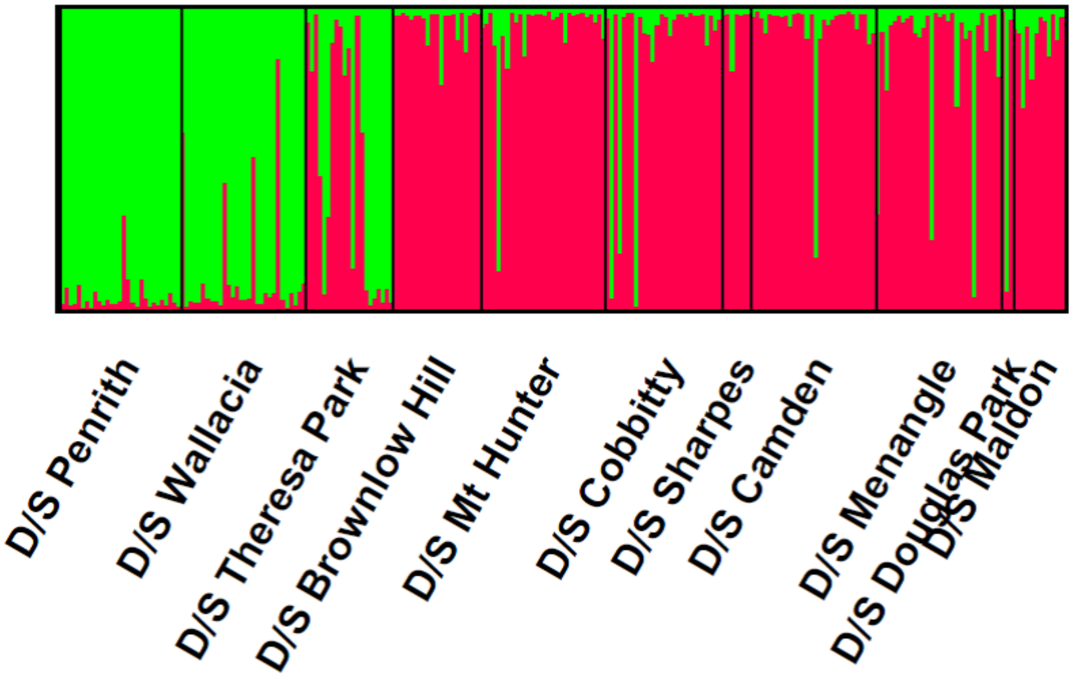


Figure 20 Proportional membership coefficient (Q) plots of Australian smelt from Nepean, Avon and Cordeaux dams for K = 2. Individual fish are represented by vertical bars and population boundaries are defined by thin vertical lines. D/S = downstream. Colours indicate the genetic population 'cluster' that each individual belongs to.

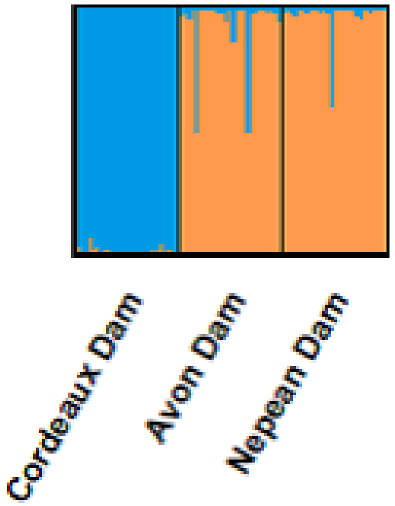


Table 23 Proportion of membership of each population of Australian smelt according to a ΔK of K = 3 for the pre-fishways data and a ΔK of K = 2 for the post-fishways data. The proportional membership of the pre-fishways population at K = 2 is also shown to allow comparison with the post-fishways data. Values > 0.6 are in bold and indicate that most individuals in the population belong to the same genetic cluster. D/S = downstream.

		Pre-fishways				Post-fishways		
			Proportion of membership (*K* = 3)*			Proportion of membership (*K* = 2)*		
Population	**Reach**	**n**	**1**	**2**	**3**	**N**	**1**	**2**
D/S Penrith	1	22	0.06	**0.79**	0.14	29	0.04	**0.96**
D/S Wallacia	2	54	0.08	**0.75**	0.16	30	0.11	**0.89**
D/S Theresa Park	3	5	0.44	0.29	0.27	21	0.48	0.52
D/S Brownlow Hill	4	10	0.41	0.13	0.47	21	**0.96**	0.04
D/S Mt Hunter	5	10	0.58	0.03	0.39	30	**0.93**	0.07
D/S Cobbitty	6	40	**0.78**	0.06	0.16	28	**0.87**	0.14
D/S Sharpes	7	11	0.49	0.08	0.43	7	**0.96**	0.04
D/S Camden	8	40	**0.74**	0.07	0.20	30	**0.94**	0.06
D/S Menangle	9	4	0.42	0.03	0.55	30	**0.86**	0.14
D/S Douglas Park	10	68	0.47	0.06	0.48	3	**0.67**	0.33
D/S Maldon	11	2	**0.91**	0.07	0.02	12	**0.90**	0.10

*****The most likely number of populuations (*K)* according to ΔK statistic.

Discussion

Nepean River genetic diversity

Before construction of 10 new fishways on weirs along the Nepean River in 2009-10, Australian smelt populations in the Nepean River had significantly lower genetic diversity (particularly allelic richness) in the upstream reaches. Following fishway construction, these significant differences were no longer present, suggesting the newly installed fishways have promoted upstream gene flow. Lower genetic diversity above barriers relative to downstream populations has been reported for many species of fish and is most likely a result of insufficient upstream gene flow to compensate for the effects of genetic drift on the isolated populations (Hänfling & Weetman 2006; Jager *et al.* 2001; Yamamoto *et al.* 2004). While there is an abundance of literature attesting to the effect of barriers on gene flow, to our knowledge, there have not been any studies to date dedicated purely to assessing the improvements to gene flow that are the result of reinstating fish passage through the installation of fishways. Nevertheless, one study on black redhorse (*Moxostoma duquesnei*) in the regulated Grand River in Ontario found some evidence that two fishways were assisting in promoting gene-flow between populations (Reid *et al.* 2008).

One limitation of this study that needs to be acknowledged is that the populations of Australian smelt between the study reach and the dams upstream were not included. Thus it is possible that the increased allelic richness in upstream populations is not due to upstream migration, but instead due to an influx of fish washed from populations further upstream - including from the dams - during high flows, which occurred during post-fishway sampling.

Genetic structure

Artificial fragmentation of populations through the construction of weirs and dams restricts migration of fish and results in substantial genetic consequences (McCraney *et al.* 2010; Yamamoto *et al.* 2004). Here we have shown that before fishway construction, Australian smelt populations in the Nepean River were spatially structured. A similar study of Australian smelt in the regulated Goulburn and Campaspe Rivers in Victoria indicated populations were also spatially structured, while the unregulated Ovens River showed no spatial structure across approximately 40 km (Woods 2008). The population of Australian smelt in the Nepean River was divided into three genetic clusters, one predominantly downstream of Wallacia Weir and the other two predominantly upstream of Wallacia Weir. The population immediately above Wallacia Weir was comprised of a small proportion (29%) of fish with membership from the downstream cluster, suggesting that few individuals were able to negotiate the original pool and weir fishway and move upstream, though not to the extent that resulted in genetic homogenisation.

Despite the small number of individuals sampled from above Wallacia pre-fishways (five fish), the data suggest Wallacia Weir was a substantial barrier to upstream movement of Australian smelt before a fishway was constructed. Post-fishways, the proportion of Australian smelt above Wallacia Weir that originated from the downstream genetic cluster increased from 29% to 52% and allelic richness also increased, suggesting that the new fishway had facilitated greater upstream movement of this species. However, based on outputs of analysis in Structure, there was only a modest increase in upstream gene flow, which suggests that the scale of migration of this species is small, that there has been insufficient time for a substantial change in genetic structure to occur, or that the Wallacia fishway is not working effectively. Fishway trapping data from the vertical-slot fishways in this study (Penrith, Theresa Park and Douglas Park fishways) demonstrate successful passage of Australian smelt, thus ineffective passage through the Wallacia Weir fishway is unlikely (given it has the same design specifications). Consequently, the data suggest insufficient numbers of individual fish have moved through the fishway and continued their migration further upstream in the short period of time that the fishways have been operational. While the species is known to attempt upstream migrations through fishways in relatively large numbers, the scale of the migration and importance to the life history is unknown (Stuart *et al.* 2008). This species is quite capable of maintaining populations in isolated lentic habitats where migration and larval displacement are not possible (Milward 1966). Thus, it has been suggested that the larger-scale movements occur over multiple generations, rather than the intra-generational migrations between freshwater and estuaries that are undertaken as a critical point in the life-cycle by species such as Australian bass (Mallen-Cooper & Brand 2007). Consequently, it may take multiple generations (several years) for the full effect of reinstating population connectivity to be reflected in more uniform genetic structure along the river.

