Population genetic structure of freshwater catfish (*Tandanus tandanus*) in the Murray-Darling Basin and coastal catchments of New South Wales: Implications for future re-stocking programs

Meaghan Rourke and Dean Gilligan

Industry & Investment NSW
Narrandera Fisheries Centre
Buckingbong Road Narrandera, NSW 2700
Australia

Recreational Freshwater Fishing Trust Project
No. DPI FT48

June 2010

Industry & Investment NSW - Fisheries Final Report Series
No. 123
ISSN 1837-2112

NSW GOVERNMENT | Industry & Investment

Recreational Fishing Trusts

Population genetic structure of freshwater catfish (*Tandanus tandanus*) in the Murray-Darling Basin and coastal catchments of New South Wales: Implications for future re-stocking programs.

June 2010

Authors:	Meaghan Rourke and Dean Gilligan
Published By:	Industry & Investment NSW (now incorporating NSW Fisheries)
Postal Address:	Narrandera Fisheries Centre, PO Box 182, Narrandera, NSW, 2700
Internet:	www.industry.nsw.gov.au

ISSN 1837-2112

Note: Prior to July 2004, this report series was published by NSW Fisheries as the 'NSW Fisheries Final Report Series' with ISSN number 1440-3544. Then, following the formation of the NSW Department of Primary Industries the report series was published as the 'NSW Department of Primary Industries – Fisheries Final Report Series' with ISSN number 1449-9967. The report series is now published by Industry & Investment NSW as the 'Industry & Investment NSW – Fisheries Final Report Series' with ISSN number 1837-2112.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

This project was funded by the Recreational Freshwater Fishing Trust, who specifically invited a project proposal on catfish genetic structure and habitat preferences in November 2006.

Fieldwork was undertaken by staff at the Narrandera Fisheries Centre (Cameron McGregor, Jarrod McPherson, Timothy McGarry, Peter McLean, Jonathon Doyle and Jamie Hutchison), Batemans Bay (Justin Stanger and Nick Thorne), Port Stephens Fisheries Institute (Matthew Timmins and Michael Rodgers) and Grafton Aquaculture Centre (Gavin Butler, Bradley Mackay and Paul Winters). Samples were also provided by Pam Clunie (Arthur Rylah Institute), Tim Page, David Sternberg, and Giovannella Carini (Griffith University), Iain Ellis, Rohan Rehwinke and Prue McGuffie (Murray-Darling Freshwater Research Centre), Steve Grammer (Namoi Valley Aquaculture), Noel Penfold (Murray-Darling Fisheries), Richard Ping Kee (Wilga Restocking), Pallamallawa and Moree Fishing Clubs, Rob Loates (angler), Max Graham (Recreational Freshwater Fishing Trust), David Morgan and Fiona McAleer (Murdoch University) and Michael Hutchison (QLD DPI).

We thank Luciano Beheregaray, then at the Molecular Ecology Laboratory at Macquarie University for sharing his expertise and allowing us to use his facility to develop the catfish microsatellite library, with significant assistance from Peter Teske and Catherine Attard. We thank Harsh Raman from the Wagga Wagga Agricultural Institute for use of the CEQ machine for microsatellite genotyping, and particularly thank Rosy Raman for her assistance with operating this machine. Finally, we would like to thank Leanne Faulks, Bob Creese, Richard Ping Kee and Cameron Westaway for their valuable comments on early drafts, and Tracey McVea for arranging formatting and printing. This work was conducted under the NSW Fisheries ACEC permit No: 05/06.

NON-TECHNICAL SUMMARY

Population genetic structure of freshwater catfish (*Tandanus tandanus*) in the Murray-Darling Basin and coastal catchments of New South Wales: Implications for future re-stocking programs

PRINCIPAL INVESTIGATORS: Meaghan Rourke and Dean Gilligan

ADDRESS: Industry and Investment NSW
Narrandera Fisheries Centre
PO Box 182
Narrandera, NSW, 2700
Telephone: 02 6959 9024 Fax: 02 6959 2935

OBJECTIVES:

(1) Identify patterns of population structure in freshwater catfish and identify management units (i.e., genetically differentiated populations).

(2) Provide recommendations regarding regions for freshwater catfish broodfish collection and fingerling release.

NON TECHNICAL SUMMARY:

Freshwater catfish (*Tandanus tandanus*) is a popular recreational freshwater fish species that was once widespread in eastern Australia. Prior to the 1980s, they provided good recreational fishing opportunities and are still considered an excellent table fish by many people. Over the last few decades, freshwater catfish have suffered a severe decline in abundance and distribution. Consequently, freshwater catfish populations within the Murray-Darling Basin are listed as an endangered population under the *Fisheries Management Act 1994* in New South Wales, threatened under the *Flora and Fauna Guarantee Act 1988* in Victoria and protected under the *Fisheries Management Act 2007* in South Australia. However, the species is still relatively common in coastal catchments north of and including the Hunter River. As a result, recreational fishing regulations relating to freshwater catfish in the Murray-Darling Basin are now very restrictive in most states, with angling prohibited in all flowing waters in western New South Wales, Victoria (apart from the Wimmera Basin) and the Murray River in South Australia, while there is a limited take of freshwater catfish in Queensland waters and some New South Wales impoundments. These regulations are likely to remain unchanged unless there is a substantial recovery in freshwater catfish numbers that may allow a recreational fishery to be re-instated.

One potential method of increasing wild population size is by stocking rivers and impoundments with hatchery-bred fish, though this can have negative genetic consequences for the wild population. Research over the last three decades has provided clear evidence that stocking wild populations with hatchery bred fish can have a range of negative outcomes that may include modification of the original population genetic structure, loss of genetic diversity, loss of local adaptations and local extinction. Nevertheless, stocking is recognised as an important tool for the management of threatened species that are incapable of natural recovery due to local extinctions. If they are carefully planned and executed, stocking programs can be successful in increasing numbers in the wild, while minimising potentially negative genetic impacts.

As a precursor to development and implementation of a freshwater catfish stocking program, detailed information is required on the natural population genetic structure across their range. This will allow the definition and establishment of broodfish genetic zones that ensure retention of

genetic biodiversity and ensure freshwater catfish are stocked into regions where they have the greatest chance of survival. Fin-clips were collected from 821 freshwater catfish sampled from 31 populations throughout the Murray-Darling Basin and coastal catchments of New South Wales. Fish were released unharmed and the fin-clips preserved in ethanol for subsequent genetic analyses. Two types of genetic markers (mitochondrial sequence data and microsatellite markers) were screened. Results confirmed the presence of an undescribed species in the Bellinger, Macleay, Hastings and Manning catchments on the mid-north coast of New South Wales, and also suggested an additional undescribed species or subspecies in the Tweed, Brunswick, Richmond and Clarence catchments. Most coastal catchments were substantially genetically differentiated from one another, owing to the lack of connection among coastal rivers. Consequently, each coastal catchment was identified as a separate broodfish genetic zone, with the exception of the Macleay and Hastings catchments, which belonged to the same zone. In contrast, there was limited genetic differentiation among most Murray-Darling Basin populations, with the exception of a small number of populations naturally isolated above waterfalls, and populations established from translocated fish.

Summary of recommendations

- That the Murray-Darling Basin be split into three broodfish genetic zones; northern (Darling River and tributaries), central (Lachlan) and lower Murray-Darling Basin (Murray River and tributaries), in recognition that freshwater catfish in vastly different habitats are likely to possess local adaptations.
- That each coastal New South Wales catchment be a separate broodfish genetic zone, with the exception of the Macleay and Hastings catchments, which belong to the same broodfish genetic zone.
- Stocked/translocated fish should originate from the same broodfish genetic zone as the population to be stocked.
- Under certain conditions, broodfish may be obtained from populations established from translocated fish in lakes and dams in Victoria, provided they are crossed with wild-caught broodfish from the same broodfish genetic zone.
- Central and south coast New South Wales populations (Karuah, Hunter, Hawkesbury and Shoalhaven catchments) should not be used as a source of broodfish for stocking any Murray-Darling Basin genetic zone.
- Stocking should not be carried out in areas where there is a healthy, self-sustaining population (e.g., most coastal catchments).
- To prevent loss of genetic diversity, inbreeding and genetic differentiation between impounded populations and the downstream river, freshwater catfish caught downstream can be translocated into impoundments.
- Freshwater catfish in major impoundments can be utilised as broodfish to stock the river below the impoundment, provided they are crossed with freshwater catfish caught below the impoundment.

1. GENERAL INTRODUCTION

1.1. Current status of *Tandanus tandanus*

Freshwater catfish (*Tandanus tandanus*) (Fig. 1.1) is a popular recreational freshwater fish species that was once widespread in eastern Australia. Prior to the 1980s, they provided good recreational fishing opportunities, and are still considered an excellent table fish by many people. There is limited data on their historical abundance, but it is clear that they were formerly much more abundant (reviewed in Clunie and Koehn 2001b). Anecdotally, *T. tandanus* numbers declined substantially following the invasion of carp (*Cyprinus carpio*) during the 1970s and 80s. In addition to carp, thermal pollution and seasonal flow reversal are also thought to have major impacts in affected reaches. *Tandanus tandanus* is now one of the rarest species of native fish in some waterways within their natural range and has virtually disappeared from southern catchments of the Murray-Darling Basin (MDB) (Fig. 1.2). In the Murray, Murrumbidgee, Lachlan, Darling and north-west catchments, they are collected on less than 1% of sampling occasions, increasing to 9% in the Macquarie, 14% in the Namoi, 20% in the Gwydir and 45% in the Border Rivers catchments (I&I NSW, Freshwater Fish Research Database). The only inland waterways that still support substantial *T. tandanus* populations are those upstream of dams or waterfalls that carp have not invaded (Fig. 1.3). *Tandanus tandanus* are still relatively common in all coastal catchments north of and including the Hunter Basin (Figs. 1.2 and 1.3), though there is genetic evidence to suggest that these may include at least one undescribed species in some coastal catchments (Musyl and Keenan 1996; Jerry 2008). They are also present in most catchments south of the Hunter as far as the Shoalhaven catchment on the southern New South Wales (NSW) coast, but some or all of these populations are believed to be translocated populations of mainly unknown origin (Harris and Battaglene 1990).

Figure 1.1. Juvenile *Tandanus tandanus*. Photo: Gunther Schmida.

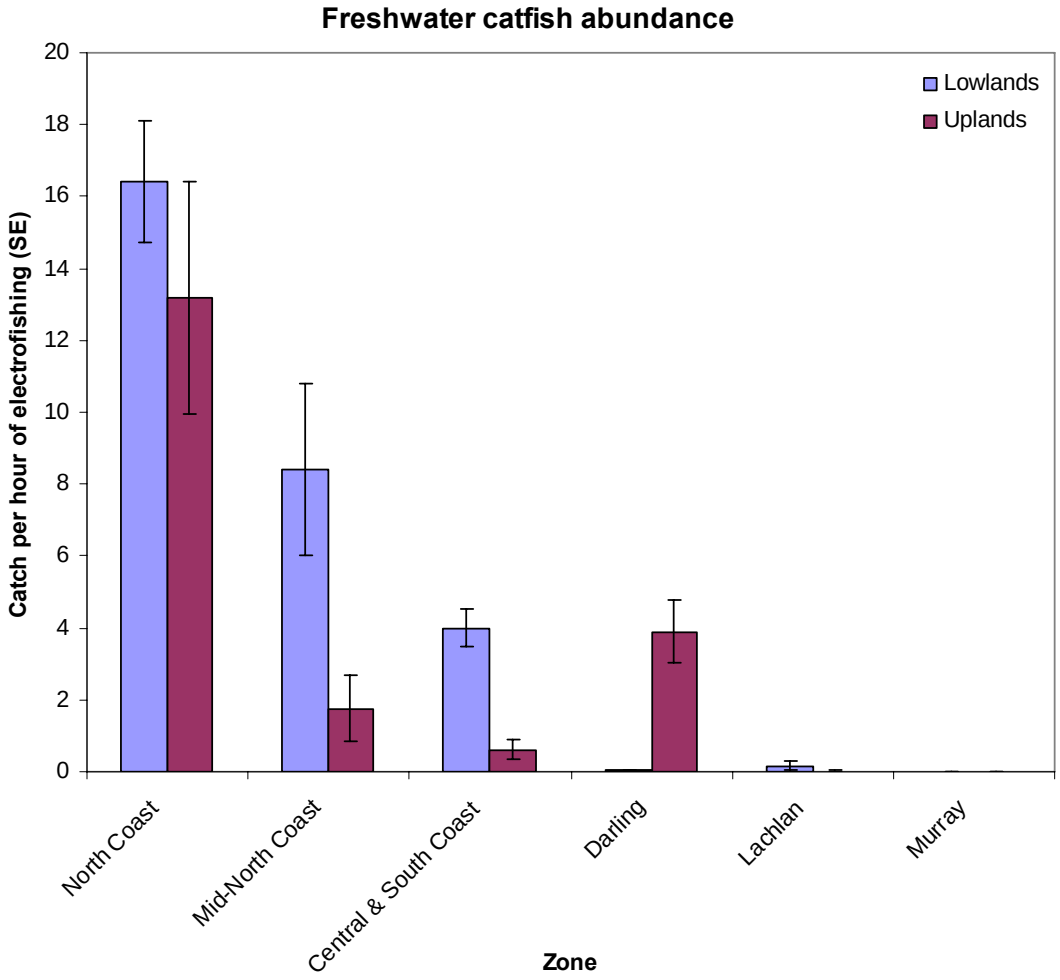

Figure 1.2. The average catch per unit effort (catch per hour of electrofishing) of *T. tandanus* within different areas of their range. Murray includes the Murrumbidgee, Darling includes all Darling tributaries, North Coast includes drainages from the Tweed to Clarence Catchments, Mid-North Coast includes Bellinger to Manning drainages and Central & South Coast includes all drainages between the Karuah and Shoalhaven. Lowlands are areas below 200 m and uplands are those above 200 m in altitude. Data collected up to 3 March 2010.

Concern over the rapid depletion of *T. tandanus* populations led to a proposal by the Fisheries Scientific Committee in May 2008 to list the Murray-Darling Basin population of *T. tandanus* as endangered, and subsequently, the MDB population of *T. tandanus* was officially listed as an endangered population in July 2009 (NSW Government Gazette no. 103). *Tandanus tandanus* is also protected under the *Fisheries Management Act 2007* in South Australia and is listed as threatened in Victoria under the *Flora and Fauna Guarantee Act 1988* in Victoria. Fishing regulations are now very restrictive, with a prohibition on take in all western flowing waters in NSW, with the exception of some impoundments, while there is also a total ban in Victoria (except for the Wimmera Basin) and South Australia. Queensland is the only state that permits the take of *T. tandanus* in all freshwater environments. The strict fishing regulations throughout most of the range of this species are likely to remain unchanged unless there is a substantial recovery in *T. tandanus* numbers that may potentially allow their endangered status to be lifted.

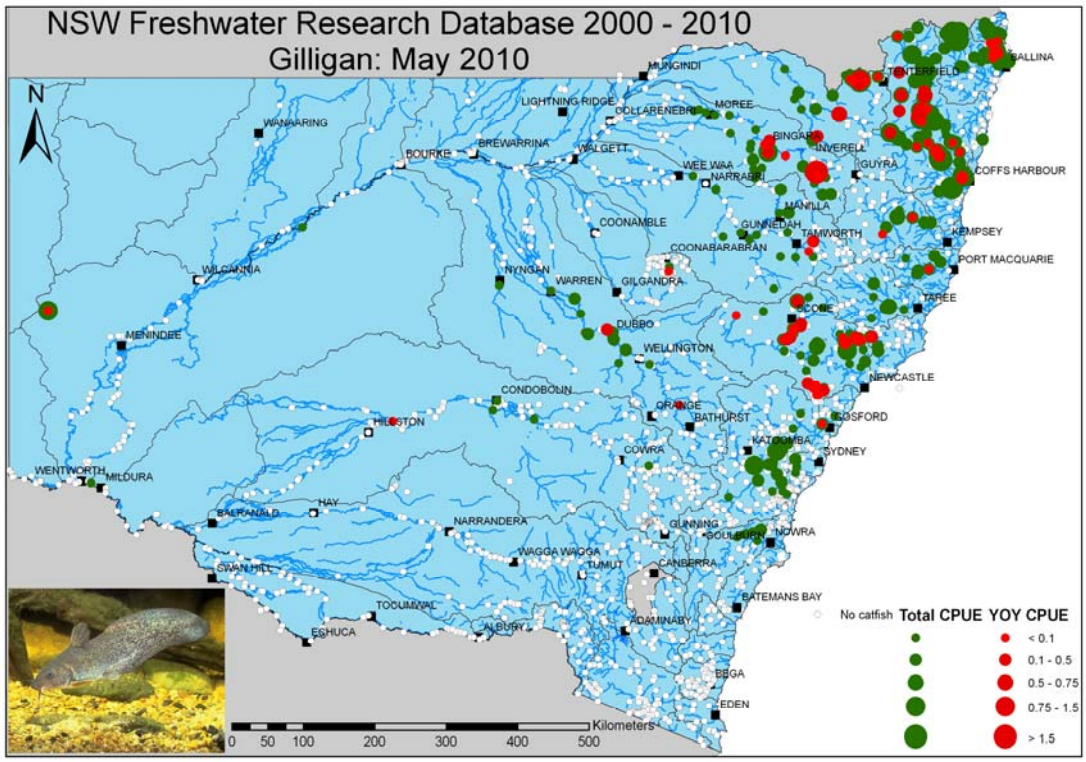

Figure 1.3. Average catch per unit effort (CPUE – catch per hour of electrofishing) of *T. tandanus* at individual sites within NSW between January 2000 and May 2010. The size of the dots represents the CPUE for the total catch (green) and the young of year (YOY) cohort (red) as indicated in the legend at bottom right. White dots represent sites sampled where no *T. tandanus* were collected.

1.2. Potential actions to promote population recovery

There are many threatening processes that are known or assumed to have had an impact on *T. tandanus* abundance and distribution, including introduced species (particularly carp), river regulation (thermal pollution, changed flow regime, barriers to fish passage), loss of in-stream and riparian vegetation, sedimentation and removal of woody debris from the river channel (Clunie and Koehn 2001b). Some of these threats have eased somewhat in some areas over recent years. For example carp populations have declined in areas of the MDB over the last few years (Nicol *et al.* 2004; Gilligan *et al.* 2010) and fishways are progressively being installed in many locations to reinstate fish passage across thousands of river kilometres (Anon 2007). In addition, plans have been proposed to remediate thermal pollution in several catchments, while the provision of environmental flows (http://www.environment.gov.au/water/australia/index.html) will lessen the negative pressure of these threatening processes upon *T. tandanus* populations. Therefore, prospects for recovery of *T. tandanus* populations in several regions of the MDB are improving. However, natural recovery cannot be relied on in many waterways given that *T. tandanus* stocks are virtually non-existent over large areas, particularly in the southern MDB. In these regions, a *T. tandanus* stocking program is a primary means of recovering the recreational fisheries for this species. Recent advances in mass production techniques have made hatchery production a viable option to produce reasonable numbers of captive reared fish for release (Trueman 2006). Prospects for success are encouraging given that past small-scale stockings have been successful in establishment of adult fish (e.g., Billabong Creek, Jerilderie).

1.3. Potential genetic impacts of stocking

Genetic studies on a range of Australian fish species have found that most are comprised of several genetically distinct populations (Moore *et al.* 2010). These populations may be adapted to their local environment, potentially gaining a fitness advantage over non-native populations of the same species (Kawecki and Ebert 2004). Given that assessing local adaptation is very difficult, the vast majority of studies have assessed neutral genetic variation under the assumption that it is associated with local adaptation (Conover *et al.* 2006). Genetic technologies have progressed considerably in recent years, and several empirical studies of marine and freshwater species have recently demonstrated that local adaptation can be substantial, even between populations where it may have been unexpected due to low neutral genetic differentiation (Saint-Laurent *et al.* 2003; Conover *et al.* 2006; Larsen *et al.* 2008). For example, two European flounder (*Platichthys flesus*) populations, one in a high-saline environment, the other in a brackish environment, exhibited extremely low neutral genetic differentiation at microsatellite loci (F_{ST} 0.006, P = 0.037), indicating a very high level of migration, but each population was locally adapted to their environment, as evidenced by the differential expression of several functional genes (Larsen *et al.* 2007). Consequently, the strength of selection for salinity tolerance was higher than the migration rate, resulting in the maintenance of the local adaptation of each population (Larsen *et al.* 2007). Therefore, even when populations are connected by high gene-flow, local adaptation cannot be ruled out (Luttikhuizen *et al.* 2003; Saint-Laurent *et al.* 2003; Conover *et al.* 2006). Given this, there are concerns that supplementing declining wild populations with large numbers of non-local fish may compromise any local adaptations that are present (Allendorf *et al.* 2001). Numerous genetic studies have been conducted in recent decades on wild populations of fish that have been supplemented with hatchery-bred individuals or received translocated individuals collected from different populations. These studies have detected genetic impacts that range from minimal (no detectable change in the wild population's genetic diversity or genetic structure), to severe (varying levels of introgression of hatchery and wild fish to extinction of the wild population) (Josefsson and Andersson 2001; Englbrecht *et al.* 2002; Hughes *et al.* 2003; Larsen *et al.* 2005; Madeira *et al.* 2005; Heggenes *et al.* 2006).