A perplexing finding of this study is that Theresa Park Weir also appears to be a substantial migration barrier to Australian smelt both pre- and post-fishways, despite trapping data from this fishway indicating that Australian smelt are the third most common species trapped in the exit of the fishway. Pre- and post-fishways, there was some genetic evidence that fish from below Wallacia were successfully migrating upstream, but there was no evidence that any of these fish were continuing their migrations further upstream past Theresa Park Weir. As suggested above, it is possible that insufficient time has passed from pre-fishway to post-fishway sampling to enable genetic changes to be detected. However, a potential natural barrier to fish movement in the form of a rocky gorge (Bents Basin) is located downstream of Theresa Park Weir. It may be that this natural barrier is the key driver responsible for the genetic fragmentation in both the pre- and post-fishway populations between Wallacia and Theresa Park Weirs. The gorge is steep and rocky with areas of fast-flowing rapids and large boulders. During very low flows, the lack of connectivity among pools may prevent migration. Similarly, during very high flows, turbulence and high water velocities may exceed the swimming ability of Australian smelt and perhaps other small-bodied species. Electrofishing and trapping data demonstrates that striped gudgeon expanded their distribution upstream of Theresa Park Weir following installation of the fishways, and thus successfully negotiated the gorge. The striped gudgeon are a small-bodied species that may have a better burst swimming speed than Australian smelt. Striped gudgeon possibly use their burst swimming ability to propel themselves from one eddy to another along the substratum. In contrast, Australian smelt rely on sustained swimming in the mid and upper water column and therefore may find it more difficult to negotiate lengthy reaches of higher velocity. Further sampling of Australian smelt is recommended in the future to determine if gene flow past Bent's Basin and Theresa Park Weir is occurring.

Downstream movement

Australian smelt populations in reaches upstream of Theresa Park Weir were relatively homogenous in genetic structure even before fishways were constructed. This may be due to fish moving upstream and downstream during weir drown-outs, or through fish moving downstream when the weirs were spilling. However, the lack of downstream movement of this species over Wallacia Weir is perplexing. Wallacia Weir rarely drowns out (Mallen-Cooper & Smit 2005), which clearly accounts for the lack of upstream movement. However, it does spill at times, presumably allowing for downstream movement. Consequently, the results of this study actually suggest that this species actively avoids being swept over the spillway given genetic structure downstream would quickly be diluted if this occurred.

Dams

The construction of dams has been implicated in the loss of genetic diversity of fish populations upstream, and significant genetic differentiation from downstream populations (Kitanishi *et al.* 2012; Yamamoto *et al.* 2004). Here we have shown that the populations of Australian smelt in the three dams have not shown a consistent response to their isolation, despite all three populations being isolated for approximately 90 years. A significant reduction in genetic diversity was not detected in the dams compared to the river populations, nor was there evidence of a recent genetic bottleneck or substantial inbreeding. Nevertheless, Cordeaux Dam had the lowest allelic richness and heterozygosity of any of the populations tested and was in fact monomorphic for three of the eight loci. This population was also substantially divergent from all other populations. The Macquarie perch population of Cordeaux Dam also displays the genetic effects of fragmentation, including low genetic diversity, evidence of a genetic bottleneck and a high degree of genetic divergence to nearby populations (Faulks *et al.* 2011).

In contrast, Australian smelt populations in nearby Nepean and Avon Dams had retained a high level of genetic diversity, were not substantially different from each other and were less divergent from downstream populations. A similar study on Australian smelt in three Victorian catchments also reported maintenance of genetic diversity in upstream sites in both regulated and non-regulated rivers (Woods 2008). The rate of genetic drift and loss of genetic diversity is directly related to effective population size (Frankham *et al.* 2002). Therefore, if a population isolated as a result of construction of a dam is sufficiently large, it is not liable to genetic drift or the loss of genetic diversity for neutral genetic markers. If the period of isolation is not long enough for the generation of new alleles through mutation, the population genetic signature of that population will change every little from its source. For example, freshwater catfish in Burrendong Dam, Copeton Dam and Keepit Dam have large catchment areas (13900, 5360 and 5700 km^2 respectively) and do not display reduced genetic variability compared to the downstream populations (Rourke & Gilligan 2010). The catchment area of Cordeaux Dam is only 91 km^2 compared to 320 km^2 and 142 km^2 above Nepean and Avon Dams respectively. In addition, there are two smaller dams upstream of Cordeaux Dam, which further reduce the habitat available to Australian smelt. In contrast, Nepean Dam can receive water transfers from the nearby Wingecarribee Reservoir (40 km^2 catchment area) on the Wingecarribee River, supplied in turn with water transfered from the Shoalhaven River, potentially introducing Australian smelt from that catchment, and thus introducing genetic diversity. Water from the Nepean Dam can also be transferred into Avon Dam, potentially allowing population connectivity between these two dams. Thus the Nepean and Avon Dams are potentially much larger populations based on available catchment area and their inter-basin connections compared to the isolated Cordeaux Dam. The latter may be too small to prevent loss of genetic diversity associated with stochastic events or loss of alleles through random genetic drift.

Smaller catchments cannot support as many individuals and are more susceptible to further population declines that may occur during droughts or disease outbreaks, possibly resulting in local extinction (Hudman & Gido 2013; Kitanishi *et al.* 2012; Morita & Yamamoto 2002; Whiteley *et al.* 2013). For example, brook trout (*Salvelinus fontinalis*) populations isolated in the smallest habitat patches above dams had substantially lower genetic diversity and were more genetically differentiated from adjacent populations than larger habitat patches (Whiteley *et al.* 2013).

An alternative explanation for the loss of genetic diversity in Cordeaux Dam may be related to water quality at the time of initial dam filling. The policy around the time of dam construction was to clear all vegetation prior to filling the storages, most likely through timber felling and removal and burning of accessible vegetation (Tony Paull, personal communication). It is likely that the water quality following initial filling would have been very poor in all three dams. Water quality conditions may have taken longer to improve in Cordeaux Dam given its small catchment size and thus longer filling time. The poor water quality may have killed many fish and the remnant populations would have been forced into the surrounding rivers and streams. Therefore, the remnant population of Australian smelt in the Cordeaux catchment may have been much smaller than in the Nepean and Avon catchments, resulting in a more dramatic loss of genetic diversity. Alternatively, they could all have suffered major bottlenecks following isolation. Nepean and Avon dam populations, however, may have subsequently recovered through immigration via the water transfers referred to above. The results presented here suggest there are potential implications for other potamodromous species isolated above dams with a small catchment area given that the persistence of a species does not necessarily indicate the population is genetically unscathed.

Management implications

There are more than 4,000 licensed weirs and dams in NSW alone (Anon 2006). A small proportion of these are high level dams or weirs with no provision for fish passage. It has been demonstrated that dams can cause a reduction in genetic diversity and or modify the genetic structure of the impounded population (Faulks *et al.* 2011; Rourke & Gilligan 2010; Yamamoto *et al.* 2004). However, there is some evidence from this and other recent studies that a large catchment area may somewhat protect a population from losing genetic diversity, though they are still susceptible to random genetic drift that results in population genetic differentiation if the effective population size of the population is small (Kitanishi *et al.* 2012; Rourke & Gilligan 2010; Whiteley *et al.* 2013). Consequently, the data presented here could have significant implications for other Australian potamodromous species above dams with only small catchment areas. This study also has implications for other rivers with mid-level weirs given that these barriers not only prevent upstream gene flow, but may also prevent downstream gene flow for species that are reluctant to pass over spillways. Consequently, for some species, upstream and downstream gene flow may only be possible under high flows that cause the barriers to completely drown-out. Thus this study has provided additional information on the importance to fishways in maintaining genetic connectivity in populations of small-bodied freshwater fish.

Synthesis and Conclusions

Background

Construction of dams and weirs in the Hawkesbury-Nepean river system during the last century inadvertently resulted in numerous barriers to migration for river dwelling animals, primarily native fish. The H-N river system historically contained a range of endemic fish species, including many with a critical requirement to migrate as part of their life cycle. Migratory fish species in the H-N river system may spend their life cycle wholly in freshwater, migrate upstream from the sea to spawn, migrate downstream to the sea or estuary to spawn, or migrate between sea and freshwater habitats, but not for the purpose of spawning. Barriers to migration affect more than half the H-N system and have obvious potential consequence for the migration, recruitment, distribution, genetic diversity and structure of endemic fish populations above and below the barriers.

The Sydney Catchment Authority completed measures at the Nepean River weirs in December 2010 that aimed to ensure delivery of environmental flows past the weirs, with a complementary aim to improve native fish passage via the installation of fishways. The subsequent ‘Nepean Fish Passage Rehabilitation Program’ was the largest ever undertaken on a coastal Australian river system and provided a valuable opportunity to evaluate the impact of reconnecting approximately 90 km of freshwater habitat to the catchments estuary on the fish community. This document reports on an intensive monitoring program that evaluated the predicted improvements to fish passage.

The three broad objectives of the monitoring program were (i) to determine if fish community diversity increases upstream as a result of the new fishways, (ii) to assess whether there is increased gene-flow among populations of Australian smelt and (iii) to determine whether environmental flows are stimulating fish movement following fishway construction.

Upstream and downstream fish community

We presented results from surveys of fish communities in 20 sites in 11 reaches spread along a 90 km section of the H-N river system pre- and post-fishway commissioning.

Prior to fishway construction, the reaches surveyed in the Nepean River exhibited a general decline in fish species richness from downstream to upstream. Following fishway commissioning, there was a slight increase in species richness in the middle reaches, but no discernible change in richness at the two most downstream and upstream sites (Figure 3). Pre-fishways, there was a significant difference in fish community structure upstream and downstream of Wallacia Weir. Post-fishways, the significant difference in fish community structure had moved upstream to Theresa Park Weir, suggesting a gradual improvement in fish community structure following fishway installation in the Wallacia to Theresa Park reach. The species that most improved their upstream distribution and relative abundance were freshwater mullet, sea mullet, freshwater herring, Australian smelt and common carp (Table 3).

Overall, there was a positive response to fishways for catadromous species (those that migrate downstream to the sea or estuary to spawn) with most species expanding their distribution upstream (freshwater herring, freshwater mullet and sea mullet). In contrast, the distributions of amphidromous species (those that migrate between sea and freshwater habitats, but not for the purpose of spawning) were very similar pre- and post-fishway commissioning. However, another likely amphidromous species, Cox’s gudgeon, were only found in the most upstream sites pre-fishways, but were detected throughout the study reach post fishways (Table 3). The most likely explanation is that the fishways coupled with the environmental flows produced environmental conditions more conducive to the habitat requirements of this species at the more downstream sites.

Potential effect of weirs on size distribution of Australian bass

Australian bass were present throughout the Nepean River prior to the fishways being installed, which could imply that their migration pathways were unaffected by the weirs. However, results indicated that pre-fishways, the upstream migration of the smallest Australian bass (young-of-year) was hindered by Theresa Park Weir as evidenced by a sharp increase in the size of the smallest fish immediately after this weir (Figure 5). Post-fishways, the smallest Australian bass were still significantly larger at the upstream sites, but the increase in fish size immediately upstream of Theresa Park Weir was less apparent, suggesting the smaller fish were using the fishway. Thus the results indicate that the small Australian bass were capable of using the old pool-and-weir fishway at Wallacia, but for reasons unknown, not the pre-existing rock ramp fishway at Theresa Park.

Passage of fish through three vertical-slot fishways in the Nepean River

We presented results from surveys of fish collected by traps placed in the entrance and exits to three fishways. Nineteen species were trapped at the fishways and all species that attempted to pass through the fishways (entrance sample) were recorded in the exit with the exception of the alien species, goldfish and eastern gambusia. However, some small-bodied native species were less successful in reaching the exit (Table 12). Fish that successfully travelled to the exit of the fishways ranged from 20 mm to 1.2 m in length, but there were significant differences in the size of fish caught at the entrance and exit of the fishways for some species. For example, larger sea mullet and freshwater mullet were not caught in the entrance of Penrith fishway. These size differences were most likely attributable to sampling bias at the entrance where the flows through the fishway were deliberately reduced at the time of entrance sampling, thus reducing the attraction flows necessary for larger-bodied individuals. The presence of the full size-range of these species during exit sampling confirmed the fishways were successfully passing small and large individuals of these species.

PIT Tagging

We documented fish migration through six fishways fitted with Passive Integrated Transponder (PIT) technology. We tagged over 3,500 medium to large-bodied fish from 14 species over the course of the study, including more than 1,500 Australian bass. Flood damage resulted in several PIT readers being inoperable for most of the study period, but the system will become operable into the future and more data will be collected. Only the Camden fishway PIT reader system operated over a sufficient period to allow fish passage analyses, and the results showed 75% efficiency in ascent of Australian bass (Table 17).

Australian bass recaptured during electrofishing or fishway trapping, as well as angler recapture data strongly suggest this species returns to a home range following the spawning migration, with 94% of recaptures during electrofishing and trapping activities at the site of original tagging, suggesting strong site fidelity following migration. In contrast, only 40% of angler recaptures were caught at the site of original tagging, suggesting only limited site fidelity. However, electrofishing and trapping were carried out when fish were expected to have completed their return migration to the river following spawning. Together, these data suggests that downstream migration is occurring and the majority of fish return to their home range following spawning.

Repair of the PIT reader system was scheduled to occur by September 2014, but still has not occurred. The data generated when tagged fish move through the fishways will enable correlations between flows and fish movement to be determined.

Genetics

We used genetic techniques to determine if weirs and dams in the Nepean River had impacted on the genetic structure of Australian in the Nepean River.

Before fishway installation there was clear differentiation of the Australian smelt population upstream and downstream of Wallacia Weir, suggesting this weir was a substantial barrier to upstream gene flow for this species. In addition, it appeared that Theresa Park Weir was also a barrier to gene flow, though the presence of a natural barrier downstream in the form of a steep rocky gorge being responsible for the genetic structure observed could not be discounted (Figure 18; Figure 19). Post-fishways, more upstream gene flow occurred at Wallacia Weir, though not at Theresa Park despite the species being the third most common species trapped in the exit of the Theresa Park fishway. This does suggest that the rocky gorge may be limiting the upstream movement of Australian smelt rather than Theresa Park Weir, though further data are required to determine if gene flow past Theresa Park Weir improves over time. To determine if the gene flow continues to improve at Wallacia and Theresa Park, further sampling and genetic analysis is recommended. These results have major implications for gene flow across fragmented populations Australia-wide and demonstrate that it is not just high-level dams that are capable of restricting gene flow.

Conclusion

The Nepean Fishways Assessment Project has shown that in only a short time since fishway installation, the fishways have been successful in improving the distribution of native fish species. It was evident from fish community composition analyses and gene flow data from Australian smelt that Wallacia was the most significant barrier in the system. This barrier has been substantially ameliorated by construction of the fishway at this weir, as evidenced by the improved movement of catadromous species and increased gene flow of Australian smelt.

Fishway trapping has shown that most species and a broad size range of fish are successfully ascending the fishways. Thus the refinements that have been made to the vertical-slot fishway design over many years of application in Australia have resulted in a design that successfully passes fish representing a wide range of life histories and swimming abilities. The results of this study will further contribute to continued improvements to fishway design for use in other systems.

This study gives an indication of the short-term response of the fish community to the new fishways. Additional monitoring over the long-term will allow additional positive changes to the fish community to be assessed.

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Appendices

Appendix 1 – Electrofishing sampling dates

	Replicate (pre-fishways)				Replicate (post-fishways)			
Site	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Devlin road	22/06/09	28/09/09	24/11/09	28/03/11	08/11/11	15/04/12	31/10/12	09/04/13
Penrith D/S	26/06/09	29/09/09	24/11/09	29/03/11	08/11/11	26/04/12	31/10/12	09/04/13
Penrith U/S	20/05/09	29/09/09	25/11/09	29/03/11	09/11/11	05/04/12	04/11/12	20/03/13
Wallacia D/S	25/06/09	17/10/09	25/11/09	30/03/11	09/11/11	03/04/12	01/11/12	20/03/13
Wallacia U/S	19/05/09	30/09/09	26/11/09	31/03/11	10/11/11	04/04/12	03/11/12	05/03/13
Theresa Park D/S	19/05/09	30/09/09	26/11/09	31/03/11	10/11/11	04/04/12	03/11/12	05/03/13
Theresa Park U/S	26/06/09	01/10/09	08/12/09	13/04/11	11/11/11	11/04/12	05/11/12	21/03/13
Brownlow Hill D/S	23/06/09	20/10/09	08/12/09	13/04/11	11/11/11	11/04/12	05/11/12	21/03/13
Brownlow Hill U/S	18/06/09	13/10/09	09/12/09	14/04/11	12/11/11	12/04/12	07/11/12	07/03/13
Mt Hunter D/S	18/06/09	13/10/09	09/12/09	14/04/11	12/11/11	12/04/12	07/11/12	07/03/13
Mt Hunter U/S	16/06/09	14/10/09	14/12/09	15/04/11	14/11/11	13/04/12	06/11/12	19/03/13
Cobbitty D/S	16/06/09	14/10/09	14/12/09	15/04/11	14/11/11	13/04/12	06/11/12	19/03/13
Cobbitty U/S	22/06/09	15/10/09	12/12/09	12/04/11	15/11/11	14/04/12	06/11/12	06/03/13
Sharpes D/S	18/05/09	15/10/09	12/12/09	12/04/11	15/11/11	14/04/12	28/11/12	06/03/13
Sharpes U/S	17/06/09	16/10/09	11/12/09	18/04/11	30/11/11	16/04/12	27/11/12	10/04/13
Camden D/S	17/06/09	16/10/09	11/12/09	18/04/11	30/11/11	16/04/12	27/11/12	10/04/13
Camden U/S	21/05/09	17/10/09	10/12/09	16/04/11	29/11/11	17/04/12	28/11/12	05/03/13
Menangle D/S	15/06/09	17/10/09	10/12/09	16/04/11	01/12/11	17/04/12	28/11/12	06/03/13
Douglas Park D/S	24/06/09	19/10/09	15/12/09	19/04/11	01/12/11	27/04/12	29/11/12	08/04/13
Maldon D/S	24/06/09	19/10/09	15/12/09	19/04/11	28/11/11	18/04/12	29/11/12	18/03/13
Warragamba River	N/A	N/A	N/A	30/03/11	29/11/11	03/04/12	01/11/12	20/03/13