Stocking programs in Australia have been instigated to support recreational fisheries and supplement populations of threatened species since the late 1970s, for species including Murray cod (*Maccullochella peelii*), trout cod (*M. macquariensis*), golden perch (*Macquaria ambigua*), Australian bass (*M. novemaculeata*), silver perch (*Bidyanus bidyanus*), barramundi (*Lates calcarifer*), southern saratoga (*Scleropages leichardti*.) and to a lesser extent, *T. tandanus*. Australian breeding programs have commonly utilised a relatively small number of wild-caught broodfish, with the exception of silver perch, which have had their breeding cycle closed (i.e., domesticated) (Rowland and Tully 2004). Prior to the recognition of the effect that non-local fish can have on the native population, fish were frequently stocked into areas away from the broodfish collection site, raising concerns for the genetic integrity of local populations. A recent study on Murray cod identified several populations that had clear evidence of stocked fish surviving in areas where there were formerly genetically distinct populations in the Border Rivers, Gwydir and Namoi River catchments (Rourke 2007). Stocked fish have also been detected in populations of Macquarie perch (*Macquaria australasica*), silver perch and Australian bass (Keenan *et al.* 1995; Jerry 1997; Bearlin 2002; Faulks *et al.* 2009).

Many genetic studies have found that the boundaries between genetically differentiated populations of freshwater fish do not necessarily coincide with an obvious physical barrier (e.g., a waterfall or catchment boundary). For example, Lake Saimaa grayling (*Thymallus thymallus*) populations separated by as little as 10 km (within the lake) and brown trout (*Salmo trutta*) populations within a single river system showed significant genetic differentiation (Carlsson *et al.* 1999; Koskinen *et al.* 2002). In addition, genetic data has also revealed that many fish species are actually a complex of two or more cryptic species (species that are not markedly morphologically distinguishable) (Musyl

and Keenan 1996; Bertozzi *et al.* 2000; Miller *et al.* 2004; Hammer *et al.* 2007). These examples illustrate how un-informed stocking programs can potentially have a negative genetic impact on biodiversity by stocking the incorrect 'genetic type' of fish. Nevertheless, stocking programs undoubtedly have an important role supporting commercial and recreational fisheries (Welcomme and Bartley 1998; Cowx 1999; De Silva and Funge-Smith 2005), and this role becomes more important as species continue to decline in abundance and distribution.

In response to concerns regarding genetic integrity of Australia's freshwater fish populations, fish stocking in NSW is undertaken in accordance with the Freshwater Fish Stocking – Fishery Management Strategy (FMS) (NSW DPI 2005). Hatcheries that wish to stock Murray cod, trout cod, golden perch and silver perch into wild populations must first be accredited to do so under the Hatchery Quality Assurance Scheme (HQAS) (NSW DPI 2008). The FMS has recognised that genetic structure needs to be fully investigated for a species prior to commencing new stocking programs for Australian freshwater fish in NSW.

1.4. *Tandanus tandanus* genetic structure

Previous genetic analysis of *T. tandanus* from the MDB and coastal catchments has suggested that at least one additional species of freshwater catfish occurs in the Bellinger, Macleay, Hastings and Manning Rivers (*Tandanus* sp. 1.) in the east coast drainages of NSW (Musyl and Keenan 1996; Jerry and Woodland 1997; Jerry 2008). This species is reported to be morphologically indistinguishable from the nominate MDB species (*Tandanus tandanus*) (Musyl and Keenan 1996). Preliminary genetic analysis of *T. tandanus* within the MDB using allozyme data suggested that there are some genetically distinct populations, coinciding predominantly with isolated impoundments, while the remainder of the riverine populations were genetically similar (Keenan *et al.* 1995). There is a need to examine the *T. tandanus* population within the MDB using more sensitive genetic markers to provide higher confidence in the genetic boundaries between *T. tandanus* populations, and to identify potential source populations for a future stocking program. In addition, we also had the secondary objective of identifying the original source populations for those populations on the central to south coast of NSW likely to be of translocated origin (non-endemic populations) (Harris and Battaglene 1990). It is possible that these translocated populations could retain genetic diversity lost from MDB stocks – primarily from the southern drainages.

1.5. Project aims

As a precursor to development and implementation of a *T. tandanus* stocking program, the NSW Freshwater Fish Stocking Fisheries Management Strategy and the *T. tandanus* recovery plan (Clunie and Koehn 2001a) require data on the natural population genetic structure of *T. tandanus* across their range. This will subsequently allow designation of broodfish genetic zones, thus ensuring stocked fish are genetically suited to their release site.

2. ISOLATION AND CHARACTERISATION OF FRESHWATER CATFISH MICROSATELLITE LOCI

*Note, this chapter is now published as:

Rourke, M.L., Teske, P.R., Attard, C.R.M., Gilligan, D.M. and Beheregaray, L.B. (2009). Isolation and characterisation of microsatellite loci in the Australian freshwater catfish (*Tandanus tandanus*). *Conservation Genetics Resources* (online December 2009: DOI 10.1007/s12686-009-9161-1).

2.1. Introduction

This project requires the development of highly sensitive genetic markers, known as microsatellites, to identify genetic differences among populations. We developed these microsatellite loci specifically for *T. tandanus* at the Molecular Ecology Laboratory at Macquarie University in mid 2008.

2.2. Methods, results and discussion

We collected a single *T. tandanus* from the Macintyre River (-29 45.754S, 151 07.226E, Border Rivers catchment, MDB) and extracted genomic deoxyribonucleic acid (DNA) from muscle tissue using a phenol-chloroform procedure (Sambrook *et al.* 1989) and a QIAGEN DNeasy tissue kit (QIAGEN). The combined products of these extractions resulted in sufficient high-molecular weight DNA for the enrichment. We followed an enrichment technique to isolate microsatellites (Fischer and Bachmann 1998, as modified in Beheregaray *et al.* 2004). Briefly, approximately 3µg of genomic DNA was digested with *Rsa*I and *Hae*III, and oligo adapters were ligated onto the blunt ends. The resultant ligation mix was annealed to CA, GA, AGAT, AACT and ACAT biotinylated probes. Fragments containing the annealed probes were purified using Streptavidin magnetic beads (Promega) and amplified in a polymerase chain reaction (PCR) using one of the oligo adaptors. The process was repeated using the PCR product of the first enrichment as a template, thus resulting in a double enrichment. Enriched DNA was purified using an UltraClean 15 DNA purification kit (MoBio Laboratories), ligated into a pCR 2.1-TOPO vector (Invitrogen), transformed into TOP10 cells (Invitrogen) and plated onto LB agar containing ampicillin (50µg/mL) and X-gal (40mg/mL).

A total of 127 positive clones were PCR amplified using M13 forward (-20) primers and sequenced using the BigDye terminator chemistry and an ABI 377 DNA automated DNA sequencer (PE Applied Biosystems). ChromasPro 1.41 (www.technelysium.com.au/ChromasPro.html) was used to assemble sequences into contigs to facilitate the identification of duplicate sequences, and to remove vector sequence. Sequences were also screened using VecScreen (http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen.html) to confirm that all the vector sequence was removed. We used PRIMER 3 (Rozen and Skaletsky 2000) to design primers for 28 microsatellite repeat sequences.

The 28 loci were tested for variability in individuals from two populations in the Murray-Darling Basin [(the Macintyre River (n = 28) and lower Border Rivers catchment (n = 28)] and in two coastal catchments [the Richmond River catchment (n = 29) and the Bellinger River catchment (n = 24)]. The latter catchment represents individuals from the undescribed species from the mid-north NSW coast as proposed elsewhere (Musyl and Keenan 1996; Jerry and Woodland 1997; Jerry 2008). PCRs were carried out in 12 µL volumes containing 5 – 20 ng DNA, 0.125 µM of forward primer, 0.25 µM of reverse primer and 0.375 µM of M13 fluorescent-labelled primer (D4, D3 or D2; CACGACGTTGTAAAACGAC, Sigma), 0.5 mM of dNTP (Astral Scientific), 2.5 mM of $MgCl_2$,

1.25 µL of 5X reaction buffer and 0.5 U of GoTaq Flexi DNA polymerase (Promega). Thermal cycling conditions consisted of 95 °C for 2 min, 30 cycles of 95 °C for 30 s, 55 °C for 45 s, and 72 °C for 1 min, and a final extension of 72 °C for 10 min. PCR products were sized using CEQ 8000 Genetic Analysis System and assessed for scoring ease and polymorphism using the CEQ 8000 software (Beckman Coulter).

Of the 28 loci screened across 109 samples of *T. tandanus*, eight were polymorphic. These loci were successfully amplified in samples from the Macintyre River, lower Border Rivers catchment and Richmond River catchment, while Tan3_27 failed to amplify in any of the Bellinger River catchment samples (undescribed species) and three loci were monomorphic (Tan1_10, Tan2_20 and Tan3_28). The mean observed heterozygosities for these four catchments respectively were 0.661, 0.676, 0.515 and 0.459; mean expected heterozygosites were 0.675, 0.721, 0.564 and 0.458; mean number of alleles per locus were 5.6, 9.5, 6.9 and 3 (Table 2.1). We used GENEPOP (Raymond and Rousset 1995) to conduct tests for Hardy-Weinberg equilibrium (HWE) at each locus, and for linkage disequilibrium between pairs of loci from each population. Three loci (Tan1_7, in the Richmond catchment, Tan2_16 in the Richmond catchment and Tan3_27 in the lower Border Rivers catchment) deviated from HWE ($P < 0.05$) after sequential Bonferroni correction (Rice 1989) due to heterozygosity deficits. However, given that these loci only departed from HWE in a single population each, null alleles are unlikely to be responsible. None of the loci-pair combinations showed evidence of linkage disequilibrium in our data set. These loci are currently being used to assess genetic diversity and population structure of *T. tandanus* across its extensive distribution to assist with the future management of the species.

Table 2.1. Primer sequences of eight *Tandanus tandanus* microsatellites, including number of individuals (N), number of alleles (N_a), observed heterozygosity (H_O), and expected heterozgosity (H_E) and P values for Hardy-Weinberg equilibrium test (*P*).

Locus	GenBank Accession no.	Repeat motif	Primer sequences (5′–3′)	Dye label	*T. tandanus* (Macintyre River MDB, *n* = 28)						*T. tandanus* (Lower Border Rivers MDB, *n* = 28)					
					Size range (bp)	N	N_a	H_O	H_E	*P*	Size range (bp)	N	N_a	H_O	H_E	*P*
Tan1_2	GQ496016	$(GT)_{20}$	F: CCGACTGTCAGTGAAAAGGAG*	D4	216 – 244	28	6	0.571	0.592	0.360	216 – 240	28	7	0.643	0.648	0.609
			R: AGGGTCTGGGAGTGAATGAG													
Tan1_7	GQ496017	$(GT)_{34}$	F: TGTATGGAGCTACTAACAAAACAGG*	D3	189 – 223	28	9	1.000	0.773	0.076	193 – 219	28	12	0.964	0.892	0.807
			R: TACTCCAGCCCTGAAGGTG													
Tan1_10	GQ496018	$(TC)_{14}$	F: TCCTGATTTCTCTCCCAAGG*	D2	308 – 310	28	2	0.357	0.408	0.643	308 – 314	28	4	0.607	0.665	0.300
			R: AGAAAGGTGGTGCATGTGTG													
Tan2_15	GQ496019	$(GA)_{15}$ †	F: CGTAGTTGTTTTTGTTTCGGAAGTAG*	D4	176 – 196	28	8	0.720	0.838	0.081	160 – 196	27	13	0.704	0.832	0.028
			R: GTTTGCACAGGAATTAACAACAG													
Tan2_16	GQ496020	$(CTAT)_{14}$	F: TGCCTGTTGTTTCTTTCTTTC*	D3	227 – 277	28	9	0.704	0.792	0.089	219 – 301	28	17	0.893	0.920	0.145
			R: ATGTTCTGCCGAGCTTGAG													
Tan2_20	GQ496021	$(GT)_{17}$	F: TCCTCTGCTCCTGCTGTTTC*	D3	265	28	1	-	-	-	263 – 267	28	3	0.286	0.304	0.620
			R: ATGGGATGCCAATTCATCAC													
Tan3_27	GQ496022	$(CT)_{17}$	F: TGTGGAAGGTTGGGGTTATG*	D2	227 – 269	28	6	0.571	0.638	0.021	217 – 269	28	14	0.714	0.810	0.006*
			R: CGTGATCATGCAAACAGATG													
Tan3_28	GQ496023	$(CT)_{18}$	F: CCCCATTTGCTTTTTCTCTG*	D2	289 – 299	27	4	0.704	0.686	0.802	289 – 299	27	6	0.593	0.694	0.324
			R: TGTTGAAAGCGGCATGTTAG													
			Average ± SD			27.87 ± 0.35	5.6 ± 3.1	0.661 ± 0.196	0.675 ± 0.147			27.7 ± 0.463	9.5 ± 5.1	0.676 ± 0.206	0.721 ± 0.197	

Table 2.1. *Continued*

Locus	GenBank Accession no.	Repeat motif	Primer sequences (5′ – 3′)	Dye label	*T. tandanus* (Richmond catchment north-coast NSW, *n* = 29)						*Tandanus sp.* (Bellinger catchment, mid-north coast NSW, *n* = 24)					
					Size range (bp)	N	N_a	H_O	H_E	*P*	Size range (bp)	N	N_a	H_O	H_E	*P*
Tan1_2	GQ496016	$(GT)_{20}$	F: CCGACTGTCAGTGAAAAGGAG*	D4	224 – 240	29	5	0.793	0.675	0.086	236 – 242	24	4	0.500	0.489	0.931
			R: AGGGTCTGGGAGTGAATGAG													
Tan1_7	GQ496017	$(GT)_{34}$	F: TGTATGGAGCTACTAACAAAACAGG*	D3	181 – 227	29	16	0.862	0.915	0.006*	181 – 183	22	2	0.091	0.087	1.000
			R: TACTCCAGCCCTGAAGGTG													
Tan1_10	GQ496018	$(TC)_{14}$	F: TCCTGATTTCTCTCCCAAGG*	D2	298 – 300	29	2	0.034	0.034	1.000	302	23	1	-	-	-
			R: AGAAAGGTGGTGCATGTGTG													
Tan2_15	GQ496019	$(GA)_{15}$ †	F: CGTAGTTGTTTTTGTTTCGGAAGTAG*	D4	160 – 186	29	11	0.759	0.786	0.397	184 – 190	16	3	0.563	0.498	0.793
			R: GTTTGCACAGGAATTAACAACAG													
Tan2_16	GQ496020	$(CTAT)_{14}$	F: TGCCTGTTGTTTCTTTCTTTC*	D3	251 – 301	24	9	0.292	0.833	0.000*	229 – 261	22	9	0.682	0.756	0.090
			R: ATGTTCTGCCGAGCTTGAG													
Tan2_20	GQ496021	$(GT)_{17}$	F: TCCTCTGCTCCTGCTGTTTC*	D3	261 – 263	29	2	0.034	0.034	1.000	269	24	1	-	-	-
			R: ATGGGATGCCAATTCATCAC													
Tan3_27	GQ496022	$(CT)_{17}$	F: TGTGGAAGGTTGGGGTTATG*	D2	215 – 223	29	4	0.690	0.613	0.506	No amplification	-	-	-	-	-
			R: CGTGATCATGCAAACAGATG													
Tan3_28	GQ496023	$(CT)_{18}$	F: CCCCATTTGCTTTTTCTCTG*	D2	291 – 301	29	6	0.655	0.620	0.954	281	23	1	-	-	-
			R: TGTTGAAAGCGGCATGTTAG													
			Average ± SD			28.4 ± 1.77	6.9 ± 4.8	0.515 ± 0.342	0.564 ± 0.343			22 ± 2.8	3 ± 2.9	0.459 ± 0.257	0.458 ± 0.276	

**5'end of each forward primer was appended with an 19-bp 'M13' sequence (CACGACGTTGTAAAACGAC, to facilitate the incorporation of a dye label during PCR), *significant at 5% level after sequential Bonferroni correction (Rice 1989), † interrupted microsatellite repeat.*

3. PHYLOGEOGRAPHIC STRUCTURE OF FRESHWATER CATFISH IN THE MURRAY-DARLING BASIN AND COASTAL CATCHMENTS OF NEW SOUTH WALES

3.1. Introduction

The distribution of freshwater fish species is strongly influenced by connectivity of riverine and floodplain habitats, as well as their physical and physiological capabilities, including migratory potential, and temperature and salt tolerances. In eastern Australia, the Great Dividing Range (GDR) is the major geological barrier between coastal catchments and inland catchments of the Murray-Darling Basin (MDB). Populations of freshwater fish in coastal catchments are typically less connected to one another given that coastal rivers drain into the ocean, resulting in restricted gene-flow and subsequent genetic differentiation among populations (Faulks *et al.* 2009; Knight 2009). In contrast, most MDB catchments are largely interconnected to one another, given that the rivers predominantly coalesce into the Murray River, allowing gene flow among some (but not necessarily all) populations (Keenan *et al.* 1995; Rourke 2007).

There are many fish genera that occur in catchments both east and west of the GDR, some of which show significant population differentiation, and in some cases, speciation, e.g., *Maccullochella* (Rowland 1985; Rowland 1993), *Mogurnda* (Faulks *et al.* 2008), *Retropinna* (Hammer *et al.* 2007), *Hypseleotris* (Thacker *et al.* 2007), *Philypnodon* (Thacker *et al.* 2008) *Macquaria* (Dufty 1986; Faulks *et al.* 2009; Faulks *et al.* 2010), *Melanotaenia* (Crowley *et al.* 1986) *Craterocephalus* (McGlashan and Hughes 2001) and *Tandanus* (Jerry 2008). However, the level of genetic differentiation is not consistent among genera, and there is evidence that gene-flow occurs sporadically in some areas. River capture via drainage rearrangements has been suggested as the mechanism for some species (Jerry, 2008; Rowland, 1993), though this may not be as common as previously thought (Bishop 1995; Unmack 2001). Craw *et al.* (2007) described several geomorphological mechanisms other than river capture that could facilitate dispersal across drainage divides. These include dispersal during flood events that allow connectivity across a divide, dispersal through the outlets of a lake or swamp that drain into catchments on both sides of the divide, or low sea-levels that allow outlets of adjacent rivers to converge when they would otherwise be isolated by high sea-levels (Craw *et al.* 2007). Dispersal across divides may also be promoted by warm and wet conditions that were dominant during interglacial periods (Faulks *et al.* 2009; Faulks *et al.* 2010). This study provides an opportunity to assess the influence of climatic conditions on the distribution of *Tandanus tandanus* in both coastal and inland catchments of south-eastern Australia (Fig. 3.1).

Tandanus tandanus from the MDB and east-coast catchments south of and including the Mary River, are morphologically uniform for those morphometric and meristic characteristics that have been assessed (Musyl 1990; Musyl and Keenan 1996). However, allozyme and mtDNA data provide evidence for an undescribed species (*Tandanus*. sp. 1) in four coastal catchments of the mid-north coast of NSW: the Bellinger, Macleay, Hastings and Manning (Musyl and Keenan 1996; Jerry and Woodland 1997; Jerry 2008). Based on allozyme data, a second undescribed species or sub species was thought to exist in the Richmond and Clarence River catchments (Musyl and Keenan 1996; Jerry and Woodland 1997), but this was not supported by analysis of mtDNA which found these fish to be consistent with *T. tandanus* from the MDB (Jerry 2008). Only *T. tandanus* has been detected within the MDB, and this species also occurs east of the GDR in coastal

catchments from south-eastern Queensland (QLD) (Jerry 2008) to the Shoalhaven River in south-eastern NSW (Fig. 3.1).