Appendix 2 – Total number of fish caught (and observed) during each electrofishing round at the Warragamba River.

Species Code	Warragamba River					Total
	1	2	3	4	5	
ANGAUS	0	0	1	0	0	1
ANGREI	2(1)	2(1)	7	3	3(3)	17(5)
CARAUR	5	0	1	0	0	6
CYPCAR	0	2	0	1	0	3
GAMHOL	1	0	0	0	0	1
GOBAUS	2	5	1	2	2	12
GOBCOX	45	0	2	6	2	55
HYPCOM	4	22(5)	11	28	1	66(5)
HYPGAL	0	0	1	2	0	3
MACNOV	31(2)	13(1)	7(1)	15	13(2)	79(6)
MUGCEP	7	45(25)	20(10)	4	0(1)	76(36)
NOTROB	0	1	0	0	0	1
ONCMYK	0	0	1	1	0	2
PHIGRA	9	19	5	5	11	49
PHIMAC	1	0	0	0	0	1
POTRIC	13	0	1	0	0	14
RETSEM	36(22)	2(3)	96(14)	2	20	156(39)
SALTRU	0	0	1	2	0	3
TANTAN	1	1	2	0	0	4
TRAPET	17	17(14)	10	23	2	69(14)
Total	**174(25)**	**129(49)**	**167(25)**	**94**	**54(6)**	**618(105)**

Appendix 3 – Rank order of sites used for RELATE analysis.

	Devlin Lane	Penrith - d/s	Penrith - u/s	Wallacia - d/s	Wallacia - u/s	Theresa Park - d/s	Theresa Park - u/s	Brownlow Hill d/s	Brownlow Hill u/s	Mt Hunter - d/s	Mt Hunter - u/s	Cobbitty d/s	Cobbitty u/s	Sharpes - d/s	Sharpes - u/s	Camden d/s	Camden u/s	Menangle - d/s	Douglas Park d/s
Devlin Lane																			
Penrith d/s	1																		
Penrith u/s	2	1																	
Wallacia d/s	3	2	1																
Wallacia u/s	4	3	2	1															
Theresa Park d/s	5	4	3	2	1														
Theresa Park u/s	6	5	4	3	2	1													
Brownlow Hill d/s	7	6	5	4	3	2	1												
Brownlow Hill u/s	8	7	6	5	4	3	2	1											
Mt Hunter d/s	9	8	7	6	5	4	3	2	1										
Mt Hunter u/s	10	9	8	7	6	5	4	3	2	1									
Cobbitty d/s	11	10	9	8	7	6	5	4	3	2	1								
Cobbitty u/s	12	11	10	9	8	7	6	5	4	3	2	1							
Sharpes d/s	13	12	11	10	9	8	7	6	5	4	3	2	1						
Sharpes u/s	14	13	12	11	10	9	8	7	6	5	4	3	2	1					
Camden d/s	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1				
Camden u/s	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1			
Menangle d/s	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1		
Douglas Park d/s	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	
Maldon d/s	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1

Appendix 4 – Individuals caught per species in the bait traps pre- and post-fishways during electrofishing sampling.

Site	**AMBAGA**		**ANGREI**		**CARAUR**		**GAMHOL**		**GOBAUS**		**GOBCOX**		**HYPCOM**		**HYPGAL**		**HYPSPP**		**PHIGRA**		**PHIMAC**	
	Pre-	**Post-**	**Pre-**	**Post-**	**Pre-**	**Post-**	**Pre-**	**Post-**	**Pre-**	**Post-**	**Pre-**	**Post-**	**Pre-**	**Post-**	**Pre-**	**Post-**	**Pre-**	**Post-**	**Pre-**	**Post-**	**Pre-**	**Post-**
Devlin Lane	-	2	-	-	1	-	6	-	-	-	-	-	1	2	-	5	-	-	1	2	-	-
Penrith D/S	-	1	-	-	-	-	2	-	-	3	-	2	1	6	2	-	-	-	7	6	1	-
Penrith U/S	-	-	-	-	-	-	1	-	-	-	-	4	2	-	-	3	-	-	20	6	1	1
Wallacia D/S	-	-	1	-	-	-	-	1	-	-	-	2	3	3	-	1	-	-	15	8	-	-
Wallacia U/S	-	-	-	-	-	-	3	-	-	-	-	-	-	-	2	6	6	-	8	2	3	-
Theresa Park D/S	-	-	-	-	-	-	1	-	-	-	-	3	-	-	3	8	5	-	10	1	3	-
Theresa Park U/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	7	1	-	12	4	-	-
Brownlow Hill D/S	-	-	-	-	-	-	-	1	-	-	-	-	-	-	11	3	-	-	11	1	2	-
Brownlow Hill U/S	-	-	-	-	-	-	2	-	-	-	-	-	-	-	7	4	3	-	4	2	-	-
Mt Hunter D/S	-	-	-	-	-	-	-	-	-	-	-	1	-	-	10	12	1	-	9	-	2	1
Mt Hunter U/S	-	-	-	-	-	-	1	-	-	-	-	2	-	-	9	11	-	-	9	8	1	-
Sharpes D/S	-	-	-	-	-	-	-	-	-	-	-	1	-	-	3	9	1	-	8	-	-	-
Sharpes U/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	5	4	-	6	1	1	1
Cobbitty D/S	-	-	-	-	-	-	-	-	-	-	-	2	-	-	7	2	2	-	4	3	1	-
Cobbitty U/S	-	-	-	-	-	-	-	1	-	-	-	-	-	-	8	17	2	-	8	4	-	-
Camden D/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3	7	-	5	1	-	-
Camden U/S	-	-	-	-	-	-	4	-	-	-	-	-	-	-	8	5	1	-	8	4	-	-
Menangle D/S	-	-	-	-	-	-	5	3	-	-	-	-	-	-	5	8	-	-	7	-	6	-
Douglas Park D/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	1	-	-	8	-	3	1
Maldon D/S	-	-	-	-	-	-	-	-	-	-	-	1	-	-	4	-	-	-	6	-	2	-
Total	**-**	**3**	**1**	**-**	**1**	**-**	**25**	**6**	**-**	**3**	**-**	**18**	**7**	**11**	**93**	**110**	**33**	**-**	**166**	**53**	**26**	**4**

Appendix 5 – Total number of fish caught (and observed) during each electrofishing round. Pre-fishway sampling was conducted during rounds 1-3 and post-fishway sampling was conducted during rounds 4-8.

Devlin Lane - Round									
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
AMBAGA	0	0	0	2	0	0	0	0	**2**
ANGREI	4(1)	3(4)	8(3)	4(1)	8(1)	3(3)	1(3)	2(1)	**33(17)**
CARAUR	11	21(1)	5	3	4	0	1	0	**45(1)**
CYPCAR	5	1	1	1(2)	7(1)	2	0	4	**21(3)**
GAMHOL	0	0	67	0	0	0	0	0	**67**
GOBAUS	10	13	0	2	2	0	2	0	**29**
GOBCOX	0	0	0	2	0	2	0	0	**4**
HYPCOM	38(10)	23	65(26)	12	21	6	5	0	**170(36)**
HYPGAL	0	0	0	6	0	0	0	0	**6**
MACCOL	0	0	0	0	0	0	0	1	**1**
MACNOV	12	9	15(1)	42(8)	58(23)	22(3)	46(4)	15	**219(39)**
MUGCEP	22	9(10)	17(3)	0	12	21	16(64)	31(7)	**128(84)**
NOTROB	6	3	2	1	0	1	1	0	**14**
PHIGRA	6	3	14	2	6	1	0	2	**34**
PHIMAC	0	1	0	0	0	1	1	0	**3**
POTRIC	0	0	9(6)	31(34)	31(15)	67(74)	11	19	**168(129)**
RETSEM	3	0	8	12	6	25	1	0	**55**
TANTAN	13(2)	1	1	3	5	5	6(2)	4(1)	**38(5)**
TRAPET	2	2(4)	7(5)	7(3)	4(1)	26(12)	15	15(2)	**78(27)**
Total	**132(13)**	**89(19)**	**219(44)**	**130(48)**	**164(41)**	**182(92)**	**106(73)**	**93(11)**	**1115(341)**

	Penrith d/s - Round								
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
AMBAGA	0	0	0	2	0	0	0	0	**2**
ANGREI	0	1(2)	5(2)	1(2)	1	1	6(1)	0	**15(7)**
CARAUR	2	1	1	2	3	0	0	0	**9**
CYPCAR	0	3	1	2	1	3(1)	3	5	**18(1)**
GAMHOL	0	6	1	0	0	0	0	0	**7**
GOBAUS	3	8	1	1	5	0	1	0	**19**
GOBCOX	0	0	0	11	1	0	0	0	**12**
HYPCOM	3	24	18	1	54	0	8	0	**108**
HYPGAL	0	1	2	0	0	0	0	0	**3**
HYPSPP	0	0	0	0	0	2	0	0	**2**
MACNOV	16	21(8)	41(13)	35(4)	69(3)	14	46	15	**257(28)**
MUGCEP	4	14(7)	7(2)	24	30(6)	39(35)	9	22(2)	**149(52)**
NOTROB	0	0	0	0	0	0	1	0	**1**
PHIGRA	2	8	27	4	12	1	5	1	**60**
PHIMAC	0	1	1	0	0	0	0	0	**2**
POTRIC	0	0	2	51	25	49(10)	19(7)	46(8)	**192(25)**
RETSEM	0	0	12	4	17	0	1	1	**35**
TANTAN	0	3	0	0	3	1	2(1)	1	**10(1)**
TRAPET	2	4	5(1)	18(5)	45(5)	5(5)	2	21(2)	**102(18)**
Total	**32**	**95(17)**	**124(18)**	**156(11)**	**266(14)**	**115(51)**	**103(9)**	**112(12)**	**1003(132)**

Penrith u/s - Round									
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	0	8(1)	4	4(1)	2(5)	1(1)	2(2)	2	**23(10)**
CARAUR	15	20	1	4	1	7(4)	1	0	**49(4)**
CYPCAR	3	7	1	2(1)	3(2)	4	10(1)	5	**35(4)**
GAMHOL	0	2	0	1	0	0	0	0	**3**
GOBAUS	1	1	0	1	0	1	2	0	**6**
GOBCOX	0	0	0	15	0	0	4	0	**19**
HYPCOM	0	1	2	0	2	1	57	1	**64**
HYPGAL	0	0	0	0	5	0	3	9	**17**
HYPSPP	7	0	0	0	0	0	0	0	**7**
MACNOV	14(4)	18(2)	16(2)	16(3)	49(14)	17	28(1)	24(1)	**182(27)**
MUGCEP	5	3(1)	13(4)	12	11(7)	16(6)	4	4	**68(18)**
NOTROB	0	0	0	0	0	0	1	0	**1**
PHIGRA	19	17	29	4	19	3	4	4	**99**
PHIMAC	0	1	1	0	1	0	0	0	**3**
POTRIC	0	5	4(5)	2(1)	2	5(1)	1	24(1)	**43(8)**
RETSEM	13(20)	1	9	2	46(40)	0	1	2	**74(60)**
TANTAN	1	1	0	3	10	3	3	2	**23**
TRAPET	6	0	2	17(1)	10(4)	1	1(1)	25(4)	**62(10)**
Total	**84(24)**	**85(4)**	**82(11)**	**83(7)**	**161(72)**	**59(12)**	**122(5)**	**102(6)**	**778(141)**

	Wallacia d/s - Round								
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	2	10	2	1(1)	3(3)	1(1)	0	0	**19(5)**
CARAUR	1	10	3	15	3	0	0	0	**32**
CYPCAR	0	1	3	1	0	9(5)	2	3	**19(5)**
GAMHOL	1	2	0	2	0	0	0	0	**5**
GOBAUS	1	0	0	0	1	0	2	0	**4**
GOBCOX	0	0	0	10	0	0	1	0	**11**
HYPCOM	3	6	1	2	5	11	5	12	**45**
HYPGAL	0	1	0	0	1	0	2	0	**4**
MACNOV	7	22(8)	30	47(14)	43(9)	13	21(2)	9(4)	**192(37)**
MUGCEP	0	7(3)	18(8)	3	1(5)	10	7	16(1)	**62(17)**
NOTROB	1	0	0	1	2	0	0	0	**4**
PHIGRA	5	30	17	4	15	2	9	9	**91**
POTRIC	0	10(1)	3	5	1(1)	8	0	22(4)	**49(6)**
RETSEM	7	28	0	7(3)	5	30	5	0(1)	**82(4)**
TANTAN	2	3(2)	3	7	7	0	4	0	**26(2)**
TRAPET	0	13	5	58(23)	9(13)	27(8)	8(6)	26(4)	**146(54)**
Total	**30**	**143(14)**	**85(8)**	**163(41)**	**96(31)**	**111(14)**	**66(8)**	**97(14)**	**791(130)**

	Wallacia u/s - Round								
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	1(2)	5(1)	5	2	3(2)	1(1)	1	0	**18(6)**
CARAUR	7(1)	1	0	3	5	2	1	3	**22(1)**
CYPCAR	3(3)	1	0	4(1)	7(1)	3(3)	7	6	**31(8)**
GAMHOL	0	5	0	0	0	0	0	0	**5**
GOBAUS	0	0	0	0	0	0	0	1	**1**
GOBCOX	0	0	0	2	0	0	1	0	**3**
HYPGAL	0	0	4	0	13	0	1	1	**19**
HYPSPP	12	0	0	0	0	0	0	0	**12**
MACNOV	7(3)	13(3)	16	4(1)	52(11)	12	29	3	**136(18)**
MUGCEP	0	0	1(2)	6	11(3)	13	15	9(1)	**55(6)**
PHIGRA	5	9	2	3	2	5	1	9	**36**
PHIMAC	3	0	0	0	0	0	0	3	**6**
POTRIC	0	4(2)	0	12(3)	16(5)	16(5)	10	5	**63(15)**
RETSEM	7	0	0	2	10(20)	0	6	14	**39(20)**
SALTRU	0	0	0	0	0	0	0	2	**2**
TANTAN	0	0	0	1	3	0	1	0	**5**
TRAPET	7(6)	0	0	7(3)	2	23(17)	2	7(3)	**48(29)**
Total	**52(15)**	**38(6)**	**28(2)**	**46(8)**	**124(42)**	**75(26)**	**75**	**63(4)**	**501(103)**

	Theresa Park d/s - Round								
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	1	4(7)	5	1(4)	0(1)	2(2)	1(3)	0	**14(17)**
CARAUR	17(2)	11(10)	8	5	2	3	0	7	**53(12)**
CYPCAR	1	5	2	7	12	8(1)	12	4	**51(1)**
GAMHOL	1	1	0	0	0	0	0	0	**2**
GOBAUS	0	0	0	0	1	0	0	0(1)	**1(1)**
GOBCOX	0	0	0	3	0	0	0	0	**3**
HYPGAL	0	0	10	4	21	2	1	2	**40**
HYPSPP	20	0	0	0	0	0	0	0	**20**
MACNOV	10	35(6)	18(3)	4(1)	36(9)	18	26	1	**148(19)**
MUGCEP	0	0	0	12(1)	6	1	9	5	**33(1)**
PHIGRA	3	11	7	0	1	1	1	6	**30**
PHIMAC	1	1	1	0	0	0	0	1	**4**
POTRIC	0	0	0	21	20	11	3	8	**63**
RETSEM	1	2	3	5	0	0	0	0	**11**
TANTAN	1	0	0	1	1	2	1	0	**6**
TRAPET	0	0	0	3(3)	10(2)	12(4)	2(3)	4(10)	**31(22)**
Total	**56(2)**	**70(23)**	**54(3)**	**66(11)**	**110(12)**	**60(12)**	**56(6)**	**38(13)**	**510(82)**