Tandanus tandanus is not thought to be naturally distributed south of the Manning River (Pollard *et al.* 1996), and populations of *T. tandanus* in at least the Hawkesbury and Hunter catchments are known to have been derived from fish translocated from the MDB (Harris and Battaglene 1990). Thus the populations in the Karuah, Hunter, Tuggerah Lakes, Hawkesbury, Port Jackson, Georges River, and Shoalhaven catchments are all probably derived from translocations of *T. tandanus* from the MDB. Nevertheless, in the absence of records for most of these catchments, it is possible that *T. tandanus* may have been native to some of these catchments, particularly the Wallis Lakes and Karuah catchments, which may represent a natural southern range extension from the adjacent Manning catchment. It is also possible that translocations may have occurred elsewhere. There is reliable anecdotal evidence that Tenterfield Fishing/Acclimatisation Group translocated *T. tandanus* from the MDB into several small tributaries of the Clarence River, including the Cataract River, during the 1930s and 1940s, and again in the 1960s and 1970s (Arnold Butler, recreational angler, pers comm.). Several Victorian populations in the southern MDB have been established from translocated fish, including Centenary Reservoir and Amphitheatre Reservoir (Clunie and Koehn 2001b). Another population exists in Cardross Lakes, which may represent a natural population, though stocking cannot be ruled out (Clunie and Koehn 2001b). All of these populations are physically isolated from one another and from the Murray River. It is also possible that illegal translocations of *T. tandanus* among catchments by some recreational anglers may still occur. Consequently, the translocation of fish may have confused the phylogeographic structure of coastal *T. tandanus* (and the undescribed *Tandanus* sp. 1).

The main aim of this component of the study is to identify genetically distinct populations (evolutionary significant units and management units) of *T. tandanus* within the MDB. In addition, we will endeavour to provide further genetic evidence to support the existence of *Tandanus* sp.1 and clarify its distribution in the Bellinger, Macleay, Hastings and Manning Rivers. Finally, we will also determine whether the NSW north coast populations are more genetically distinct than currently thought, and clarify the influence of translocated fish from the MDB. Finally, we will test the hypothesis that four NSW central and south coast populations (Karuah, Hunter, Hawkesbury and Shoalhaven) are not part of the species natural distribution, and whether they have a MDB origin.

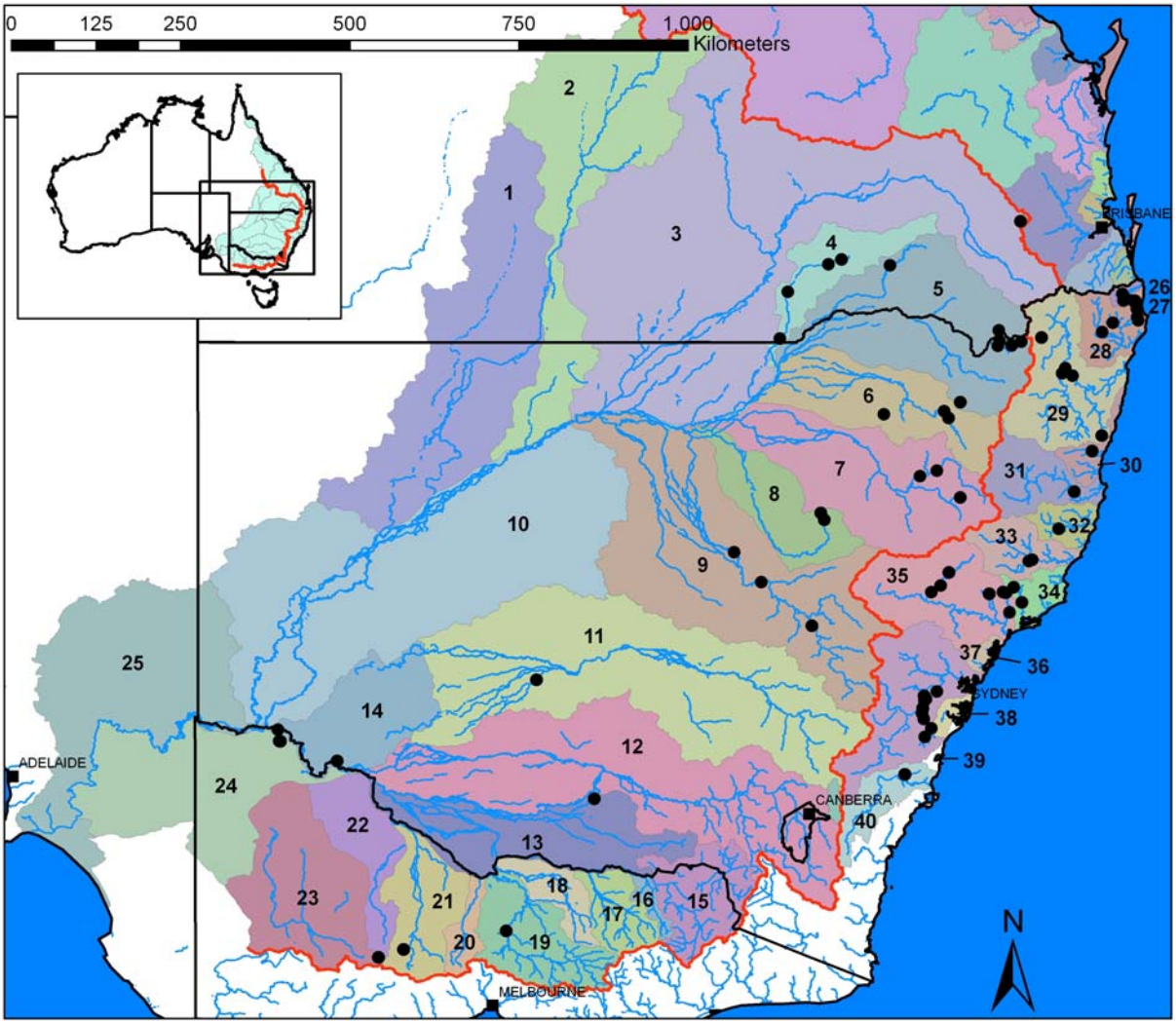

Figure 3.1. Catchments historically and/or currently occupied by *Tandanus* species in south eastern Australia. The locations where genetic samples were collected for mtDNA analysis are indicated by black points. State borders are indicated by black lines and the ridge of the Great Dividing Range by a red line. Numbers represent the following basins: 1. Paroo, 2. Warrego, 3. Condamine-Culgoa, 4. Moonie, 5. Border Rivers, 6. Gwydir, 7. Namoi, 8. Castlereagh, 9. Macquarie-Bogan, 10. Barwon-Darling, 11. Lachlan, 12. Murrumbidgee, 13, Murray Riverina, 14. Benanee, 15. Upper Murray, 16. Kiewa, 17. Ovens, 18. Broken, 19. Goulburn, 20. Campaspe, 21. Loddon, 22. Avoca, 23. Wimmera, 24. Mallee, 25. Lower Murray, 26. Tweed, 27. Brunswick, 28. Richmond, 29. Clarence, 30. Bellinger, 31. Macleay, 32. Hastings, 33. Manning, 34. Karuah, 35. Hunter, 36. Tuggerah Lakes-Lake Macquarie, 37. Hawkesbury, 38. Port Jackson – Botany Bay – Port Hacking, 39. Lake Illawarra, 40. Shoalhaven.

3.2. Methods

3.2.1. Sampling and DNA extraction

Fin-clip samples were collected from a total of 240 individuals from 28 populations. These comprised almost all of the drainages where *T. tandanus* are known to remain in NSW, including four catchments occupied by the proposed undescribed species (*Tandanus* sp. 1) on the NSW mid-north coast (Bellinger, Macleay, Hastings and Manning), eight other NSW coastal populations (Tweed, Brunswick, Richmond, Clarence, Karuah, Hunter, Hawkesbury and Shoalhaven) and 16 populations within the MDB (Condamine, Moonie, above Macintyre Falls, above Tenterfield Falls, Lower Border Rivers, Gwydir, Namoi, Castlereagh, Lachlan, Macquarie, Benanee, Murray Riverina, Centenary Lake, Cardross Lakes, Amphitheatre and Tahbilk Lagoon) (Fig. 3.1). The MDB catchments within the historical range of *T. tandanus* where no samples were collected or otherwise available were the Lower Murray River, Murrumbidgee, Darling, Paroo, Warrego, Campaspe, Broken, Ovens, Kiewa and Upper Murray. We did not sample any populations from coastal catchments in Queensland. Fish were captured using a variety of methods including boat and backpack electrofishing, fyke nets and gill nets. Samples from wild caught broodfish from the Lachlan and Gwydir catchments were supplied by two hatcheries (Murray Darling Fisheries and Namoi Valley Aquafarming). Fin-clip samples were preserved in 100% ethanol and stored at -20ºC. DNA was extracted using the Jet Quick DNA extraction kit (Genomed) following the manufacturer's instructions.

3.2.2. Laboratory procedures

A ~ 400 bp fragment of the mitochondrial DNA (mtDNA) control region encompassing part of the tRNA-PRO locus and the central conserved region was amplified by polymerase chain reaction (PCR) using the primers Primer 'A' and Primer 'E' (Lee *et al.* 1995) (Table 1). PCR reactions were performed in a 20 µl volume containing 5 – 20 ng DNA, 1.4 µM of Primer 'A' and Primer 'E', 1 mM dNTP (Astral Scientific), 2 mM of $MgCl_2$, 2.4 µL of 5 X reaction buffer, and 1 U of GoTaq Flexi DNA polymerase (Promega). PCR cycling conditions consisted of an initial denaturation step at 96 ºC for 1 min, 30 cycles of 96 ºC for 10 secs, 50 ºC for 5 sec, 60 ºC for 4 mins, and a 10 ºC hold. PCR products were purified using an AxyPrep PCR clean-up kit (Axygen) following the manufacturer's instructions and samples were sequenced at the Australian Genome Research Facility, University of Queensland.

3.2.3. Data analysis

Raw DNA sequence files were edited and trimmed in SeqScanner (Applied Biosystems), converted into FASTA format and aligned using MEGA 4.0 (Tamura *et al.* 2007). Haplotype and nucleotide diversity were calculated in Arlequin v3.11 (Excoffier *et al.* 2005). The genealogical relationships among *T. tandanus* and *Tandanus* sp. 1 were assessed by constructing a haplotype network using the program TCS, which uses a statistical parsimony approach to determine connections between haplotypes (Clement *et al.* 2000). The most suitable of 56 models of sequence evolution was determined using MODELTEST 3.7 and PAUP* 4.0b10 (Posada and Crandall 1998; Swofford 2003) using likelihood ratio tests. Results indicated that the Hasegawa, Kishino, and Yano (HKY) model of evolution with equal rates for all sites best fitted the mtDNA data (Hasegawa *et al.* 1985). Bayesian Markov Chain Monte Carlo (MCMC) analysis was conducted in BEAST v1.4.8 (Drummond and Rambaut 2007) to reconstruct the phylogeny of *T. tandanus* and *Tandanus.* sp. 1. The freshwater cobbler (*Tandanus bostocki*), Hyrtl's tandan (*Neosilurus hyrtlii*), Cooper Creek catfish (*Neosiluroides cooperensis*) and silver tandan (*Porochilus argenteus*) were used as outgroup taxa. Given that *T. tandanus* is thought to have undergone a range expansion from the

coastal catchments to the MDB (Jerry 2008), we assumed an expansion-growth tree prior distribution and applied the uncorrelated relaxed lognormal model of lineage variation and the HKY substitution model. Two replicate runs of the MCMC were conducted for 10,000,000 steps and sampled every 1,000 steps. The output of both runs were imported into Tracer v1.4.1 (Rambaut and Drummond 2007) to assess whether the number of MCMC steps was sufficient to bring effective sample sizes (ESS) above the minimum threshold of 200, as recommended in the BEAST documentation. All ESS values greatly exceeded 200 and the ESS for posterior probability was more than 1,500 for both analyses. Consequently, it was deemed appropriate to combine the output of both runs in LogCombiner v1.4.8 (distributed with the BEAST package) and viewed in Tracer v1.4.1, resulting in a combined ESS for posterior probability of 4,014 and very high ESS values for all other parameters. Tree files from both runs were combined in LogCombiner v1.4.8 and condensed into a single maximum clade credibility tree in TreeAnnotator v1.4.8 (distributed with the BEAST package) and visualised in FigTree v1.2 (Rambaut 2006).

Tajima's *D* test (Tajima 1989a; Tajima 1989b) was conducted in Arlequin v3.11 to determine if mutations were selectively neutral, and to test whether the populations had increased or decreased in size (a significant positive or negative value of Tajima's *D* respectively). This test was applied to three groupings of the data as identified from phylogeographic analyses: 1) all populations, 2) *Tandanus* sp. 1, and 3) NSW north coast populations. We also tested the NSW central and south coast populations given their putative origin from MDB fish. Mismatch distributions (Arlequin v3.11) were constructed for the same three groupings of the data as a second method of determining whether the population was increasing or decreasing in size (Rogers and Harpending 1992). This analysis plots the frequency distribution of the pairwise differences between haplotypes with the distribution expected under a model of population expansion. In order to calculate approximate times of divergence between major groupings of the data, we used DNAsp (Rozas and Rozas 2009) to calculate the net nucleotide divergence (Da) between: 1) *Tandanus* sp. 1 and NSW north-coast populations, 2) between *Tandanus* sp. 1 and the MDB population, and 3) between the NSW north-coast and MDB populations. For this analysis, all stocked populations on the NSW central and south coast (Karuah, Hunter, Hawkesbury and Shoalhaven) and in the MDB (Centenary, Cardross and Amphitheatre) were excluded, as were the seven fish with MDB haplotypes in the Clarence (see results). We calculated divergence time from the net nucleotide divergence assuming an average mutation rate for fish mtDNA control regions of 3.6% per million years (Donaldson and Wilson 1999). We acknowledge that there may be some error associated with our estimated dates of divergence given that mtDNA mutation rates can be highly variable (Avise 2004).

To determine whether populations were genetically structured we used analysis of molecular variance (AMOVA) to partition the molecular variance at several hierarchical levels (Arlequin v3.11). We performed the analyses excluding populations with a known or presumed extensive stocking/translocation history (Karuah, Hunter, Hawkesbury, Shoalhaven, Centenary, Cardross and Amphitheatre), and also removed two fish from the central and south coast populations and one fish from the Lachlan population that had north coast haplotypes (see results). The seven putative translocated fish in the Clarence population were designated a separate population assigned to the MDB lineage. The groups tested were: among MDB, among *Tandanus* sp. 1, among NSW north coast populations, between *Tandanus* sp. 1 and NSW north coast populations, between *Tandanus* sp. 1 and MDB populations, between NSW central and south coast and MDB populations, and among *Tandanus* sp. 1, the north coast and non-stocked MDB populations.

3.3. Results

3.3.1. Genetic variation

A total of 408 bp of the mtDNA control region were obtained and aligned for 240 *T. tandanus* and *Tandanus* sp. 1 samples from 28 populations. Twenty six haplotypes were identified and deposited in GenBank along with the four haplotypes from the outgroup taxa (GenBank Accession No.s GQ495986 – GQ496015). Seven haplotypes were unique to the MDB, 13 were unique to coastal populations and five haplotypes were shared between MDB and coastal catchments (Clarence, Karuah, Hunter, Hawkesbury and Shoalhaven; all with a stocking/translocation history) (Table 3.1). The undescribed species (*Tandanus* sp. 1) in the Bellinger, Macleay, Hastings and Manning populations had five haplotypes not found in any other population. Haplotype and nucleotide diversities were 0.842 ± 0.016 and 0.021 ± 0.011 respectively, and there were a total of 36 variable sites, 35 of which were parsimony informative (Table 3.2).

3.3.2. Phylogenetic structure

The Bayesian consensus tree revealed three major clades within the *Tandanus* populations studied (Fig. 3.2). The first was comprised of haplotypes found only in the Bellinger, Macleay, Hastings and Manning, corresponding to the undescribed species of *Tandanus* sp. 1 (clade 1). The second was comprised of haplotypes that were from the MDB populations, the two coastal populations reportedly established from MDB stock (Hunter, Hawkesbury), a further two coastal populations with an uncertain history (Karuah and Shoalhaven), and a population known to have received translocated MDB *T. tandanus* (Clarence) (clade 2). Clade 3 was comprised predominantly of populations collected from the north coast NSW, with the exception of a single haplotype from the Hunter and Hawkesbury catchments, and a single haplotype only found in the Lachlan (MDB) population.

The haplotype network indicates fine-scale structuring across *T. tandanus* populations, and also distinguishes *Tandanus* sp. 1 (Fig. 3.3). Although there are separate clusters of the MDB and coastal haplotypes, there is some overlap, consistent with the Bayesian consensus tree. From the Bayesian tree and haplotype network it is evident that haplotype C (found in two fish, one from the Hunter and one from the Hawkesbury population) is more closely related to NSW north coast haplotypes, and does not support our hypothesis for a solely MDB origin for central and south coast populations. Furthermore, a single haplotype (Haplotype E) from a single Lachlan River (MDB) sample clustered with the north coast group.

Tajima's *D* tests for neutrality were not significant, thus the model of population expansion could not be rejected ($D = 0.032$ $P = 0.620$, $D = 0.433$ $P = 0.715$, $D = 0.471$ $P = 0.720$, and $D = 1.26$ $P = 0.894$, for all populations combined, *Tandanus* sp. 1, the north coast and central and south coast populations respectively). Results from the mismatch distributions similarly failed to reject a model of demographic expansion for all population groups tested (sum of squares differences, SSD, and raggedness, r, both $P > 0.05$). The only exception was for *Tandanus* sp. 1 (SSD = 0.113 $P = 0.034$, raggedness, $r = 0.390$ $P = 0.005$), suggesting that this group has not undergone an historical expansion. The net nucleotide divergence between *Tandanus* sp. 1 and NSW north-coast (0.046 ± 0.008), and *Tandanus* sp. 1 and MDB (0.059 ± 0.008) were similar and equate to divergence times of ~1.29 mya, and ~ 1.64 mya, respectively. In contrast, the net nucleotide divergence between the NSW north-coast and MDB was much smaller (0.0067 ± 0.002) indicating a more recent divergence time ~186 kya.

Table 3.1. Haplotype frequencies in each population. The seven probable MDB fish detected in the Clarence catchment are allocated to a separate population [Clarence (translocated)].

	Haplotype																								
Population (sample size)	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**	**W**	**X**	**Y**	**Z**
NSW North-Coast																										
Tweed (4)	-	-	-	0.25	-	-	-	-	0.75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brunswick (5)	-	-	-	-	-	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Richmond (4)	-	-	-	-	-	-	0.75	0.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clarence (4)	-	-	-	-	-	-	-	-	-	0.25	0.5	0.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clarence (translocated, 7)	0.29	0.71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***Tandanus* sp. 1**																										
Bellinger (4)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.25	-	-	-	0.75
Macleay (5)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.80	0.20	-	-	-
Hastings (4)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.50	0.25	0.25	-	-
Manning (5)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-
NSW Central and South Coast																										
Karuah (5)	0.40	0.20	-	-	-	-	-	-	-	-	-	-	-	-	0.40	-	-	-	-	-	-	-	-	-	-	-
Hunter (23)	0.70	-	0.04	-	-	-	-	-	-	-	-	-	0.09	-	0.13	-	0.04	-	-	-	-	-	-	-	-	-
Hawkesbury (28)	0.96	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shoalhaven (26)	0.35	-	-	-	-	-	-	-	-	-	-	-	-	-	0.65	-	-	-	-	-	-	-	-	-	-	-
Murray-Darling Basin																										
Condamine (3)	-	-	-	-	-	-	-	-	-	-	-	-	-	0.66	-	-	0.34	-	-	-	-	-	-	-	-	-
Moonie (5)	-	-	-	-	-	-	-	-	-	-	-	-	0.20	-	0.40	-	0.20	-	-	0.20	-	-	-	-	-	-
Macintyre Falls (5)	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	-	-	-	-	-	-	-	-
Tenterfield Falls (5)	-	-	-	-	-	-	-	-	-	-	-	-	-	0.40	-	-	-	0.60	-	-	-	-	-	-	-	-
Lower Border Rivers (4)	0.25	0.25	-	-	-	-	-	-	-	-	-	-	-	0.25	0.25	-	-	-	-	-	-	-	-	-	-	-
Gwydir (3)	-	-	-	-	-	-	-	-	-	-	-	-	0.33	0.33	-	-	-	-	-	-	0.33	-	-	-	-	-
Namoi (4)	0.25	-	-	-	-	-	-	-	-	-	-	-	0.25	-	0.25	-	0.25	-	-	-	-	-	-	-	-	-
Castlereagh (5)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.60	-	0.40	-	-	-	-	-	-	-	-	-
Macquarie (5)	-	-	-	-	-	-	-	-	-	-	-	-	0.60	0.40	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.1. *Continued*

	Haplotype																								
Population (sample size)	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**	**W**	**X**	**Y**	**Z**
Lachlan (5)	0.40	-	-	-	0.20	-	-	-	-	-	-	-	-	-	0.40	-	-	-	-	-	-	-	-	-	-	
Benanee (4)	0.75	-	-	-	-	-	-	-	-	-	-	-	-	0.25	-	-	-	-	-	-	-	-	-	-	-	-
Murray Riverina (5)	0.80	-	-	-	-	-	-	-	-	-	-	-	-	0.20	-	-	-	-	-	-	-	-	-	-	-	-
Centenary (26)	0.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.80	-	-	-	-	-	-	-	-	-	-
Cardross (12)	0.16	-	-	-	-	-	-	-	-	-	-	-	-	0.50	-	-	-	-	0.34	-	-	-	-	-	-	-
Amphitheatre (20)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	-	-	-	-	-	-
Tahbilk (4)	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.2. Aligned haplotypes from 240 *T. tandanus* and *Tandanus* sp. 1 mtDNA control region sequences. Invariant sites were excluded.