	Theresa Park u/s - Round								
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	2	4(2)	7(3)	2(5)	2(4)	1(2)	5(1)	1(5)	**24(22)**
CARAUR	0	0	0	2	3	0	1	0	**6**
CYPCAR	1	9	1(1)	5	8	4(1)	11(3)	3(1)	**42(6)**
GOBCOX	0	0	0	1	1	1	1	0	**4**
HYPGAL	0	4	11	1	23	1	0	0	**40**
HYPSPP	1	0	0	0	0	0	0	0	**1**
MACNOV	8	5(2)	5(3)	9(4)	21(2)	7(2)	17(2)	14(3)	**86(18)**
MUGCEP	0	0	0	0	1	1	0	2	**4**
PHIGRA	1	8	21	2	3	2	0	2	**39**
POTRIC	0	0	0	2	4(1)	10(3)	2	7	**25(4)**
RETSEM	0	0	0	0	0	10	0	2	**12**
TANTAN	2	0	3	2	3	1	0	0(1)	**11**
TRAPET	0	0	0	0	0	2	0	4(1)	**6(1)**
Total	**15**	**30(4)**	**48(7)**	**26(9)**	**69(7)**	**40(8)**	**36(6)**	**35(11)**	**300(52)**

	Brownlow Hill d/s - Round								
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	6(1)	6	9(3)	2(1)	1(4)	0	5(2)	0(2)	**29(13)**
CARAUR	0	0	0	0	3	0	0	0	**3**
CYPCAR	1	6	3	1	4	14(5)	6	0	**35(5)**
GAMHOL	0	0	0	0	17	0	0	0	**17**
GOBCOX	0	0	0	0	0	1	1	0	**2**
HYPGAL	0	13	3	2	2	0	5	2	**27**
MACNOV	4(1)	11	11(1)	7(1)	17(3)	13	14(1)	10	**87(7)**
MUGCEP	0	0	0	2	2	2(3)	0	1	**7(3)**
PHIGRA	2	6	11	3	0	0	0	1	**23**
PHIMAC	1	1	0	0	0	0	0	0	**2**
POTRIC	0	0	0	5(2)	0	7	2	8(1)	**22(3)**
RETSEM	2	0	0	1	3	2(1)	7	0	**15(1)**
TANTAN	1	2	0	2	0	0	2	1	**8**
TRAPET	0	0	0	0	0	1	1	6	**8**
Total	**17(2)**	**45**	**37(4)**	**25(4)**	**49(7)**	**40(9)**	**43(3)**	**29(3)**	**285(32)**

	Brownlow Hill u/s - Round								
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	1	0	11(5)	0	2(2)	2(2)	2(1)	2(2)	**20(12)**
CARAUR	0	6(3)	1	1	2	3	6	2	**21(3)**
CYPCAR	3	1	4	12	12(4)	0(1)	2	2(3)	**36(8)**
GAMHOL	0	0	5	0	0	0	0	0	**5**
GOBCOX	0	0	0	0	0	3	1	0	**4**
HYPGAL	0	9	1	2	3	0	0	0	**15**
HYPSPP	3	0	0	0	0	0	0	0	**3**
MACNOV	6	8(1)	12(2)	5	11(1)	6	11(2)	2	**61(6)**
MUGCEP	0	0	0	0	0	3	0	0	**3**
PHIGRA	2	2	5	3	1	0	0	4	**17**
POTRIC	0	0	0	9	1	1(1)	0	1(2)	**12(3)**
RETSEM	0	1	0	0	0	3	0	0	**4**
TANTAN	2	1	1	1	2	2	3	1(1)	**13(1)**
TRAPET	0	0	0	0	0	2	2	1	**5**
Total	**17**	**28(4)**	**40(7)**	**33**	**34(7)**	**25(4)**	**27(3)**	**115(8)**	**219(33)**

Mt Hunter d/s - Round									
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	6	8(7)	11(4)	0(2)	3(1)	2(2)	6	0(4)	**36(20)**
CARAUR	4	3	1	1	1	0	1	0	**11**
CYPCAR	1	6	1	5	6(2)	2	0	3	**24(2)**
GAMHOL	0	0	0	1	0	0	0	0	**1**
GOBCOX	0	0	0	1	1	2	3	0	**7**
HYPGAL	0	20	34	12	30	0	3	0	**99**
HYPSPP	2	0	0	0	0	0	0	0	**2**
MACNOV	7	5(1)	10(2)	5(2)	7(2)	3(2)	15	3	**55(9)**
MUGCEP	0	0	0	5	1	4	1	1	**12**
PHIGRA	3	4	17	1	6	1	1	2	**35**
PHIMAC	1	0	1	1	0	0	0	0	**3**
POTRIC	0	0	0	3	0	1	0	1	**5**
RETSEM	2	9	0	8	0	19	25	20	**83**
TANTAN	6	1(1)	1	1	4	2	5	2	**22(1)**
Total	**32**	**56(9)**	**76(6)**	**44(4)**	**59(5)**	**36(4)**	**60**	**32(4)**	**395(32)**

Mt Hunter u/s -Round									
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	1(2)	5(2)	798)	1	3(1)	0(1)	1(4)	0(4)	**18(22)**
CARAUR	0	9	0(1)	1	3(1)	0	1	0	**14(2)**
CYPCAR		3(2)	1	2	2	3(2)	3	8	**22(4)**
GAMHOL	1	0	2	8	0	0	0	0	**11**
GOBCOX	0	0	0	4	0	1	0	1	**6**
HYPGAL		9	16	5	28		1	2	**61**
MACNOV	1	5	6(6)	3(2)	5	4(2)	29(1)	3	**56(11)**
PHIGRA	1	1	14	5	7		1	7	**36**
PHIMAC	0	0	1	0	0	1	0	0	**2**
POTRIC	0	0	0	0	0	3	1	5	**9**
RETSEM	17(5)	6	1	1	4	14	2	3	**48(5)**
TANTAN	0	0	4(2)	1	3	1	5	1	**15(2)**
TRAPET	0	0	0	0	0	6	0	0	**6**
Total	**21(7)**	**38(4)**	**52(17)**	**31(2)**	**55(2)**	**33(5)**	**44(5)**	**30(4)**	**304(46)**

Cobbitty d/s - Round									
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	3	1	6	4(1)	5(3)	3(1)	5(4)	2	**29(9)**
CARAUR	2	1	0	5	0	1	3	1	**13**
CYPCAR	5	2	6	6	4	3(2)	3	3(3)	**32(5)**
GAMHOL	0	0	0	0	0	2	0	0	**2**
GOBCOX	0	0	0	3	0	2	0	2	**7**
HYPGAL	0	0	22	2	0	0	0	0	**24**
HYPSPP	2	0	0	0	0	0	0	0	**2**
MACNOV	6	9(2)	7	18(2)	13(2)	10	6	3(1)	**72(7)**
MUGCEP	0	0	0	0	0	1	5	0	**6**
PHIGRA	0	0	8	4	0	1	1	0	**14**
PHIMAC	0	0	1	0	0	0	0	1	**2**
POTRIC	0	3	1	2	0	12	0	9(3)	**27(3)**
RETSEM	0	6	85	6(2)	1	16	1	3	**118(2)**
TANTAN	2	5	1	4	2	2	3	0	**19**
TRAPET	0	0	0	0	0	1	0	2	**3**
Total	**20**	**27(2)**	**137**	**54(5)**	**25(5)**	**54(3)**	**27(4)**	**26(7)**	**370(26)**

Cobbitty u/s - Round									
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	2	5	17(5)	3(1)	7(7)	2(6)	1(2)	1(2)	**38(23)**
CARAUR	19	10	1	2	7	0(1)	0	0	**39(1)**
CYPCAR	3	6(2)	4(5)	6(2)	0	2(1)	6	4(5)	**31(15)**
GOBCOX	0	0	0	0	2	0	0	1	**3**
HYPGAL	0	20	7	24	36	0	1	3	**91**
HYPSPP	7	0	0	0	0	0	0	0	**7**
MACNOV	9	16	7(1)	6(3)	27	14(1)	23	3	**105(5)**
MUGCEP	0	0	1	0	1	4	0	0	**6**
PHIGRA	0	7	7	0	5	0	0	5	**24**
POTRIC	0	0	0	2	0	1	1	16(3)	**20(3)**
RETSEM	0	3	1	0	0	4	0	3	**11**
TANTAN	3	1	9	0	6(1)	2	3	1	**25(1)**
TRAPET	0	0	0	2	0	1	0	1	**4**
Total	**43**	**68(2)**	**54(11)**	**45(6)**	**91(8)**	**30(9)**	**35(2)**	**38(10)**	**404(48)**