Haplotype				
N	TGCTACGTTC	GGCGAAGAAG	GCAAGATTCG	AGTTGG
A	.......C..	......A...	A.........	......
B	.A.....C..	......A...	A.........	......
C	.A...T....	......A...	AT........	......
D	.A..GT....	......A...	AT........	......
E	.A...T....	......A...	AT.G......	......
F	.A..GT....	......A...	AT...G....	......
G	.A...T...T	......A...	AT.G......	......
H	.A...TA..T	......A...	AT.G......	......
I	.A...T...T	......A...	AT.G...C..	......
J	.A...T...T	......A...	.T.G.GC...	......
K	.A...T..CT	......A...	.T.G.G....	......
L	.A......CT	......A...	.T.G.G....	......
M	.......C..	......A...	....	......
O	.A.....C..	....	....	......
P	.A.....C..	.A........	....	......
Q	.A.C...C..	....	....	......
R	.A.....C..	....	...G......	......
S	.A.....C..	....	....	....T.
T	CA.C...C..	....	.T........	......
U	....	......A...	A.........	......
V	CAT.GTA..T	..TAGGA.GA	CTTTA.C...	G.CC.A
W	CAT.GTA..T	..TAGGA.GA	CTTTA.C...	GTCC.A
X	CAT.GTA..T	A.TAGGA.GA	CTTTA.C...	GTCC.A
Y	CAT.GTA..T	..TAGGAGGA	CTTTA.C.AA	G.CC.A
Z	.AT.GTA..T	..TAGGA.GA	CTTTA.C...	G.CC.A

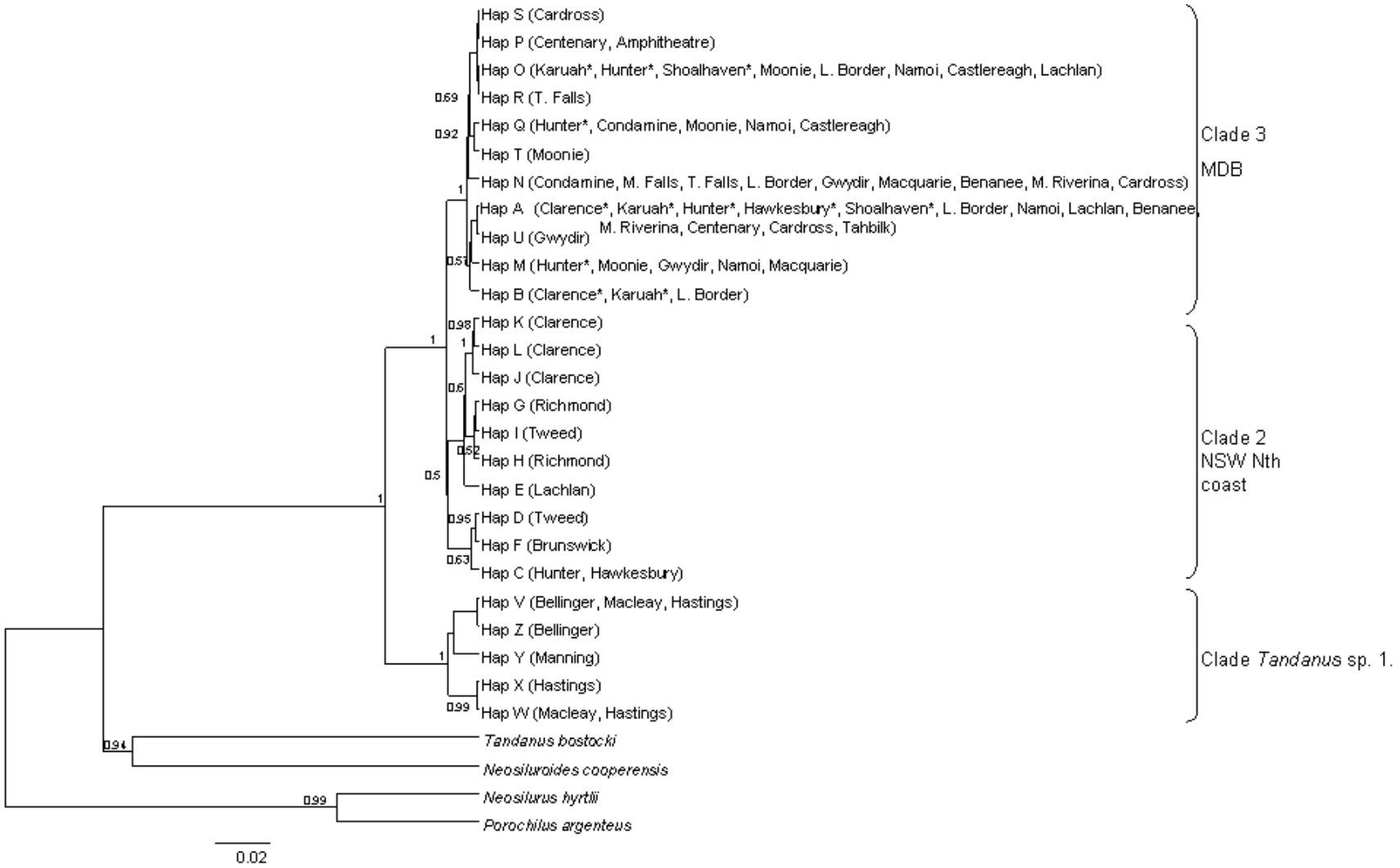

Figure 3.2. Bayesian maximum clade credibility tree for *T. tandanus* and *Tandanus* sp. 1 based on 408 bp of mtDNA control region. Nodes are labelled with the posterior probability of monophyly for that particular clade and the scale is percentage sequence divergence. * putative MDB haplotype found in coastal catchments (due to translocation).

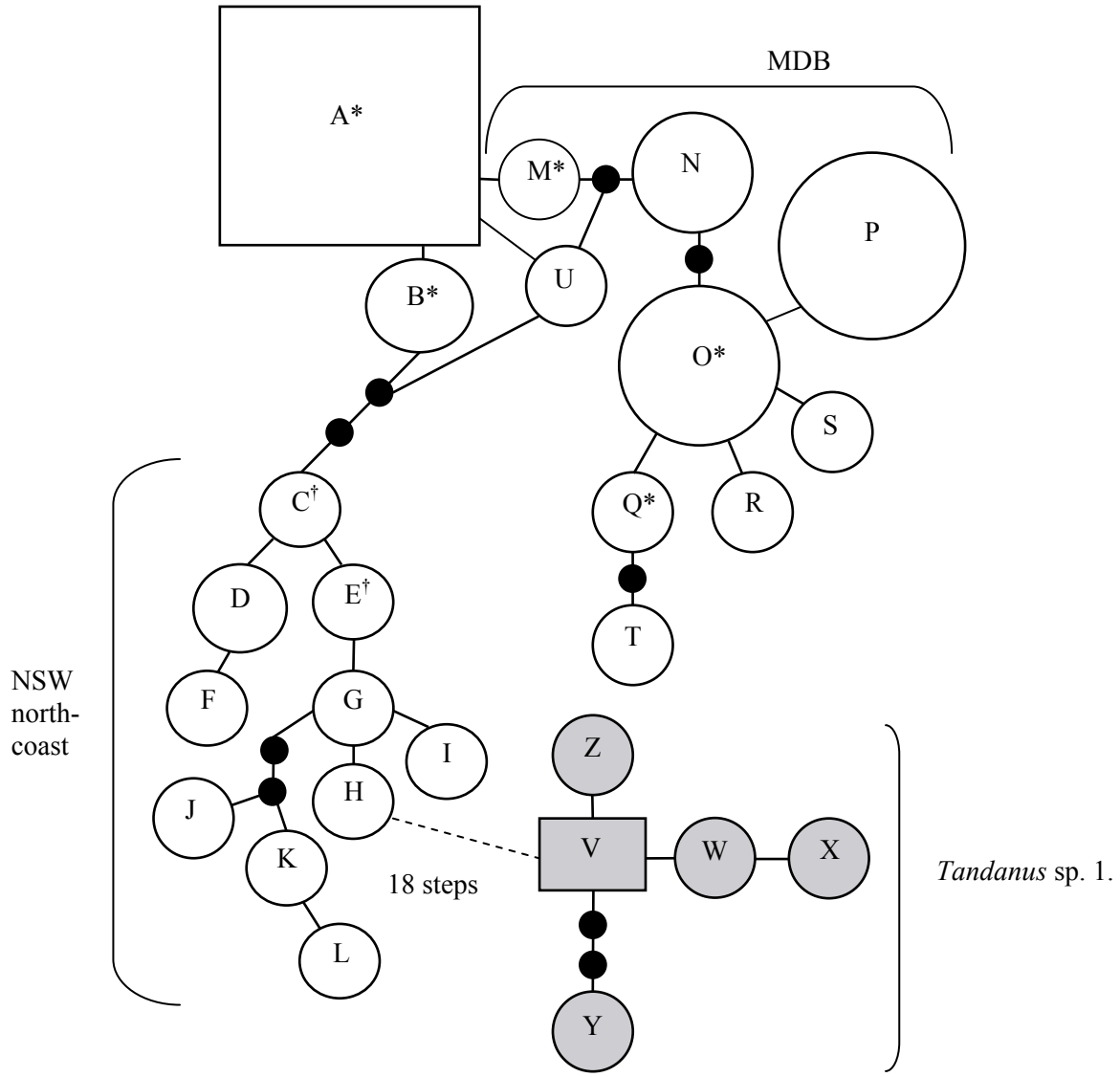

Figure 3.3. Minimum spanning network based on the mtDNA control region showing the relationship among *T. tandanus* and *Tandanus* sp. 1 populations. Haplotypes are designated by letters and correspond to those in Table 3.1. The size of circles and squares are relative to the frequency of the haplotype. Haplotypes separated by a single line are one mutation apart and black dots are missing haplotypes (un-sampled or ancestral haplotypes). Square haplotypes are those that are likely to be the most ancient and shaded haplotypes are those representing *Tandanus* sp. 1 The minimum number of steps between the lineage V (undescribed species) and lineage H is 18 (not supported by statistical parsimony). *putative MDB haplotype also found in some coast populations, †north coast haplotypes also found in a MDB population (C) and two south coast populations (E) (Table 3.1).

AMOVA results indicate that there is a substantial amount of genetic structuring at all hierarchical levels (Table 3.3). There was a high level of differentiation among the non-stocked MDB, *Tandanus* sp. 1, and the NSW north coast populations (F_{ST} = 0.342, P = < 0.0001; F_{ST} = 0.801, P = < 0.0001; F_{ST} = 0.767, P = < 0.0001 respectively). There was also substantial differentiation between *Tandanus* sp. 1 and the NSW north coast (F_{CT} = 0.848, P = < 0.05), and between *Tandanus* sp. 1 and non-stocked MDB populations (F_{CT} = 0.891, P = < 0.01). The lowest level of differentiation was among the non-stocked MDB populations (F_{ST} = 0.342, P = < 0.0001) and between the non-stocked MDB and NSW central and south coast populations (F_{CT} = 0.400, P = < 0.0001).

Table 3.3. Summary of AMOVA results for *T. tandanus* in the Murray-Darling Basin (MDB) and coastal catchments showing the level of genetic differentiation among populations (FST) and between groups (FCT). Excludes known stocked populations in the MDB and NSW central and south coast, and translocated NSW north coast individuals in the Lachlan, Hunter and Hawkesbury populations. The seven putative MDB fish identified in the Clarence were included as a separate population within the MDB.

Population comparisons	**No. pops**	**No. groups**	F_{ST}	F_{CT}
Among MDB populations	14	1	0.342***	
Among *Tandanus* sp. 1	4	1	0.801***	
Among NSW north coast	4	1	0.767***	
Between *Tandanus* sp. 1 and NSW north coast	8	2	0.967***	0.848*
Between *Tandanus* sp.1 and MDB	18	2	0.937***	0.891**
Between NSW central and south coast and MDB	18	2	0.413***	0.400***
Among *Tandanus* sp.1 NSW north coast and MDB	21	3	0.912***	0.826***

*Significant F_{ST} and F_{CT} and values are indicated by *** P < 0.0001, **P < 0.01 and * P < 0.05.*

3.4. Discussion

The current project has expanded upon earlier studies (Musyl and Keenan 1996; Jerry and Woodland 1997; Jerry 2008) by increasing sample sizes and sampling distribution in order to clarify the phylogeny of the *Tandanus* species in south eastern Australia, and assesses phylogeographic structure. We identified three well supported clades: one containing haplotypes from *Tandanus* sp. 1 [clade 1: clade II of Jerry (2008)], one containing predominantly NSW north-coast haplotypes (clade 2), and one containing predominantly MDB and NSW central and south coast haplotypes (clade 3). In contrast, our clades 2 and 3 were not distinguished by Jerry (2008) who grouped them together into clade I within his classification.

The phylogenetic position of *Tandanus* sp. 1 observed here is similar to that of previous studies (Musyl and Keenan 1996; Jerry and Woodland 1997; Jerry 2008), and provides additional strong support for the formal taxonomic recognition of this taxon as a distinct species. Pending the formal taxonomic process, we propose that this species be referred to as *Willung*, the traditional name used by the Aboriginal people of the Dhanggati, Gumbaynggirr and Birrbay tribes whose country encompassed the entire range of the species (Muurrbay Aboriginal Language and Culture Co-operative, pers. comm.). This included the Bellinger, Macleay, Hastings and Manning catchment on the mid-north coast of NSW.

Although clades 2 and 3 represent distinct divisions between the NSW north coast catchments and the Murray-Darling Basin, both are clearly lineages of *T. tandanus*. However, the phylogeographic structure within clades 2 and 3 has been compromised by the presence of translocated fish, potentially influencing the interpretation of the species' evolutionary history. These translocations are taken into consideration in the following discussion of clades 2 and 3. Clade 2 was largely restricted to the Tweed, Brunswick, Richmond and Clarence catchments on the NSW north coast. The exceptions were two haplotypes, C and E, which were sampled from a single individual from the Lachlan River (MDB, haplotype E), and two fish from the Hunter and Hawkesbury populations (NSW central and south coast, haplotype C) (Fig. 3.2 and 3.3). Interestingly, neither of these clade 2 haplotypes was collected within the four NSW north coast populations sampled. The most parsimonious explanation for the presence of 'north coast' haplotypes in the MDB (one individual) and the NSW central and south coast catchments (two individuals) is that they had been translocated into these two areas. This is feasible given the extensive translocation history of *T. tandanus* between drainages both within and outside its natural range (Clunie and Koehn 2001b, Gavin Butler, NSW I&I, pers comm.). Alternatively, the fact that we did not sample haplotype E or C in any of the NSW north coast populations can be explained by insufficient sampling (only four or five individuals genotyped from each north coast catchment), or that these two haplotypes may be present in clade 2 populations occurring in south-east Queensland rather than those catchments sampled in NSW. The latter hypothesis is supported by unsubstantiated reports of the illegal release of fingerlings purchased in Queensland into a waterway in the Hunter catchment (Rodney Hardwick, Hunter – Central Rivers CMA, pers. comm.).

Clade 3 encompassed all 116 samples collected from the MDB (with the exception of the aforementioned Lachlan River fish that grouped with clade 2), and 49 of 51 fish collected from the Hunter and Hawkesbury populations, as we expected from translocation records (Harris and Battaglene 1990). Similarly, all 31 samples from the Karuah and Shoalhaven catchments contained clade 3 haplotypes, suggesting they too are not natural populations but were also translocated from the MDB. Clade 3 haplotypes were also detected in seven of the 11 samples genotyped from the Clarence catchment on the north coast. Six of these were collected from Cataract River, a tributary of the Clarence River known to have received translocated MDB fish (Arnold Butler, recreational angler, pers comm.), and the seventh fish was sampled ~150 river kilometres downstream in the Clarence River. We suggest that these fish carrying clade 3 haplotypes are translocated fish from the MDB (or their descendants). The documented history of translocation of MDB fish into some central and south coast populations, coupled with the phylogenetic evidence presented here leaves us in no doubt that NSW central and south coast populations are non-native. A similar conclusion to ours was drawn by Faulks *et al.* (2009) in their study of Macquarie perch, where MDB haplotypes were recorded in some coastal locations, consistent with translocation records for that species.

3.4.1. Biogeography

Our estimates of divergence indicate that clade 1 (*Tandanus* sp. 1) diverged from clade 3 (MDB) ~1.64 mya, while clade 2 (NSW north coast) diverged from clade 3 ~186 kya. The GDR is the major barrier between coastal clades and the MDB, and it began to form approximately 90 mya (Wellman 1979). Thus, the separation of the MDB and coastal clades occurred far more recently than this major vicariant event, and an alternative explanation for the current phylogeographic pattern is needed. River capture is one mechanism that may have allowed *T. tandanus* to cross the GDR in the vicinity of the Clarence River, given that this is an area of relatively low elevation (Jerry 2008). Alternatively, at the time of the most recent contact between the MDB and coastal populations, ~186 kya, Australia was at the end of an interglacial period (OIS 7), where warm and wet conditions would have prevailed (Imbrie *et al.* 1984; Kershaw *et al.* 2003). The wetter interglacial climate may have facilitated dispersal across the drainage divide, possibly via floodwaters, or swampy areas near the border of the drainage divide (Ollier and Haworth 1994;

Unmack 2001; Craw *et al.* 2007; Burridge *et al.* 2008). Irrespective of the mechanism/s by which the GDR was crossed, there is mounting evidence that the Clarence River was an important site of relatively recent connection between coastal and inland catchments for several freshwater fish species, and previously at various times within the Pleistocene. These include: southern purple spotted gudgeon (*Mogurnda adspersa*) populations that were estimated to have diverged ~ 1.4 – 1.8 million years ago (Faulks *et al.* 2008), carp gudgeon (*Hypseleotris* sp. 3) populations (no date of divergence was given) (Thacker *et al.* 2007) and eastern freshwater cod (*Macullochella peelii ikei*) diverged from Murray cod ~1.7 mya (Rowland 1993). Alternatively, if *T. tandanus* haplotypes C or E are found to also occur in the Fitzroy or Burnett catchments in Queensland, this would provide tentative support for a connection between the coastal and MDB drainage divisions being via these catchments.

Surprisingly, there has been more recent contact between clades 2 and 3 (186 kya), which are separated by the GDR, than between clades 1 and 2 (1.29 mya) in adjacent coastal catchments. Jerry (2008) suggested that *Tandanus s*p. 1. populations became isolated from coastal *T. tandanus* populations in northern QLD after low-lying interfluvial habitat was reduced by continental drying coupled with rising sea levels during the early Pliocene. While this mechanism may also be responsible for the lack of connectivity between the two coastal clades identified in this study, it is also possible that the Great Escarpment that runs between the Clarence and Bellinger catchments (Ollier 1982b; Ollier 1982a), is a significant barrier separating the two clades.

There was an extremely high degree of population structuring within clades 1 and 2 (F_{ST} = 0.801, P < 0.0001, F_{ST} = 0.767 P < 0.0001 respectively), indicating greatly reduced gene-flow among populations within each clade. In clade 1, the level of population structuring is due to high variation in the frequency of haplotypes across the four populations, and the unique haplotypes in the Manning population. However, in clade 2 every population possessed unique haplotypes (haplotypes A and B were excluded given their probable MDB origin). This pattern of high differentiation among coastal catchments is more than likely due to the historical and current isolation of rivers within coastal catchments by high sea levels. Further, these rivers were unlikely to have connected even during much lower sea levels due to narrow continental shelf margins (Unmack 2001). A similar pattern of high population structuring among coastal populations was detected in the Oxleyan pygmy perch (*Nannoperca oxleyana*) (Knight 2009) and Macquarie perch (Faulks *et al.* 2009), and was also attributed to the limited connectivity of coastal catchments. In contrast, the natural populations within the MDB are not as highly differentiated (F_{ST} = 0.342 P < 0.0001) and only six of the 12 MDB haplotypes were isolated to a single population (Table 3.1), despite these populations encompassing eleven catchments across an area far larger than the coastal populations. This pattern is likely due to the greater connectivity of MDB catchments, as most rivers ultimately coalesce in the Murray River. Similarly, Murray cod and golden perch have very similar distributions to *T. tandanus* within the MDB, and display very limited population differentiation, with the exception of some catchments isolated by wetlands (Rourke 2007; Faulks *et al.* 2010). Similarly, some populations of *T. tandanus* are likely to be intermittently isolated by wetland and waterfall barriers, e.g., the Lachlan River (terminates at the Great Cumbung Swamp), the Macquarie River (flows into the non-terminal Macquarie Marshes) (O'Brien and Burne 1994; Brock 1998; Kingsford *et al.* 2004), Tenterfield Creek above Tenterfield Falls, and the Macintyre River above Macintyre Falls (Fig. 3.1). However, only two of these populations showed evidence of isolation: the population above Macintyre Falls was fixed for a single haplotype, though this was a common haplotype found in many populations, and the Tenterfield Falls population had a unique haplotype, but it also possessed another common haplotype. More extensive sampling of populations within the MDB and the analysis of microsatellite loci is required to identify fine-scale structure to determine the full extent of population differentiation in the MDB (Chapter 4).

3.4.2. Management implications

A national recovery plan for *T. tandanus* has made a range of recommendations to assist with increasing *T. tandanus* numbers to a more sustainable level (Clunie and Koehn 2001a). Stocking and/or translocation programs were identified as a high priority action to reintroduce *T. tandanus* into suitable sites (Clunie and Koehn 2001a). Stocking programs typically involve removing a subset of adult fish from the wild, facilitating spawning in captivity and then releasing their progeny back into the wild, while translocation programs remove a subset of the population (adult or juveniles) and relocate them to a new area. In recognition of the potential negative genetic impacts that these stocking/translocation programs can have (Hindar *et al.* 1991; Utter 2004), the recovery plan also stipulated that stocking should not be carried out between drainage basins where different genetic stocks have been identified.

Tandanus sp. 1. (clade 1) is historically isolated and monophyletic, fulfilling the criteria of an Evolutionary Significant Unit (ESU) (Moritz 1994; Fraser and Bernatchez 2001). Similarly, *T. tandanus* from the NSW north-coast (clade 2) and the MDB (clade 3) are monophyletic (when stocked fish are removed) and have been isolated for a substantial period of time, thus also qualifying as ESUs (Moritz 1994; Fraser and Bernatchez 2001). Further, given the high degree of physical isolation of coastal populations (Unmack 2001), and the high level of genetic divergence among populations within clades 1 and 2 (as evidenced by F_{ST} values), we suggest that each coastal population be designated as a separate Management Unit (MU – significant divergence at nuclear or mtDNA loci). These MUs may in future be elevated to full ESUs if microsatellite data corroborates the distinctiveness of these clades. It is more difficult to assign MUs within the MDB given most rivers are interconnected and haplotypes are shared over a broader area. Again, microsatellite data may help identify if different genetic stocks are in fact present (Chapter 4).

This study has demonstrated that there is a high degree of genetic differentiation among the three clades, and thus we do not recommend any inter-clade stocking/translocation. Further, we also recommend that each ESU and MU identified by this study be managed separately with regards to stocking programs. In practice, this will involve only releasing fish into the same ESU or MU from which their parents were collected in order to avoid introducing different genetic stocks that may hybridise with the local population. By taking these precautions, the potential negative genetic impacts of stocking/translocation, e.g., outbreeding depression, loss of genetic diversity and loss of between-population variation (Hallerman 2003; Miller and Kapuscinski 2003), can be minimised. The results of this study demonstrate how genetic methods can contribute in a positive way to help inform management plans for species of conservation concern.

4. MICROSATELLITE POPULATION GENETIC STRUCTURE OF FRESHWATER CATFISH WITHIN THE MURRAY-DARLING BASIN AND COASTAL CATCHMENTS OF NSW

4.1. Introduction

The consensus of former genetic assessments of freshwater catfish populations (allozyme and mtDNA) is that a unique undescribed species is present in the Bellinger, Macleay, Hastings and Manning Rivers of the NSW mid-north coast (*Tandanus* sp.1) and that although they diverged relatively recently (~ 186 kya), populations of *T. tandanus* on the NSW north coast (Clarence, Richmond, Brunswick and Tweed catchments) are distinctly different from those in the MDB. The north coast population may represent a different sub-species, incorporating populations from south-east Queensland catchments north to at least the Burdekin (Musyl 1990; Jerry and Woodland 1997; Jerry 2008, Chapter 3). Within the MDB, analysis of seven allozyme loci in samples from nine impounded sites and six riverine sites indicated that there was a central population comprised mostly of riverine fish, and that five of the impoundment populations were divergent (Keenan *et al.* 1995). Similarly, our mtDNA analysis suggested little population genetic structure within the MDB. Based on these collective data, it was recommended that each coastal clade be recognised as an evolutionary significant unit (ESU), and each coastal catchment be managed as an independent management unit (MU), while the MDB be managed as a single ESU (Chapter 3). However, while it appears that most of the populations in the MDB belong to a single ESU based on mtDNA data, some additional but as yet inadequately resolved population structure may be present.

It is this unresolved population structure within the MDB that is of most relevance to fisheries management as the MDB population is the only population of *T. tandanus* that is listed as threatened and consequently is most in need of population enhancement. A recovery plan for *T. tandanus* prepared by the Arthur Rylah Institute outlines a range of recovery actions needed to reverse the decline of this species and return numbers to more sustainable levels in the MDB (Clunie and Koehn 2001a). Several of the recommendations in the recovery plan involve the implementation of an appropriately managed stocking program. Recent advances in mass production techniques for *Tandanus* mean that hatchery production is now a viable option to produce reasonable numbers of captive reared fish for release (Trueman 2006). In response to concerns regarding genetic integrity of Australia's freshwater fish populations, fish stocking in NSW is undertaken in accordance with the Freshwater Fish Stocking – Fishery Management Strategy (FMS) (NSW DPI 2005). The FMS has recognised that genetic structure needs to be fully investigated for a species prior to commencing new stocking programs on Australian freshwater fish. In order to resolve the uncertainty regarding the population structure of *T. tandanus* populations within the MDB, there is a need to utilise more sensitive genetic markers than allozyme and mtDNA loci. Assessment of microsatellite markers will allow identification of fine-scale genetic structure that may be present in the MDB, and consequently, provide additional information to designate ESUs and MUs.

The results of our mtDNA analysis (Chapter 3) demonstrated that the populations of *T. tandanus* in coastal catchments south of and including the Karuah River are derived from the translocation of MDB fish. The few reports of translocation that exist suggest that the Hawkesbury River population was established with fish translocated from the Macquarie River (MDB), and the Hunter

River population was established with fish translocated from Keepit Dam (MDB) (Harris and Battaglene 1990). While the Macquarie and Namoi populations are two of the most abundant populations remaining within the NSW portion of the MDB, and not in imminent risk of further decline, it is possible that some of the fish used to found other translocated coastal populations could have been sourced from southern MDB rivers where *T. tandanus* populations have declined so significantly that broodstock collection may place undue pressure on the remnant stocks, i.e., the Murrumbidgee, Murray and Darling Rivers. If population substructure is detected within the MDB, we may be able to identify the source population of each of the translocated populations in coastal rivers. And if so, the coastal populations may provide a ready source of broodfish and/or translocates for recovery programs within MDB rivers.

Consequently, this part of the study has three main aims:
(1) Use microsatellite loci to build upon previous genetic work *(*Keenan *et al. 1995;* Musyl and Keenan *1996;* Jerry *2008)* and thoroughly describe the population genetic structure of *T. tandanus* and *Tandanus* sp.1 across the MDB and coastal catchments of NSW,
(2) clarify whether fish previously identified as of MDB origin in the Clarence population have introgressed with native Clarence fish and,
(3) use microsatellite loci to test whether NSW central and south coast populations originate from the MDB and, if possible, identify the likely population of origin.

4.2. Methods

4.2.1. Sample collection

A total of 821 samples were collected using a variety of methods, including boat and backpack electrofishing, fyke nets and gill nets. Samples were also collected by other individuals, State Government agencies and universities (Namoi Valley Aquaculture, Queensland Department of Primary Industries and Fisheries, Murray-Darling Fisheries, Department of Sustainability and Environment, Murray-Darling Freshwater Research Centre, Griffith University, Murdoch University, Bingera Fishing Club, Narrabri Fishing Club, Pallamallawa Fishing Club and several private anglers). We attempted to obtain samples from at least 30 individuals from each population, but in some cases this was not possible due to low abundances. Fin-clip samples were collected from a total of 18 un-stocked populations within the MDB (Condamine, Moonie, above Tenterfield Falls, above Macintyre Falls, lower Border Rivers, Gwydir above Copeton Dam, Gwydir below Copeton Dam, Berrygill Creek, Namoi above Keepit Dam, Namoi below Keepit Dam, Castlereagh, Lachlan, Macquarie above Burrendong Dam, Macquarie below Burrendong Dam, Benanee, Murray Riverina and Tahbilk Lagoon), five stocked populations within the MDB (Imperial Lake, Centenary Reservoir, Cardross Lakes, Amphitheatre Reservoir and Wimmera), four NSW north coast populations (Tweed, Brunswick, Richmond and Clarence), four populations of *Tandanus* sp.1 (Bellinger, Macleay, Hastings and Manning) (Musyl and Keenan 1996; Jerry 2008), and four NSW central to south coast populations (Karuah, Hunter, Hawkesbury and Shoalhaven) that were established from fish translocated from the MDB (Fig. 4.1). Fin-clips were preserved in the field in 100% ethanol, followed by storage at -20^{o}C. Fish were released unharmed immediately following sampling.

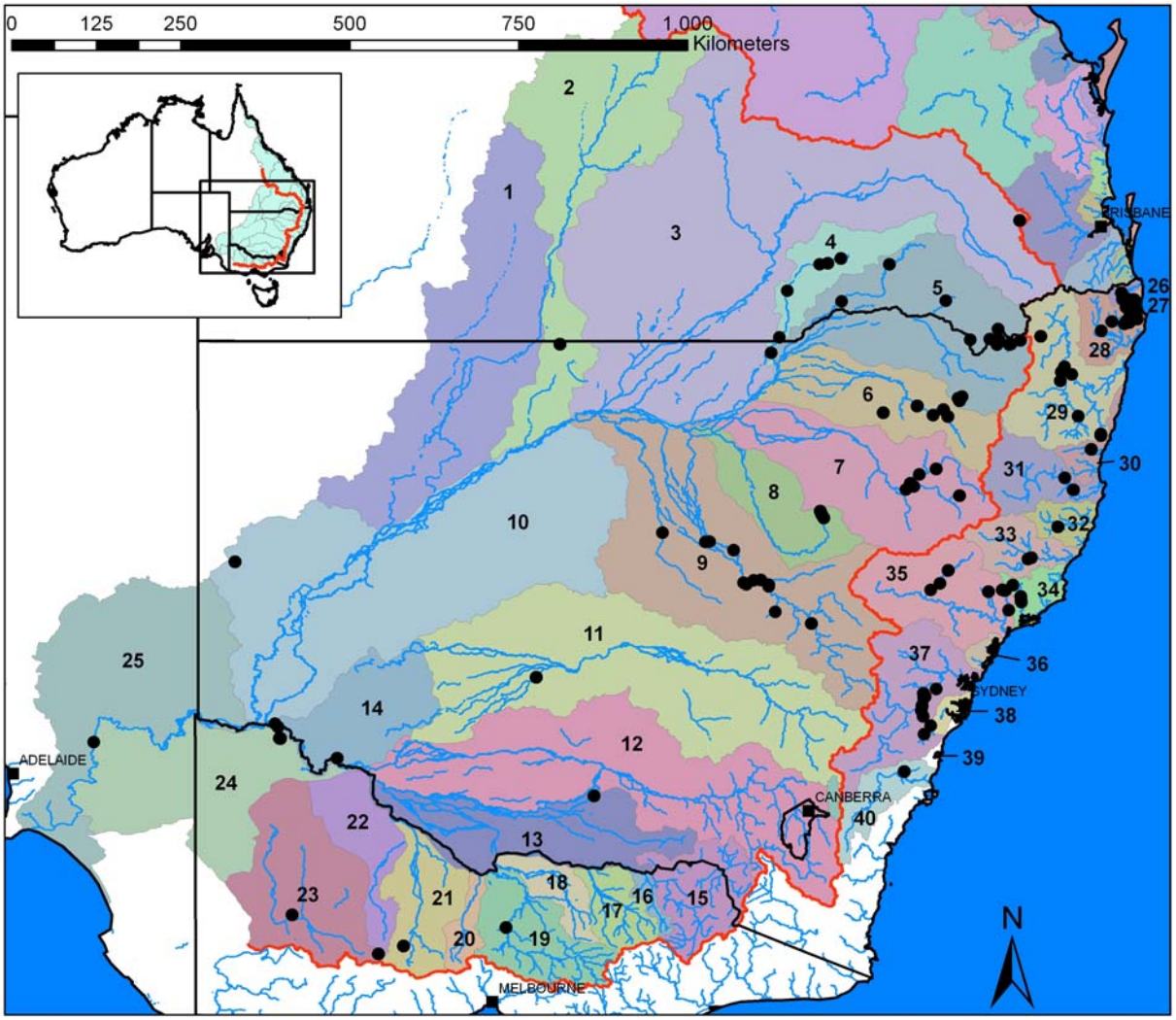

Figure 4.1. Sampling locations for the microsatellite DNA analysis are indicated by black points. State borders are indicated by black lines and the ridge of the Great Dividing Range by a red line. Numbers correspond to basin numbers as described in Figure 3.1.

4.2.2. DNA extraction and microsatellite genotyping

DNA was extracted using the Jet Quick DNA (Genomed) extraction kit following the manufacturer's instructions. Eight microsatellite loci were selected for analysis (dye labels in parentheses): Tan1_2 (D4), Tan1_7 (D3) and Tan1_10 (D2), Tan2_16 (D4), Tan2_15 (D4), Tan2_20 (D3), Tan3_27 (D2), and Tan3_28 (D2) (Chapter 2, GenBank Accession numbers GQ496016 – GQ496023). PCR was performed 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 $MgCl_2$ 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 (Tan1_2, Tan1_7

and Tan1_10), bin2 (Tan2_16 and Tan 3_27) and bin 3 (Tan 2_15, Tan2_20 and Tan3_28), 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 accurately sized, raw allele lengths were assigned into bins using FLEXIBIN (Amos *et al.* 2007).

4.2.3. Genetic diversity and differentiation

GENEPOP version 3.3 (Raymond and Rousset 1995) was used to check for conformance to Hardy-Weinberg expectations (HWE) using exact probability tests and 10,000 permutations. Tests for linkage disequilibrium between each pair of loci in all populations were also carried out in GENEPOP 3.3 (10,000 permutations). Observed heterozygosity (H_O) and the number of private alleles in each population were calculated using GENALEX 6. Allelic richness (A_R – allelic diversity corrected for sample size) and expected heterozygosity (H_E) were calculated in FSTAT 2.93 (Goudet 1995). In addition, permutation tests (15,000 permutations) were conducted to determine if there were significant differences in genetic diversity (A_R and H_E) between several groupings of the data: central and south coast stocked populations, MDB stocked populations, non-stocked MDB populations, *Tandanus* sp.1 populations, NSW north coast populations and MDB populations isolated by a waterfall (above Tenterfield Falls and above Macintyre Falls) or a dam (above Copeton, Keepit and Burrendong Dams). Pairwise F_{ST} values among the sample sites were calculated in ARLEQUIN 3.01 (Excoffier *et al.* 2005) and the significance was calculated from 10,000 permutations of the data. Given the putative MDB origin of seven Clarence samples (six from Cataract Creek and one from the Clarence River), and the genetic distinctiveness of seven fish from the Boral Dam site within the Castlereagh catchment based on *Structure* analysis (see results), we isolated these two groups of samples from the remainder of samples representing the Clarence and Castlereagh populations for the F_{ST} analysis. The resulting matrix was used to construct a multi-dimensional scaling plot (MDS) using PRIMER v6 (Clarke and Gorley 2006) to aid in visualisation of the data.

4.2.4. Population structure

Population genetic boundaries are often difficult to define, particularly for fish populations in interconnected rivers. The program *Structure* 2.1 (Pritchard *et al.* 2000) does not require pre-defined populations as input. Instead, the program assumes *K* genetic clusters and uses allele frequency data to 'assign' individuals to one or more genetic clusters (*K*). It is also possible to identify potential immigrants from genetically distinct populations and hybrid individuals, although the power to do the latter accurately with eight loci is limited (Vähä and Primmer 2006). *Structure* 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* ($K = 1 – 30$) in order to examine the consistency of results. Estimation of the most likely number of populations (*K*) was achieved in two ways. Firstly, the average likelihood [P(X/K)] of each *K* was plotted to determine the value with the highest likelihood. Secondly, the method of Evanno *et al.* (2005) was used to estimate the value of ΔK, which is based on the second order rate of change of the log probability [P(X/K)].

4.2.5. Assignment tests

One aim of this study was to determine whether the NSW central and south coast populations and the seven 'suspect' fish from the Clarence population were translocated MDB fish. We used GENECLASS2, Version 2.0 (Piry *et al.* 2004) to assign individuals to their most likely population of origin. The program was run using Bayesian assignment and Monte-Carlo re-sampling methods (Rannala and Mountain 1997) to assess the probability of population membership with 10,000 simulated individuals and $\alpha = 0.05$. We created a reference file that contained all populations

except the central and south coast populations and ran the program to detect whether the central and south coast populations were more similar to native coastal or to MDB populations.

4.2.6. Population bottleneck

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 loose 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 and Luikart 1996). For example, a bottleneck resulting in an effective population size (N_e) of 50 is likely to be detectable for approximately 25 – 250 generations following the initiation of population decline, after which time the loci return to mutation-drift equilibrium (Cornuet and Luikart 1996). We used the two-phase mutation model (TPM) given that it performs best with microsatellite data (Di Rienzo *et al.* 1994). The Wilcoxon signed-rank test was used to assess the significance of the results. TPM settings were 90% one-step mutations and 10% multiple-step mutations and a variance among multiple steps of 12, and the program was run for 5,000 iterations. The program performs optimally with a minimum of 12 loci and 30 individuals (Piry *et al.* 1999). Therefore, we tested for bottlenecks in five main groups, the NSW north-coast, *Tandanus* sp.1, the MDB populations that have not been or are minimally stocked, NSW central and south coast stocked populations and MDB stocked populations. We acknowledge that there may be a reduction in statistical power to detect recent bottlenecks given that we have used only eight loci.

4.2.7. Effective population size

We estimated both the long term and contemporary effective population size (N_e) for the same five groupings of the data as for the bottleneck tests. The long-term N_e was estimated using a heterozygosity based method (Ohta and Kimura 1973) according to a stepwise-mutation model (SMM) as, $N_e = [1/(1- H_E)2-1]/8\mu$, where H_E is expected heterozygosity and μ is the mutation rate. This model assumes that the microsatellite mutations involve changes of one repeat unit (Jarne and Lagoda 1996). Given that our data may not conform with this mutation model, we also estimated long-term N_e using an infinite alleles model (IAM) as, $N_e = H_E /4\mu(1- H_E)$ (Kimura and Crow 1963). We applied a microsatellite mutation rate of $\mu = 5 \times 10^{-4}$ to both methods, which is that commonly used for fishes (Lippé *et al.* 2006), and used FSTAT to calculate H_E for each population group. We estimated contemporary effective population size for the same populations using a linkage disequilibrium method that returns an estimate for the parental generation using the program NeEstimator 1.3 (Peel *et al.* 2004). However, this method has recently been shown to be biased when the sample size is less than the true N_e (England *et al.* 2006). Therefore, our data set presents an opportunity to test an updated method of estimating N_e that also uses the linkage disequilibrium method but incorporates an empirical correction method to eliminate bias, LDNE (Waples and Do 2008). This program is capable of producing both parametric confidence intervals (CI) and jackknife CI, the latter method purportedly encompasses the true N_e more often (Waples and Do 2008). However, for data sets using relatively few loci, the parametric method may be more suitable, and thus was used to evaluate our estimates. We selected a model of random mating and evaluated results for a P_{crit} of 0.05.