Sharpes d/s - Round									
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	2(1)	5	4(2)	2(1)	3(2)	0(3)	6	1(1)	**23(10)**
CARAUR	2(4)	5	1	2	2	5	2	5	**24(4)**
CYPCAR	2	7(2)	2	8(2)	3	4(4)	5	2	**33(8)**
GOBCOX	0	0	0	3	1	0	0	5	**9**
HYPGAL	0	9	1	10	2(1)	0	7	1	**30(1)**
HYPSPP	1	0	0	0	0	0	0	0	**1**
MACNOV	3(6)	9	21(5)	7(3)	15	12	10	3	**80(14)**
MUGCEP	0	0	0	0	3	4	1	0	**8**
PHIGRA	1	7	4	0	0(1)	0	0	2	**14(1)**
POTRIC	1	0	0	0	0	7(4)	0	3	**11(4)**
RETSEM	0	8	4	1	0	7	0	0	**20**
TANTAN	2	2	3	3	2	1	1	2(1)	**16(1)**
TRAPET	0	0	0	0	0	3	1	4(4)	**8(4)**
Total	**14(11)**	**52(2)**	**40(7)**	**36(6)**	**31(4)**	**43(11)**	**33**	**28(6)**	**277(47)**

Sharpes u/s - Round									
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	1	3	7	2(3)	0	2(3)	5(1)	0(1)	**20(8)**
CARAUR	0	3	3	1	1(1)	0	0	0	**8(1)**
CYPCAR	0(2)	5	5(1)	5	5(1)	2(4)	0	0	**22(8)**
GAMHOL	0	0	0	0	0	0	0	1	**1**
GOBCOX	0	0	0	0	0	3	2	0	**5**
HYPGAL	0	7	0	0	13	0	3	2	**25**
HYPSPP	6	0	0	0	0	0	0	0	**6**
MACNOV	8(5)	9	11(2)	11(4)	16	5	14(1)	8(1)	**82(13)**
MUGCEP	0	0	0	1	2(1)	5	0	0	**8(1)**
PHIGRA	3	1	12	0	2	2	1	3	**24**
PHIMAC	1	0	0	1	0	0	0	0	**2**
POTRIC	0	0	0	0	0	6(4)	3	1	**10(4)**
RETSEM	23	0	1	0	0	3	7	4	**38**
TANTAN	2	2	5	2	1	2	3(1)	3	**20(1)**
TRAPET	0	0	0	0	0	5	0	2	**7**
Total	**44(7)**	**30**	**44(3)**	**23(7)**	**40(3)**	**35(11)**	**38(3)**	**24(2)**	**278(36)**

Camden d/s -Round									
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	7(2)	6	10	4(6)	2(1)	0(3)	4(4)	3	**36(16)**
CARAUR	7	6	7	1	1	0	0	0	**22**
CYPCAR	1	8	1	6(2)	9(2)	0	1	0	**24(6)**
GOBCOX	0	0	0	3	0	1	1	0	**5**
HYPGAL	0	1	0	5	0	0	13	4	**23**
HYPSPP	1	0	0	0	0	0	0	0	**1**
MACNOV	10	22	17	12(1)	13	13	16(2)	6(2)	**109(5)**
MUGCEP	0	0	0	6(1)	0	1	0	0	**7(1)**
PHIGRA	1	4	6	2	1	1	2	1	**18**
POTRIC	0	0	0	0	0	3(4)	0	0(1)	**3(5)**
RETSEM	0	22	0	17	0	10	1	39	**89**
TANTAN	4	7	8(2)	6(1)	1	4	7	2	**39(3)**
TRAPET	0	0	0	0	0	5(2)	0	0	**5(2)**
Total	**31(2)**	**76(3)**	**49(2)**	**62(11)**	**27(3)**	**38(9)**	**45(6)**	**55(3)**	**383(36)**

Camden u/s - Round									
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	0	4	5(4)	2	1(2)	2(4)	4	0	**18(10)**
CARAUR	11	7	5	8	0	5(3)	0	2	**38(3)**
CYPCAR	0	0	0	1	0	0	0	0	**1**
GAMHOL	3	1	13	1	0	0	0	0	**18**
HYPGAL	0	3	29	3	16	0	1	5	**57**
HYPSPP	50	0	0	0	0	0	0	0	**50**
MACNOV	7(3)	10	3(2)	7	7	11(1)	10	5	**60(6)**
MUGCEP	0	0	0	0	0	0	1	0	**1**
PHIGRA	10	2	12	1	6	3	1	8	**43**
PHIMAC	0	0	1	0	0	0	0	0	**1**
POTRIC	0	0	0	0	0	0	0	1	**1**
RETSEM	0	2	2(1)	0	0	7	5	27	**43(1)**
TANTAN	6	5	7	5	5	7	6	1(1)	**42(1)**
TRAPET	0	0	0	0	0	3	0	2	**5**
Total	**87(3)**	**34**	**77(7)**	**28**	**35(2)**	**38(8)**	**28**	**51(1)**	**378(21)**

Menangle d/s - Round

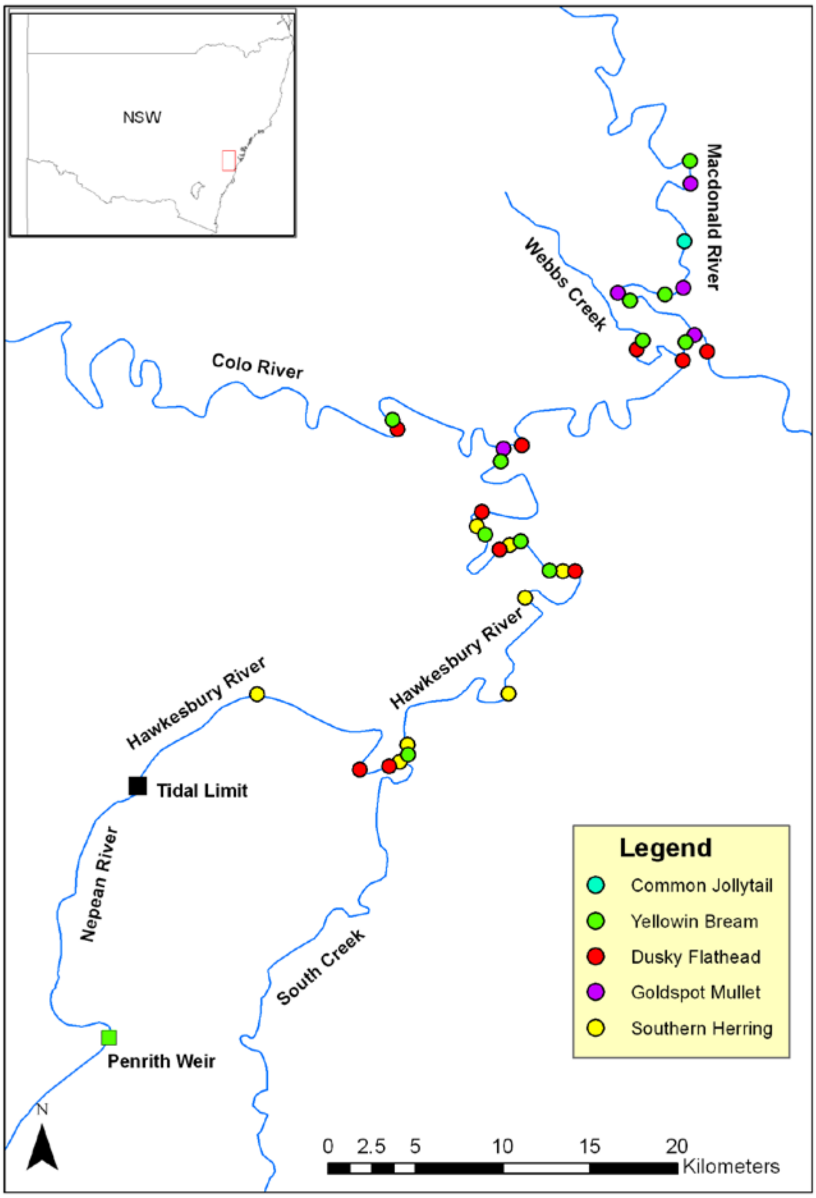
Species	1	2	3	4	5	6	7	8	Total
ANGREI	1(1)	7	6	3(1)	3	2(1)	4	1	**27(3)**
BIDBID	0	0	0	0	0	1	0	0	**1**
CARAUR	16	3	4	3	2	0	2	0	**30**
CYPCAR	0	2(1)	0(1)	1	0	0	1	0	**4(2)**
GAMHOL	0	1	15	23	0	1	0	0	**40**
GOBCOX	0	0	1	1	2	3	2	8	**17**
HYPGAL	0	1	29	19	11	0	9	1	**70**
MACNOV	8(2)	10(1)	9	13	6	10(1)	12	7(1)	**75(5)**
PHIGRA	1	2	8	1	7	2	4	10	**35**
PHIMAC	6	1	0	0	0	2	0	0	**9**
RETSEM	2	0	1	12	23(7)	33(9)	14	10	**95(16)**
TANTAN	9(1)	4	7(3)	5	5	6	5	3	**44(4)**
TRAPET	0	0	0	1	0	3(1)	0(1)	692)	**10(4)**
Total	**43(4)**	**31(2)**	**80(4)**	**82(1)**	**59(7)**	**63(12)**	**53(1)**	**46(3)**	**457(34)**