4.3. Results

4.3.1. Hardy-Weinberg and genotypic disequilibrium

All but one locus successfully amplified in all populations. The exception was Tan2_27 which did not amplify in populations of *Tandanus* sp.1. Data for each population was analysed both with and without this locus and results were consistent. Therefore the locus was included in all subsequent analyses, with the exception of the calculation of allelic richness. Four loci in the Bellinger (Tan1_7, Tan1_10, Tan2_20 and Tan3_28), and one in each of the Macleay (Tan1_10), Hastings (Tan1_10) and Manning (Tan3_28) were monomorphic. After sequential Bonferroni correction (Rice 1989) (table adjusted to 1 population x 28 locus pairs) only 15 of 952 (1.6%) locus pairs across the four populations were significant for Linkage Disequilibrium (LD) ($P < 0.05$). All loci were considered suitable for use given that no locus pair was consistently linked across populations. After sequential Bonferroni correction (table adjusted to 1 locus x 34 populations), 12 of 272 (4.4%) locus/population combinations still departed from HWE, and all were due to a deficit of heterozygotes (Table 4.1). A deficiency of heterozygotes could result from the presence of null alleles – alleles that fail to amplify due to mutations in the primer binding site (Jarne and Lagoda 1996), inbreeding or the existence of population structure (Frankham *et al.* 2002). Close inspection of our data showed that Tan2-16 was responsible for eight of the 12 deviations in all four NSW north coast populations, two of the NSW central and south coast populations established by translocated fish and in two MDB populations – one isolated above Burrendong Dam, and the other from an almost certainly inbred population from an isolated dam in the Castlereagh catchment (Boral Dam). Given that this locus did not depart from HWE in the remaining 26 populations it is most likely that the deviations were due to either inbreeding or the presence of unresolved population structure within these population units (Wahlund effect). We excluded this locus from the data set and ran the *Structure* analysis again (see below) as the program partly relies upon HW deviations to allocate individuals to a genetic cluster (Pritchard *et al.* 2000). Results were highly consistent whether Tan2-16 was included or not (data not shown), thus we retained this locus for all analyses.

4.3.2. Genetic diversity

Genetic diversity varied widely among populations and ranged from $H_E = 0.23$ (Bellinger) to $H_E = 0.73$ (Imperial Lake) and $A_R = 2.17$ (Bellinger) to $A_R = 4.85$ (lower Border Rivers) (Table 4.1, Fig 4.2). The two main groups of populations established from translocated fish, the NSW central and south coast and MDB stocked populations had not lost a significant amount of allelic richness and heterozygosity compared to the combined non-stocked MDB populations (A_R, $P = 0.167$; H_E, $P = 0.391$ and A_R, $P = 0.486$; H_E, $P = 0.347$ respectively), despite having gone through demographic bottlenecks. *Tandanus* sp. 1 populations had a significantly lower heterozygosity and a near significant reduction in allelic richness compared to the un-stocked MDB populations (A_R, $P = 0.051$; H_E, $P = 0.014$), but not compared to the NSW north coast populations (A_R, $P = 0.497$; H_E, $P = 0.410$). Nor were there significant differences between the NSW north coast and the MDB populations (A_R, $P = 0.307$; H_E, $P = 0.160$), or populations above waterfalls compared to the remaining MDB (A_R, $P = 0.900$; H_E, $P = 0.892$ respectively). Populations isolated above dams had not lost genetic diversity relative to inter-connected riverine populations in the MDB (A_R, $P = 0.769$; H_E, $P = 0.937$). Allele frequency differences among nearly every population combination were significantly different ($F_{ST} < 0.05$, Table 4.2), suggesting gene flow is restricted even among geographically close populations.

Table 4.1. Sample size N, number of alleles N_a, allelic richness A_R, observed H_O and expected H_E heterozygosity, loci deviating from Hardy-Weinberg expectations (HW disequilibrium).

Population	N	N_a	Number private alleles	A_R†	H_O	H_E	HW disequilibrium
NSW North Coast *T. tandanus*							
Tweed	30	49	2	3.97	0.45	0.55	Tan2_16
Brunswick	29	31		2.89	0.36	0.48	Tan2_16
Richmond	29	55	2	3.95	0.51	0.56	Tan2_16
Clarence	30	65	2	4.32	0.52	0.63	Tan1_10, Tan2_16, Tan2_20
NSW Mid-North Coast *Tandanus* sp. 1.							
Bellinger	24	22		2.17	0.23	0.23	-
Macleay	27	45	5	3.72	0.53	0.52	-
Hastings	17	39	3	3.81	0.47	0.49	-
Manning	14	30	4	3.25	0.49	0.48	-
NSW Central and South Coast *T. tandanus*							
Karuah*	30	28		2.79	0.47	0.45	-
Hunter*	28	56		4.32	0.59	0.67	Tan2_16
Hawkesbury*	30	45	1	3.55	0.51	0.61	Tan2_16, Tan2_20
Shoalhaven*	30	30		3.16	0.56	0.54	-
Murray-Darling Basin *T. tandanus*							
Condamine	14	44	1	4.07	0.56	0.55	-
Moonie	15	53		4.28	0.65	0.65	-
Macintyre Falls	28	45	3	3.62	0.58	0.59	-
Tenterfield Falls	28	58	2	4.54	0.72	0.70	-
Lower Border Rivers	25	70	3	4.85	0.69	0.72	-
Gwydir catchment, above Copeton Dam	24	36		3.31	0.53	0.58	-
Gwydir catchment, below Copeton Dam	25	67		4.78	0.68	0.69	-
Gwydir catchment, Berrigill Creek	19	24	1	2.50	0.47	0.43	-
Namoi catchment, above Keepit Dam	24	55		4.07	0.64	0.63	-
Namoi catchment, below Keepit Dam	28	60	1	4.67	0.67	0.69	-
Castlereagh	20	54	4	4.29	0.48	0.64	Tan2_15, Tan2_16
Lachlan	31	52		3.83	0.57	0.61	-
Macquarie catchment, above Burrendong Dam	27	60	2	4.60	0.65	0.69	Tan2_16
Macquarie catchment, below Burrendong Dam	16	51		4.32	0.66	0.66	-
Imperial Lake*	25	57	3	4.60	0.70	0.73	-
Benanee	26	56	2	4.10	0.62	0.66	-
Murray Riverina	17	47	1	3.91	0.62	0.60	-
Centenary Reservoir*	28	38		3.06	0.46	0.46	-
Cardross Lakes*	22	54	1	4.25	0.66	0.66	-
Amphitheatre Reservoir*	29	31	4	2.65	0.35	0.38	-
Wimmera*	13	43		4.17	0.67	0.62	-
Tahbilk Lagoon	19	27	1	2.46	0.44	0.42	-

**Indicates population was most likely established from translocated fish from the MDB (see text).*

† A_R does not include data from Tan 3_27 as it did not amplify in the Bellinger, Macleay, Hastings or Manning populations.

4.3.3. Genetic structure

An MDS plot clearly shows the three anticipated major groups of populations: a MDB group, a NSW north coast group and a *Tandanus* sp. 1 group (Fig. 4.3). Furthermore, the NSW central and south coast populations, which were known or hypothesised to originate from translocated MDB fish (Karuah, Hawkesbury, Hunter and Shoalhaven, Chapter 3), also cluster with the MDB group. The MDS analysis also demonstrates that the six individuals with putative MDB mtDNA haplotypes collected from Cataract Creek in the Clarence catchment (NSW north coast) (Chapter 3) also clustered with the MDB group based on nuclear data (Fig.4.3). The seventh fish with a MDB mtDNA haplotype in the Clarence catchment had a nuclear genotype consistent with Clarence fish, and therefore was included in the Clarence population (see section 4.3.5). *Tandanus* sp. 1 was strongly divergent from both north coast and MDB populations, and also exhibited high intraspecific genetic structure, with the Bellinger population exhibiting very high F_{ST} values when compared to the Macleay, Hastings and Manning populations (F_{ST} = 0.25, F_{ST} = 0.35 and F_{ST} = 0.49 respectively Table 4.2), suggesting the Bellinger population has been isolated from the other three *Tandanus.* sp.1 populations for a considerable amount of time. Similarly, the four NSW north coast catchments have high F_{ST} among populations, indicating limited gene-flow between the Tweed, Brunswick, Richmond and Clarence catchments. The level of divergence among MDB populations was substantially lower, even among populations separated by many thousands of river kilometres. However, all above-dam samples (Copeton, Keepit and Burrendong Dams) were significantly differentiated from populations immediately downstream, albeit at a low level (F_{ST} = 0.08, F_{ST} = 0.05 and F_{ST} = 0.01 respectively, $P < 0.05$), potentially indicating that the above-dam populations have undergone genetic drift or inbreeding since being isolated from downstream populations. Similarly, the two populations above waterfalls (Tenterfield Falls and Macquarie Falls), also expressed low but significant differences to their downstream population in the lower Border Rivers (F_{ST} = 0.02 and F_{ST} = 0.06 respectively, $P < 0.05$). F_{ST} differentiation among stocked and un-stocked populations was typically higher than among un-stocked populations.

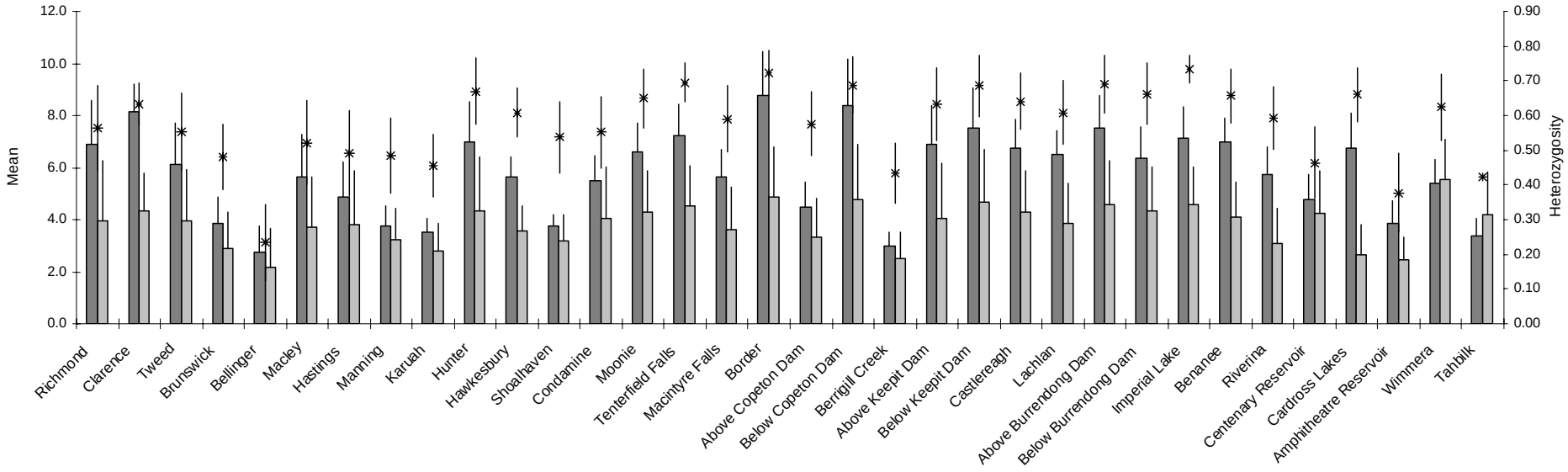

Figure 4.2. Mean number of alleles (dark grey bars), allelic richness (light grey bars) and expected heterozygosity (stars) for each population of *T. tandanus* and *Tandanus* sp. 1. Standard error bars are shown.

Table 4.2. Pairwise FST among *T. tandanus* and *Tandanus* sp. 1 populations based on seven microsatellite loci (Tan3_28 had too much missing data). Seven probable stocked individuals from the Clarence population (Clarence A) and Boral Creek (Castlereagh) samples were included separately. The dark and light-shaded boxed areas indicate populations sampled from the NSW north-coast and from *Tandanus* sp. 1 populations respectively. All results were significant ($P < 0.05$) based on 10,000 permutations of the data, with the exception of those in bold.

		RIC	CLA(A)	CLA	TWE	BRU	BEL	MAC	HAS	MAN	KAR	HUN	HAW	SHO	CON	MOO	TEN	MAF	BOR	ACO	BCO	BER	AKE	BKE	CAS	BORAL	LAC	BBU	ABU	DAR	BEN	RIV	CEN	CAR	AMP	GOU
Clarence (A)	CLA(A)	0.43																																		
Clarence	CLA	0.12	0.38																																	
Tweed	TWE	0.24	0.39	0.17																																
Brunswick	BRU	0.14	0.44	0.09	0.18																															
Bellinger	BEL	0.55	0.67	0.57	0.55	0.59																														
Macleay	MAC	0.39	0.43	0.39	0.37	0.41	0.25																													
Hastings	HAS	0.42	0.47	0.43	0.40	0.45	0.35	0.01																												
Manning	MAN	0.42	0.43	0.41	0.38	0.43	0.49	0.21	0.16																											
Karuah	KAR	0.41	0.35	0.43	0.43	0.46	0.60	0.45	0.46	0.40																										
Hunter	HUN	0.32	0.17	0.27	0.29	0.32	0.50	0.35	0.35	0.29	0.16																									
Hawkesbury	HAW	0.35	0.21	0.31	0.34	0.35	0.53	0.37	0.39	0.34	0.21	0.11																								
Shoalhaven	SHO	0.37	0.17	0.33	0.34	0.38	0.54	0.38	0.39	0.34	0.13	0.08	0.13																							
Condamine	CON	0.39	0.15	0.36	0.36	0.40	0.59	0.39	0.41	0.36	0.30	0.09	0.17	0.16																						
Moonie	MOO	0.34	0.17	0.31	0.34	0.37	0.55	0.36	0.37	0.32	0.21	0.06	0.10	0.09	0.12																					
Tenterfield Falls	TEN	0.32	0.11	0.26	0.28	0.31	0.48	0.33	0.33	0.30	0.18	0.07	0.11	0.09	0.09	0.07																				
Macintyre Falls	MAF	0.38	0.16	0.34	0.37	0.40	0.55	0.39	0.41	0.37	0.29	0.10	0.17	0.17	0.07	0.12	0.10																			
Border	BOR	0.30	0.09	0.26	0.28	0.30	0.48	0.32	0.32	0.28	0.16	0.04	0.08	0.07	0.06	0.03	0.02	0.06																		
Above Copeton Dam	ACO	0.40	0.20	0.36	0.37	0.40	0.55	0.39	0.40	0.36	0.26	0.11	0.18	0.16	0.09	0.11	0.09	0.09	0.08																	
Below Copeton Dam	BCO	0.30	0.15	0.29	0.30	0.32	0.49	0.33	0.33	0.29	0.14	0.03	0.09	0.07	0.08	0.02	0.05	0.07	0.01	0.08																
Berrigill Creek	BER	0.42	0.38	0.43	0.47	0.49	0.64	0.45	0.47	0.44	0.26	0.20	0.21	0.22	0.29	0.15	0.21	0.26	0.16	0.24	0.15															
Above Keepit Dam	AKE	0.36	0.22	0.34	0.36	0.39	0.54	0.38	0.38	0.32	0.16	0.04	0.16	0.11	0.13	0.08	0.12	0.14	0.07	0.11	0.05	0.20														
Below Keepit Dam	BKE	0.32	0.13	0.28	0.29	0.32	0.48	0.33	0.33	0.28	0.14	0.02	0.09	0.07	0.08	0.04	0.03	0.08	**0.01**	0.08	0.02	0.18	0.05													
Castlereagh	CAS	0.34	0.15	0.30	0.31	0.34	0.55	0.35	0.36	0.30	0.15	0.03	0.11	0.06	0.07	0.06	0.06	0.10	0.03	0.10	0.04	0.23	0.05	0.03												
Boral Dam (Castlereagh)	BORAL	0.52	0.41	0.49	0.46	0.52	0.74	0.51	0.57	0.53	0.44	0.32	0.33	0.32	0.41	0.32	0.24	0.37	0.26	0.37	0.29	0.50	0.37	0.27	0.33											
Lachlan	LAC	0.34	0.13	0.30	0.32	0.34	0.53	0.38	0.39	0.35	0.16	0.09	0.11	0.07	0.14	0.07	0.05	0.16	0.05	0.15	0.07	0.23	0.12	0.05	0.08	0.25										
Below Burrendong Dam	BBU	0.33	0.10	0.30	0.32	0.35	0.53	0.34	0.35	0.31	0.18	0.05	0.09	0.09	0.10	0.05	0.05	0.10	0.03	0.13	0.02	0.22	0.08	0.02	0.07	0.26	0.05									
Above Burrendong Dam	ABU	0.31	0.13	0.28	0.30	0.32	0.48	0.32	0.32	0.29	0.17	0.05	0.10	0.08	0.09	0.04	0.05	0.09	0.04	0.10	0.02	0.20	0.07	0.03	0.05	0.28	0.05	0.01								
Imperial Lake	DAR	0.17	0.17	0.14	0.17	0.15	0.46	0.30	0.30	0.27	0.22	0.12	0.15	0.13	0.16	0.11	0.12	0.19	0.10	0.18	0.11	0.25	0.17	0.11	0.11	0.31	0.13	0.11	0.11							
Benanee	BEN	0.34	0.11	0.28	0.29	0.32	0.52	0.36	0.36	0.33	0.23	0.09	0.08	0.10	0.09	0.06	0.05	0.12	0.04	0.11	0.06	0.20	0.13	0.06	0.08	0.24	0.05	0.05	0.05	0.11						
Riverina	RIV	0.38	0.22	0.34	0.36	0.38	0.55	0.38	0.38	0.33	0.22	0.07	0.10	0.09	0.11	0.07	0.09	0.15	0.08	0.11	0.07	0.20	0.09	0.07	0.06	0.37	0.11	0.10	0.07	0.14	0.07					
Centenary Reservoir	CEN	0.43	0.24	0.37	0.40	0.41	0.60	0.45	0.46	0.43	0.32	0.20	0.15	0.20	0.25	0.18	0.16	0.24	0.17	0.26	0.20	0.37	0.25	0.18	0.17	0.38	0.17	0.19	0.18	0.22	0.14	0.15				
Cardross Lakes	CAR	0.33	0.11	0.28	0.31	0.33	0.52	0.35	0.35	0.31	0.20	0.06	0.08	0.08	0.08	0.05	0.03	0.09	**0.01**	0.10	0.03	0.18	0.10	0.03	0.06	0.24	0.06	0.03	0.05	0.10	0.03	0.08	0.15			
Amphitheatre Reservoir	AMP	0.47	0.30	0.41	0.43	0.45	0.64	0.48	0.50	0.47	0.36	0.26	0.21	0.24	0.33	0.26	0.21	0.31	0.23	0.33	0.26	0.43	0.31	0.24	0.24	0.43	0.22	0.25	0.23	0.25	0.20	0.21	0.02	0.20		
Tahbilk Lagoon	GOU	0.43	0.25	0.41	0.42	0.46	0.63	0.44	0.47	0.43	0.30	0.20	0.18	0.19	0.23	0.17	0.15	0.24	0.14	0.26	0.16	0.32	0.25	0.17	0.18	0.34	0.12	0.14	0.14	0.23	0.14	0.20	0.27	0.16	0.34	
Wimmera	WIM	0.36	0.18	0.33	0.34	0.37	0.57	0.36	0.38	0.32	0.23	0.05	0.15	0.08	0.08	0.06	0.08	0.09	0.04	0.09	0.05	0.24	0.09	0.03	**0.02**	0.33	0.11	0.07	0.06	0.13	0.10	0.08	0.23	0.08	0.31	0.17

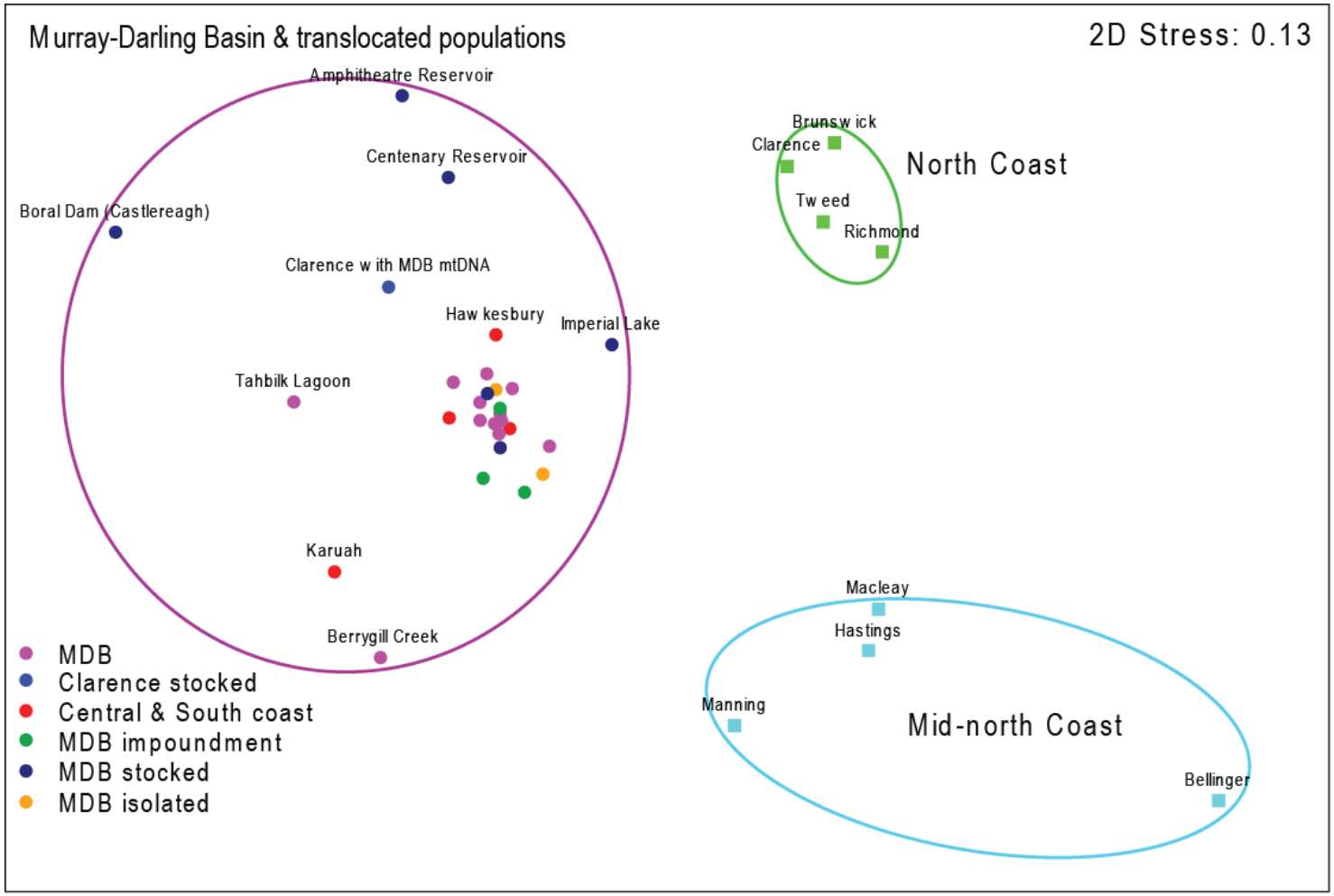

Figure 4.3. Multi-dimensional scaling ordination of pairwise F_{ST} for *T. tandanus* and *Tandanus* sp. 1 showing the three major population groups. Six of the seven fish from the Clarence displaying a MDB mtDNA haplotype (Chapter 3) are indicated as a separate population. The seventh fish had Clarence nuclear DNA and was therefore included in the Clarence group.

4.3.4. Population structure

Estimating the most likely value of *K* (the number of discrete genetic units amongst the populations sampled) was not straightforward. The initial *Structure* run indicated that the posterior probability [P(X|*K*)] was maximal at *K* = 19, while the Δ*K* estimate was maximal at *K* = 3 (Fig. 4.4). The method of Evanno *et al.* (2005) detects the uppermost hierarchical structure present in the data. This is evidently occurring in our data at *K* = 3, with *Structure* clearly dividing our samples into three groups with population membership values nearly all > 0.9; 1) populations from the NSW north coast, 2) *Tandanus* sp.1, and 3) populations from the MDB (including the translocated NSW central and south coast populations and six of the seven translocated samples from in the Clarence) (Fig 4.5). Therefore, we split the data set into these three subsets and ran *Structure* again using the original parameters and estimated Δ*K* for each group. Firstly, we removed the NSW north coast samples that possessed MDB mtDNA (Chapter 3) (likely translocated from the MDB) given that they clearly do not group with the other Clarence fish as evidenced by this analysis (Fig. 4.5) and the F_{ST} analysis (Table 4.2), and are likely to interfere with estimating the most likely value of *K*. For the NSW north coast, P(X|*K*) was maximal at *K* = 5, while the Δ*K* estimate was at *K* = 2 (Fig. 4.6 a). The results for the NSW mid-north coast showed that P(X|*K*) and Δ*K* were both maximal at *K* = 3 (Fig. 4.6 b). The MDB and NSW central and south coast populations had a maximal P(X|*K*) of 16 and a Δ*K* of *K* = 2 (Fig 4.6 c). Thus, with the exception of the NSW mid-north coast populations, the Δ*K* method of estimating the true number of *K* populations tends to indicate fewer genetic populations than suggested by P(X|*K*). In addition, two out of our three major groups continued to resolve genetic clusters that corresponded to sampled populations at higher values than Δ*K*. Therefore, we considered it appropriate to use maximal posterior probability estimates [P(X|*K*)] to examine the relationships between each population within the three major groups.

At *K* = 5, the NSW north coast was divided into five main groups, three that directly corresponded to the Clarence, Tweed and Brunswick catchments, each showing a degree of admixed individuals. The fourth and fifth group consisted of samples from the Richmond catchment (Fig. 4.7 a). There was no pattern to the sampling location of these two clusters, and there was not a high degree of admixture between the two clusters. Thus it is possible that there are translocated individuals in the Richmond from another catchment further north (SE Queensland) that was not included in this study.

Tandanus sp. 1 was divided into three well supported clusters, the Bellinger catchment and Manning catchment were identified as separate clusters at population membership values of $q > 0.97$, while the Macleay and Hastings catchments were allocated to a single cluster at a population membership values of $q > 0.94$. Higher values of *K* did not alter the pattern (Fig. 4.7 b). The populations of the MDB and NSW central and south coast showed a much higher degree of intra-population admixture than the other two groups (*K* = 16) (Fig. 4.7 c). Most natural MDB populations were not allocated strongly to any genetic cluster within the MDB (Condamine, Moonie, lower Border Rivers, Gwydir below Copeton Dam, Namoi above Keepit Dam, Namoi below Keepit Dam, Castlereagh, Lachlan, Macquarie above Burrendong Dam, Macquarie below Burrendong, Benanee and Murray Riverina), suggesting a high degree of gene-flow. The exceptions were the Berrygill Creek and Tahbilk Lagoon populations that each had population membership to their own genetic cluster at a high level ($q = 0.88$ and $q = 0.87$ respectively, Table 4.3). The populations above Tenterfield and Macintyre Falls also each had reasonably high population membership in separate genetic clusters ($q = 0.48$ and $q = 0.68$ respectively, Table 4.3). Seven fish from the Castlereagh population had very high individual *q* values ($q > 0.82$) allocating them to a single cluster. These fish were all collected at the same site, Boral Dam, which is a small dam at a quarry site and isolated from rivers and creeks in the Castlereagh catchment. It is not known where these translocated fish originated from. Thus, the population differentiation could be attributed to founder effects and subsequent inbreeding and genetic drift. The only above-dam

population to show substantial membership to one cluster was Copeton Dam (q = 0.79). Several translocated populations showed high membership to individual clusters not represented in any other population including the Karuah catchment (q = 0.80), Hunter catchment (q = 0.42) Hawkesbury catchment (q = 0.61), Shoalhaven catchment (q = 0.74), Imperial Lake (q = 0.76) and Centenary Reservoir and Amphitheatre Reservoir (to the same cluster, q = 0.73 and q = 0.88 respectively) (Table 4.3).

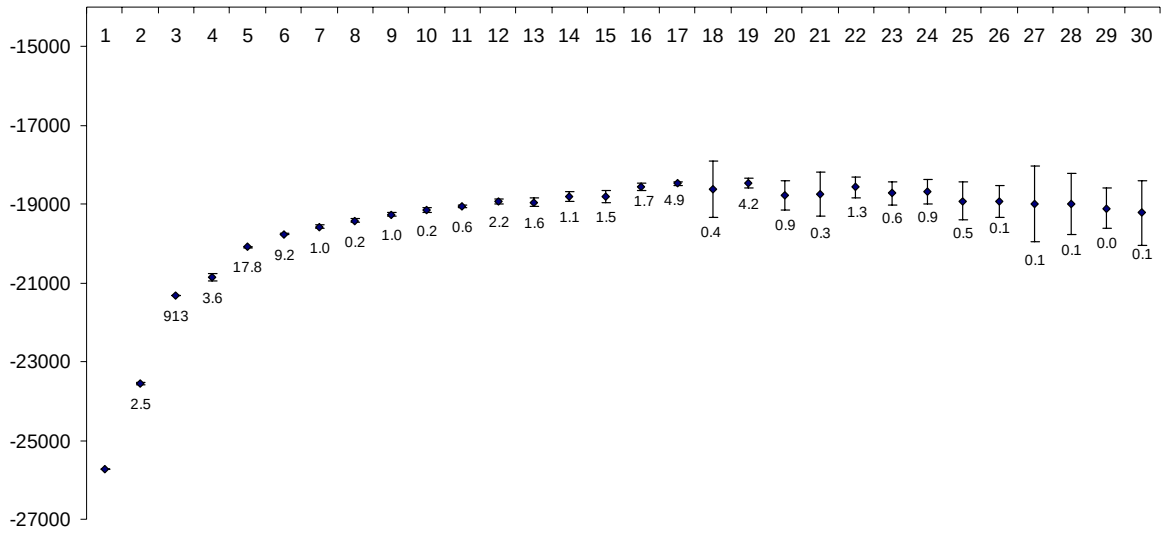

Figure 4.4. a Log probability LnP(X|K) for different numbers of genetic clusters K, calculated by Structure analysis for all populations. Points and error bars show the mean and standard deviation averaged across 5 runs at each K. ΔK values are shown as numerals within the figure.

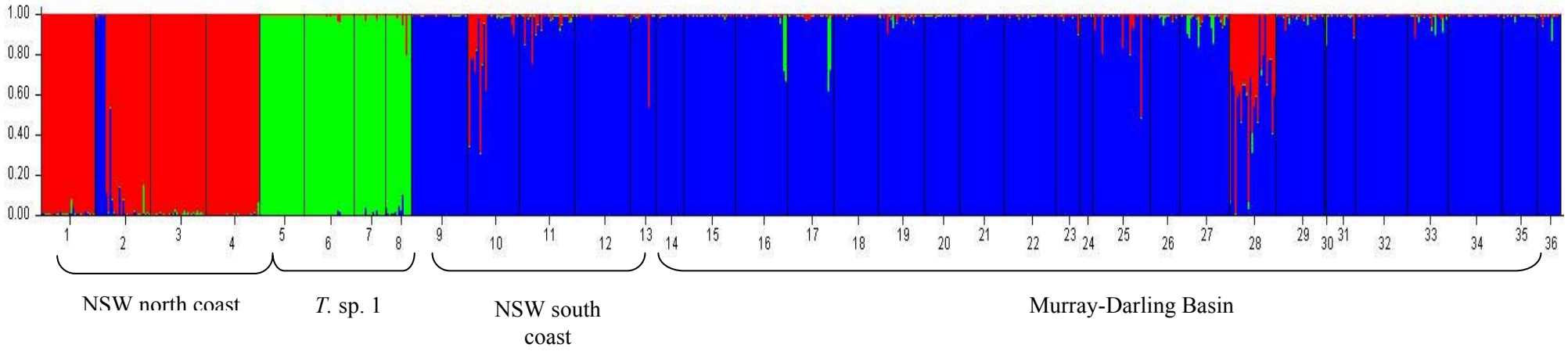

Figure 4.5. Population structure of *T. tandanus* and *Tandanus* sp. 1 as inferred by *Structure* across all populations in this study for $K = 3$ (max ΔK). Individuals are represented by vertical lines and population boundaries are defined by a thin vertical black line. Several samples from the Castlereagh population (Boral Dam, population 24) are also indicated. A single sample was collected from the Lower Murray population, and was subsequently grouped with the Murray Riverina population for all other analyses given it was genetically indistinguishable. Note that the first seven samples from the Clarence population are those with MDB haplotypes (Chapter 3). Population codes are as follows 1 Richmond, 2 Clarence, 3 Tweed, 4 Brunswick, 5 Bellinger, 6 Macleay, 7 Hastings, 8 Manning, 9 Karuah, 10 Hunter, 11 Hawkesbury, 12 Shoalhaven, 13 Condamine, 14 Moonie, 15 Above Macintyre Falls, 16 Above Tenterfield Falls, 17 Lower Border Rivers, 18 Above Copeton Dam, 19 Below Copeton Dam, 20 Berrygill Creek, 21 Above Keepit Dam, 22 Below Keepit Dam, 23 Castlereagh, 24 Boral Dam, 25 Lachlan, 26 Below Burrendong Dam, 27 Above Burrendong Dam, 28 Imperial Lake, 29 Benanee, 30 Lower Murray, 31 Murray Riverina, 32 Centenary Reservoir, 33 Cardross Lakes, 34 Amphitheatre Reservoir, 35 Tahbilk Lagoon, 36 Wimmera.

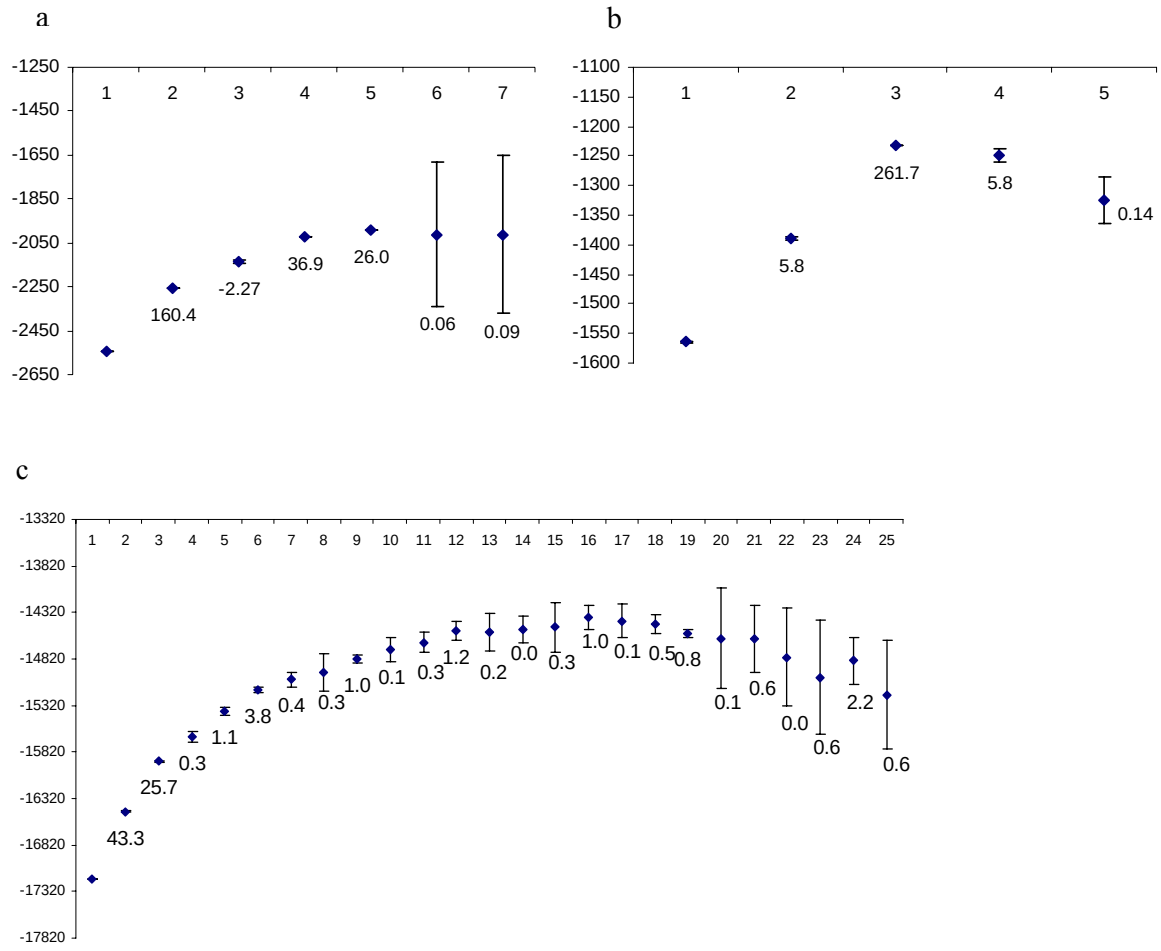

Figure 4.6. Log probability LnP(X|*K*) for different numbers of genetic clusters *K*, calculated by Structure analysis for a) NSW north coast populations, b) NSW mid-north coast populations and c) MDB and NSW central and south coast populations. Points and error bars show the mean and standard deviation averaged across 5 runs at each *K*. Δ*K* values are shown as numerals within the figure.

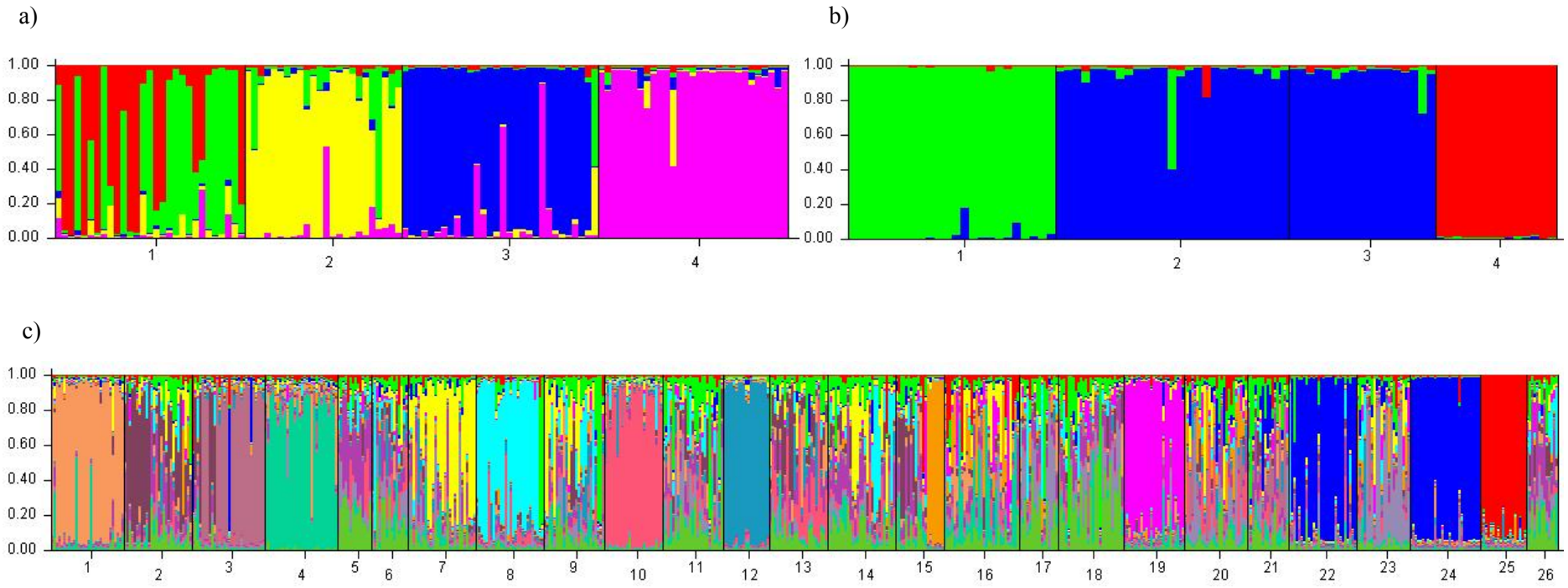

Figure 4.7. a) The four NSW north coast populations (*T. tandanus*) for the maximal value of P(X|*K*), *K* = 5. Population codes: 1, Richmond, 2, Clarence (samples with MDB mtDNA removed), 3, Tweed, 4, Brunswick. b) The four NSW mid-north coast populations (*Tandanus* sp.1) for the maximal value of P(X|*K*), *K* = 3. Population codes: 1 Bellinger, 2 Macleay, 3 Hastings, 4 Manning. c) The MDB and NSW central and south coast populations (*T. tandanus*) for the maximal value of P(X|*K*), *K* = 16. Population codes: 1 Karuah, 2 Hunter, 3 Hawkesbury, 4 Shoalhaven, 5 Condamine, 6 Moonie, 7 Above Tenterfield Falls, 8 Above Macintyre Falls, 9 Lower Border Rivers, 10 Above Copeton Dam, 11 Below Copeton Dam, 12 Berrygill Creek, 13 Above Keepit Dam, 14 Below, Keepit Dam, 15 Castlereagh, 16 Lachlan, 17 Below Burrendong Dam, 18 Above Burrendong Dam, 19 Imperial Lake, 20 Benanee, 21 Murray Riverina, 22 Centenary Reservoir, 23 Cardross Lakes, 24 Amphitheatre Reservoir, 25 Tahbilk Lagoon, 26 Wimmera.