Douglas Park d/s - Round

Species	1	2	3	4	5	6	7	8	Total
ANGREI	8(4)	15(1)	16(1)	8(5)	6	1(1)	2(9)	4(14)	**60(35)**
CARAUR	2	5	4	6	8(1)	4(1)	7(1)	0	**36(3)**
CYPCAR	1	0	2	2	7	0	0	0	**12**
GAMHOL	0	0	4	0	0	0	0	0	**4**
GOBCOX	0	1	1	0	2	0	1	2(2)	**7(2)**
HYPGAL	0	1	25	0	2	0	1	2	**31**
MACNOV	12(5)	10(2)	11(2)	20(6)	12(2)	6(2)	22(3)	10(7)	**103(29)**
MUGCEP	0	0	0	3	0	0	1	1	**5**
PHIGRA	1	7	11	0	0	1	0	2	**22**
PHIMAC	2	3	4	0	1	1	0	0	**11**
RETSEM	37(28)	2	14	0	0	0	0	3	**56(28)**
TANTAN	2	1	1	6	1	1	1	2(1)	**15(1)**
TRAPET	0	0	0	0	1	0	0	9	**10**
Total	**65(37)**	**45(3)**	**93(3)**	**45(11)**	**40(3)**	**14(4)**	**35(13)**	**35(24)**	**372(98)**

				Maldon d/s - Round					
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
ANGREI	0	10(3)	15	3	1(1)	2(2)	3(12)	2(2)	**36(19)**
CARAUR	0	0	1	0	2	3	6	0	**12**
CYPCAR	1	1	1	0	0	0	0	0	**3**
GOBCOX	0	1	2	6	2	1	3	4	**19**
HYPGAL	0	3	2	0	0	0	0	7	**12**
MACNOV	2	2	2	12	1	5(2)	10	9(2)	**43(4)**
MUGCEP	0	0	0	0	0	0	0	1	**1**
PHIGRA	2	3	11	0	1	0	1	5	**23**
PHIMAC	0	0	2	0	0	1	0	0	**3**
RETSEM	36(9)	2	3	39	2	1(4)	0	10	**93(13)**
TANTAN	0	0	0	0	0	2	1	1	**4**
Total	**41(9)**	**22(3)**	**39**	**60**	**9(1)**	**15(7)**	**24(12)**	**39(4)**	**249(36)**

Appendix 6 – Species caught downstream of the study reach between 1992 and 2007. Data extracted from the NSW DPI Freshwater Fish Database.



Appendix 7 – Fishway trapping dates

Set number	Penrith	Entrance / Exit	Theresa Park	Entrance / Exit	Douglas Park	Entrance / Exit
1	24/02/10	EXIT	N/A	N/A	N/A	N/A
2	25/02/10	ENTRANCE	N/A	N/A	N/A	N/A
3	22/03/10	EXIT	N/A	N/A	N/A	N/A
4	23/03/10	EXIT	N/A	N/A	N/A	N/A
5	24/08/10	ENTRANCE	24/08/10	ENTRANCE	N/A	N/A
6	25/08/10	EXIT	25/08/10	EXIT	N/A	N/A
7	26/08/10	ENTRANCE	26/08/10	ENTRANCE	N/A	N/A
8	27/08/10	EXIT	27/08/10	EXIT	N/A	N/A
9	29/08/10	ENTRANCE	29/08/10	ENTRANCE	N/A	N/A
10	30/08/10	EXIT	30/08/10	EXIT	N/A	N/A
11	31/08/10	ENTRANCE	31/08/10	ENTRANCE	N/A	N/A
12	01/09/10	EXIT	01/09/10	EXIT	N/A	N/A
13	26/10/10	ENTRANCE	26/10/10	ENTRANCE	26/10/10	ENTRANCE
14	27/10/10	EXIT	27/10/10	EXIT	27/10/10	EXIT
15	28/10/10	ENTRANCE	28/10/10	ENTRANCE	28/10/10	ENTRANCE
16	29/10/10	EXIT	29/10/10	EXIT	29/10/10	EXIT
17	31/10/10	ENTRANCE	31/10/10	ENTRANCE	31/10/10	ENTRANCE
18	01/11/10	EXIT	01/11/10	EXIT	01/11/10	EXIT
19	02/11/10	EXIT	02/11/10	EXIT	02/11/10	EXIT
20	03/11/10	ENTRANCE	03/11/10	ENTRANCE	03/11/10	ENTRANCE
21	01/02/11	ENTRANCE	01/02/11	ENTRANCE	01/02/11	ENTRANCE
22	02/02/11	EXIT	02/02/11	EXIT	02/02/11	EXIT
23	03/02/11	EXIT	03/02/11	EXIT	03/02/11	EXIT
24	N/A	N/A	04/02/11	ENTRANCE	04/02/11	ENTRANCE
25	06/02/11	EXIT	06/02/11	ENTRANCE	06/02/11	ENTRANCE
26	07/02/11	ENTRANCE	07/02/11	EXIT	07/02/11	EXIT
27	08/02/11	ENTRANCE	08/02/11	EXIT	08/02/11	EXIT
28	09/02/11	EXIT	09/02/11	ENTRANCE	09/02/11	ENTRANCE
29	13/09/11	ENTRANCE	13/09/11	ENTRANCE	13/09/11	ENTRANCE
30	14/09/11	EXIT	14/09/11	EXIT	14/09/11	EXIT
31	15/09/11	EXIT	15/09/11	EXIT	15/09/11	EXIT
32	16/09/11	ENTRANCE	16/09/11	ENTRANCE	16/09/11	ENTRANCE
33	16/10/11	ENTRANCE	16/10/11	ENTRANCE	16/10/11	ENTRANCE

Set number	Penrith	Entrance / Exit	Theresa Park	Entrance / Exit	Douglas Park	Entrance / Exit
34	17/10/11	EXIT	17/10/11	EXIT	17/10/11	EXIT
35	18/10/11	EXIT	18/10/11	EXIT	18/10/11	EXIT
36	19/10/11	ENTRANCE	19/10/11	ENTRANCE	19/10/11	ENTRANCE
37	13/12/11	ENTRANCE	13/12/11	ENTRANCE	13/12/11	EXIT
38	14/12/11	EXIT	14/12/11	EXIT	14/12/11	ENTRANCE
39	15/12/11	EXIT	15/12/11	EXIT	15/12/11	ENTRANCE
40	16/12/11	ENTRANCE	16/12/11	ENTRANCE	16/12/11	EXIT
41	10/01/12	ENTRANCE	10/01/12	EXIT	10/01/12	EXIT
42	11/01/12	EXIT	11/01/12	ENTRANCE	11/01/12	ENTRANCE
43	12/01/12	EXIT	12/01/12	ENTRANCE	12/01/12	ENTRANCE
44	13/01/12	ENTRANCE	13/01/12	EXIT	13/01/12	EXIT
45	31/01/12	EXIT	31/01/12	EXIT	31/01/12	EXIT
46	01/02/12	ENTRANCE	01/02/12	ENTRANCE	01/02/12	ENTRANCE
47	02/02/12	ENTRANCE	02/02/12	ENTRANCE	02/02/12	ENTRANCE
48	03/02/12	EXIT	03/02/12	EXIT	03/02/12	EXIT
49	04/02/12	EXIT	04/02/12	EXIT	04/02/12	EXIT
50	05/12/12	ENTRANCE	05/12/12	ENTRANCE	05/12/12	ENTRANCE
51	06/12/12	ENTRANCE	06/12/12	ENTRANCE	06/12/12	ENTRANCE
52	07/12/12	EXIT	07/12/12	EXIT	07/12/12	EXIT
53	13/01/13	EXIT	13/01/13	EXIT	13/01/13	EXIT
54	14/01/13	ENTRANCE	14/01/13	ENTRANCE	14/01/13	ENTRANCE
55	15/01/13	ENTRANCE	15/01/13		15/01/13	ENTRANCE
56	16/01/13	EXIT	16/01/13	EXIT	16/01/13	EXIT
57	12/02/13	EXIT	12/02/13	EXIT	12/02/13	EXIT
58	13/02/13	ENTRANCE	13/02/13	ENTRANCE	13/02/13	ENTRANCE
59	14/02/13	ENTRANCE	14/02/13	ENTRANCE	14/02/13	ENTRANCE
60	15/02/13	EXIT	15/02/13	EXIT	15/02/13	EXIT

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