Table 4.3. Proportion of membership of each population of *T. tandanus* from the NSW central and south coast and MDB populations assuming 16 genetic clusters inferred by Structure. Values > 0.45 for a population to an individual cluster are in bold.

Population	Cluster membership															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Karuah	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	**0.80**	0.01	0.01	0.02	0.01	0.01	0.06	0.01
Hunter	0.02	0.07	0.01	0.07	0.02	0.04	0.03	0.42	0.03	0.02	0.01	0.10	0.03	0.02	0.02	0.07
Hawkesbury	0.03	0.01	0.06	0.01	0.01	0.02	0.01	0.11	0.01	0.02	**0.61**	0.02	0.01	0.02	0.02	0.02
Shoalhaven	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.07	0.01	0.02	0.02	0.01	0.02	**0.74**	0.01
Condamine	0.03	0.05	0.01	0.03	0.02	0.06	0.01	0.04	0.01	0.03	0.01	0.30	0.08	0.10	0.02	0.21
Moonie	0.02	0.06	0.02	0.06	0.02	0.10	0.02	0.08	0.02	0.07	0.04	0.11	0.08	0.10	0.05	0.15
Tenterfield Falls	0.02	0.04	0.02	**0.48**	0.01	0.02	0.01	0.02	0.02	0.03	0.03	0.08	0.03	0.09	0.05	0.06
Macintyre Falls	0.02	0.08	0.01	0.01	0.01	**0.68**	0.01	0.02	0.01	0.02	0.01	0.02	0.06	0.02	0.01	0.02
Border	0.02	0.14	0.02	0.21	0.03	0.12	0.02	0.06	0.04	0.07	0.03	0.06	0.03	0.07	0.05	0.07
Above Copeton	0.01	0.01	0.01	0.02	0.01	0.03	0.01	0.01	0.01	0.04	0.02	0.01	**0.79**	0.01	0.01	0.01
Below Copeton	0.02	0.19	0.02	0.04	0.02	0.07	0.01	0.08	0.06	0.03	0.05	0.07	0.05	0.12	0.05	0.12
Berrigill Creek	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	**0.89**	0.01	0.01	0.01	0.01	0.01	0.01
Above Keepit Dam	0.01	0.10	0.02	0.05	0.01	0.03	0.01	0.17	0.08	0.07	0.01	0.09	0.20	0.04	0.03	0.08
Below Keepit Dam	0.02	0.10	0.02	0.16	0.01	0.14	0.02	0.07	0.03	0.02	0.02	0.15	0.05	0.05	0.04	0.12
Castlereagh	0.04	0.06	0.02	0.02	0.04	0.04	0.33	0.14	0.04	0.01	0.02	0.11	0.01	0.02	0.04	0.06
Lachlan	0.08	0.04	0.02	0.11	0.10	0.01	0.11	0.03	0.07	0.02	0.02	0.06	0.02	0.07	0.19	0.06
Below Burrendong Dam	0.03	0.11	0.01	0.04	0.02	0.11	0.05	0.03	0.03	0.02	0.02	0.09	0.02	0.21	0.03	0.18
Above Burrendong Dam	0.03	0.14	0.02	0.03	0.03	0.05	0.01	0.04	0.03	0.02	0.03	0.13	0.02	0.12	0.05	0.27
Imperial Lake	0.01	0.01	0.02	0.02	**0.76**	0.01	0.01	0.01	0.03	0.01	0.01	0.02	0.01	0.02	0.04	0.02
Benanee	0.04	0.07	0.05	0.08	0.04	0.03	0.14	0.02	0.01	0.04	0.09	0.06	0.11	0.13	0.03	0.07
Riverina	0.03	0.16	0.10	0.03	0.04	0.02	0.02	0.04	0.04	0.03	0.13	0.11	0.05	0.08	0.04	0.09
Centenary Reservoir	0.01	0.03	**0.73**	0.01	0.01	0.05	0.01	0.01	0.01	0.01	0.02	0.03	0.02	0.02	0.01	0.03
Cardross Lakes	0.02	0.05	0.04	0.08	0.05	0.05	0.08	0.02	0.01	0.04	0.06	0.04	0.03	0.36	0.03	0.03
Amphitheatre	0.01	0.01	**0.89**	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01
Tahbilk Lagoon	**0.87**	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Wimmera	0.04	0.08	0.01	0.03	0.04	0.09	0.02	0.08	0.03	0.01	0.03	0.22	0.02	0.08	0.08	0.15

4.3.5. Assignment tests

Assignment tests for the Clarence, Richmond, Tweed and Brunswick confirmed that six of the seven translocated individuals from the Clarence population were assigned to the Border Rivers catchment with probabilities $P > 0.05$. The seventh individual with a MDB mtDNA haplotype however, was assigned to the Clarence based on its microsatellite genotype. A single individual from the Brunswick population was assigned to the Clarence population. The four NSW central and south coast populations were all overwhelmingly assigned to MDB populations, apart from a single fish from the Hunter assigned to the Clarence population (Table 4.4). This is consistent with the mtDNA evidence for these populations being established from the MDB. The majority of the central and south coast populations were assigned to three MDB populations, the Border Rivers catchment, the Gwydir catchment below Copeton Dam and the Namoi catchment below Keepit Dam (Table 4.4). Four of the Hunter samples fell below the assignment threshold ($P = > 0.05$) and were therefore unable to be assigned to any population.

Table 4.4. Percentage of NSW central and south coast fish assigned to MDB and NSW north coast populations.

Assigned to	**Central and south coast populations**			
	Karuah (30 individuals)	Hunter (28 individuals)	Hawkesbury (30 individuals)	Shoalhaven (30 individuals)
Clarence		3.6		
Moonie			6.7	3.3
Above Tenterfield Falls	3.3			6.7
Lower Border Rivers	20.0	21.4	23.3	53.3
Gwydir catchment below Copeton Dam	36.7	21.4	23.3	13.3
Namoi catchment above Keepit Dam			3.3	
Namoi catchment below Keepit Dam	33.3	28.6	16.7	10.0
Castlereagh	6.7	3.6	6.7	
Lachlan				6.7
Macquarie catchment below Burrendong Dam			13.3	
Macquarie catchment above Burrendong Dam		3.6		6.7
Darling		3.6		
Benanee			3.3	
Murray Riverina			3.3	
Not assigned ($P = < 0.05$)		14.3		

4.3.6. Population bottleneck

There was no evidence of population bottlenecks in the five groups tested (NSW north coast, *Tandanus* sp. 1, MDB, stocked MDB and NSW central and south coast, $P = > 0.05$). However, there was a significant heterozygosity deficiency in four of the five groups (NSW north coast, *Tandanus* sp. 1, MDB and stocked MDB, $P = 0.009$, $P = 0.027$, $P = 0.004$ and $P = 0.002$ respectively), which may indicate either an influx of rare alleles from a divergent population, or a recent population expansion (Maruyama and Fuerst 1985; Cornuet and Luikart 1996).

4.3.7. Effective population size

The long-term estimates of effective population size across all populations under the SMM ranged from N_e = 1,481 to 2,667, while the IAM estimates were lower, ranging from 816 to 1,207 (Table 4.5). The most accurate long-term N_e estimates are likely to be those produced by the SMM given that microsatellites typically conform to this model (Zhivotovsky and Feldman 1995). The two methods utilised to estimate contemporary N_e were over an order of magnitude lower than long-term estimates ranging from 29.8 to 203.4 for NeEstimator and 6.0 to 318.6 for LDNE. The LDNE estimates for the un-stocked populations were higher than those of NeEstimator, but were much lower for the two stocked populations (NSW central and south coast and MDB stocked) (Table 4.5). Confidence intervals (95%) could not be placed around the LDNE estimate for the NSW mid-north coast estimate of 42.4 (14.4 – infinity), potentially because the sample size was less than 90 (Bartley *et al.* 1992). Regardless of the method used, the MDB populations historically had the largest N_e, but estimates are now substantially lower. The NSW north coast and NSW mid-north coast have suffered much more serious reductions in N_e. Contemporary estimates of N_e for the stocked fish in the MDB and NSW coastal populations are not dissimilar to those from natural populations elsewhere, ranging from 6.0 to 33.7 and 16.6 to 34.3 respectively.

Table 4.5. Estimates of long-term effective population size N_e, for three groups of populations based on expected heterozygosity, H_E, using a stepwise-mutation model, SMM, and an infinite-allele model, IAM, and contemporary effective population size estimated from two linkage disequilibrium methods in NeEstimator and LDNE. Number of individuals, N.

Population	**N**	**H_E**	**Long-term N_e**		**Contemporary N_e (95% CI)**	
			SMM	**IAM**	**NeEstimator**	**LDNE**
NSW north coast	118	0.67	2,102	1,034	31.5 (28.7 – 34.7)	61.1 (31.8 – 206.0)
Tandanus. sp. 1	82	0.62	1,481	816	29.8 (25.8 – 34.7)	42.4 (14.4 – infinity)
NSW central and south coast (stocked)	118	0.64	1,734	908	34.3 (30.4 – 38.7)	16.6 (13.2 – 20.6)
MDB	386	0.71	2,667	1207	203.4 (186.3 – 222.8)	318.6 (168.2 – 1204.4)
MDB (stocked)	117	0.66	1,938	979	33.7 (31.0 – 36.6)	6.0 (4.0 – 7.7)

4.4. Discussion

4.4.1. Population structure and genetic diversity

Microsatellite analysis of 30 *T. tandanus* and four *Tandanus* sp. 1 populations sampled from a broad geographic area has confirmed the three genetically divergent groups identified in Chapter 3: a MDB *T. tandanus* group containing all MDB populations and translocated populations on the NSW central and south coast, a NSW north coast *T. tandanus* group, and a *Tandanus* sp. 1 group.

4.4.2. MDB group

Although populations of *T. tandanus* within the MDB were nearly all significantly genetically differentiated based on F_{ST} analysis, the differences among populations were small (most $F_{ST} < 0.10$) and there was little or no pattern of relationships within the MDB. As expected, the greatest level of genetic differentiation among populations within the MDB was in those that were established from translocations, isolated by dams or waterfalls, or in small waterways with limited connectivity to a major river (Tahbilk Lagoon and Berrygill Creek). Thus, microsatellite data largely concurs with allozyme data (Keenan *et al.* 1995), where a single panmictic population of *T. tandanus* was identified in interconnected rivers below dams, while above-dam populations were usually genetically differentiated. Historically, *T. tandanus* populations in the MDB most likely exhibited very little genetic differentiation, with the exception of a few small populations isolated by natural barriers. This is in stark contrast to the high level of genetic structuring evident over a very limited geographical scale in coastal populations of *T. tandanus* and *Tandanus* sp. 1, which typically showed greatly reduced gene-flow among populations.

There was a relatively high level of genetic diversity in the un-stocked MDB populations (H_E = 0.62 ± 0.09) (marginally higher than a published average for freshwater fishes (H_E = 0.54 ± 0.25) (DeWoody and Avise 2000). Furthermore, no recent genetic bottleneck could be detected within the MDB, despite the substantial decline of *T. tandanus* throughout much of the basin. However, there was a substantial reduction in the contemporary estimate of N_e [N_e = 318.6 (168.2 – 1204.4)], down from long-term estimates of as high as N_e = 2,667 (Table 4.5). Despite the decline, the contemporary N_e estimate is consistent with the median N_e estimate for natural populations of N_e = ~260 (based on 83 studies using the temporal method) (Palstra and Ruzzante 2008), and highly consistent to the estimate for Murray cod in the southern MDB [N_e = 322 (195 – 828)] (Rourke *et al.* 2009). Nevertheless, *T. tandanus* N_e is still substantially lower than the estimate of N_e = 500 required for populations to retain evolutionary potential (Franklin 1980; Franklin and Frankham 1998). The stocked MDB populations had far more drastic reductions in N_e as expected for introduced populations [N_e = 6.0 (4.0 – 7.7)].

4.4.3. NSW north coast

A previous study of allozyme loci suggested that the Nymboida River population (Clarence catchment) is a separate subspecies of *T. tandanus* (Musyl and Keenan 1996) and further allozyme work tentatively extended the range of this sub-species to the Tweed and Richmond Rivers (Jerry and Woodland 1997). A later mtDNA study by Jerry (2008) did not recognise this sub-species, but instead grouped all MDB, NSW north coast and south-east Queensland populations sampled into a single clade. In contrast, mtDNA data reported in Chapter 3 shows that the NSW north coast should be recognised as a separate clade. The apparent sharing of haplotypes with the MDB reported by Jerry (2008) may have been due to the presence of translocated fish in his samples. Our microsatellite data supports this assertion and also shows that the Brunswick population should be grouped with the Richmond, Clarence and Tweed populations, and that this complex is

significantly different from the MDB. Furthermore, there is restricted gene-flow among these four catchments, with F_{ST} ranging from 0.09 between the Clarence and Brunswick, to 0.24 between the Richmond and Tweed. Although there was evidence of some first generation immigrants into the Clarence, Tweed and Brunswick from other north coast populations, the degree of genetic structuring among the four NSW north coast populations indicates limited gene-flow. This is also highly consistent with mtDNA data that show no shared haplotypes across catchment boundaries (Chapter 3).

Whether the NSW north coast populations qualify as a separate sub-species or species is a difficult question given that no morphological differentiation has been detected (Musyl 1990; Musyl and Keenan 1996). However, the north coast populations are genetically almost as different from the MDB as the undescribed species based on F_{ST} analysis, and have been isolated from the MDB for ~186 ky. They possess mtDNA haplotypes not found anywhere else (when translocated fish are excluded, see discussion below). Based on their allozyme analysis, Musyl (1990) and Musyl and Keenan (1996) suggested the north coast populations were genetically differentiated at least to the level of sub-species. The results of the present study support this suggestion that NSW north coast populations of *T. tandanus* represent an additional undescribed sub-species and perhaps even a separate species.

4.4.4. Tandanus sp. 1

We have presented further evidence that the NSW mid-north coast catchments (Bellinger, Macleay, Hastings and Manning) are occupied by a distinct species *Tandanus* sp. 1 (Willung). This species is strongly genetically divergent from all other populations, thus supporting previous allozyme and mtDNA evidence (Musyl 1990; Musyl and Keenan 1996; Jerry and Woodland 1997; Jerry 2008, Chapter 3). Estimates from mtDNA nucleotide divergence places the estimated date of divergence of *Tandanus* sp. 1 and the NSW north coast at ~1.29 mya and *Tandanus* sp. 1 and the MDB at ~1.64 mya (Chapter 3). Although *Tandanus s*p.1 is reportedly morphologically indistinguishable from *T. tandanus* (Musyl and Keenan 1996), it has been reported that 93% of nests constructed in the Bellinger River are a "U" shape, while only 7% are circular as is typical for populations in the MDB (Bishop, pers. comm. as cited in Musyl and Keenan 1996). However, recent observations by one of the authors (DG) questions this observation as all nests he observed in the Hastings and Macleay Rivers were circular. Not only is *Tandanus* sp.1 highly divergent from *T. tandanus*, there is a striking level of genetic differentiation among most populations, with three strongly supported genetic clusters identified: one represented by the Bellinger population, one by the Manning population and a third by the combined Macleay and Hastings populations. Previous mtDNA data did not reveal the extent of this population subdivision, as haplotypes were shared among all populations except for the Manning (Jerry 2008, Chapter 3). Similarly, allozyme data were unable to distinguish the four populations and led the authors to conclude high gene-flow among populations (Jerry and Woodland 1997). Our data suggest that gene-flow is far more restricted within this species and has been so for a considerable amount of time. The level of genetic diversity of *Tandanus* sp.1 populations ($H_E = 0.43 \pm 0.13$) was significantly lower than that observed in the MDB. The reduced heterozygosity was largely driven by the Bellinger population, which was monomorphic for four of seven loci that amplified. Thus the combination of isolation and small effective population size may have resulted in a loss of alleles in this population (Frankham *et al.* 2002). Given the loss of heterozygosity and that N_e had declined markedly to 42.4 (14.4 – infinity), it was interesting that no genetic bottleneck was detected in *Tandanus* sp. 1. While confidence intervals could not be placed around the contemporary N_e estimate for *Tandanus* sp.1, the true value is likely to be low given the consistency with the estimate from NeEstimator [29.8 (25.8 – 34.7)]. Given that the long-term historical N_e for *Tandanus* sp. 1 was as high as 1,481, there are concerns for the long-term persistence of *Tandanus* sp.1. Such a low N_e exposes the population to stochastic genetic factors such as inbreeding and genetic drift, making it susceptible to future loss of genetic diversity and ultimately increasing its chance of extinction (Frankham *et al.* 2002).

4.4.5. Translocation history in the NSW central and south coast and Clarence populations

A suite of genetic analyses provided additional evidence that all four central and south coast populations (Karuah, Hunter, Hawkesbury and Shoalhaven) are more genetically related to the MDB than any coastal population, thus verifying their origin from MDB fish (Chapter 3). However, the exact MDB catchment of origin could not be determined, but was largely a mix of the lower Border Rivers, Gwydir catchment below Copeton Dam and the Namoi catchment below Keepit Dam. Despite presumably being derived from relatively few translocated fish and exposed to a demographic bottleneck during their establishment, these populations have not lost a significant amount of genetic diversity in comparison to non-stocked MDB populations. This, combined with the retention of genetic diversity, may indicate that the initial introductions captured a substantial amount of the original genetic diversity, and that the newly established populations underwent a rapid population expansion in the unoccupied habitats (Zenger *et al.* 2003). Nevertheless, N_e estimates were extremely low [N_e = 16.6 (13.2 – 20.6)], indicating the populations have not completely escaped the negative genetic implications of being established from a low number of founders. At establishment, each population would have had different allele frequencies due to chance alone (Frankham *et al.* 2002). The lack of homogenisation of these catchments suggests that there is minimal gene-flow among them, similar to the situation in northern coastal catchments.

Previous mtDNA analysis revealed shared haplotypes between the MDB and Clarence populations, which was potentially due to either river capture that enabled gene-flow across the GDR, or to recent translocations (Jerry 2008, Chapter 3). The microsatellite data provide additional support to the findings in Chapter 3 and confirms that the shared haplotypes were due to translocations of MDB fish, most likely from the Border Rivers catchment. Further, six out of the seven MDB fish identified showed no evidence of hybridisation between Clarence and MDB fish. The lack of substantial introgression despite several introductions is intriguing. It is possible that the Clarence fish display "immigrant inviability", whereby strong reproductive barriers exist between divergent populations, thus restricting gene-flow (Nosil *et al.* 2005). Alternatively, crosses of divergent populations can result in severe outbreeding depression, whereby co-adapted gene complexes are broken down, potentially leading to reduced fitness in the F1 generation (Hallerman 2003). Thus, our data suggest early introductions probably failed, and the majority of MDB fish identified here are the result of far more recent introductions from the lower Border Rivers catchment.

4.4.6. Biogeography of coastal and MDB catchments

The GDR is clearly a significant barrier to contemporary gene-flow of *T. tandanus* between coastal and inland catchments given the presence of an undescribed species (*Tandanus* sp. 1) and possibly an undescribed species or subspecies in the NSW north coast drainages. The GDR in the region of the current study is also a barrier to gene-flow in several other fish genera including *Maccullochella* (Rowland 1985; Rowland 1993), *Mogurnda* (Faulks *et al.* 2008), *Retropinna* (Hammer *et al.* 2007), *Hypseleotris* (Thacker *et al.* 2007), *Philypnodon* (Thacker *et al.* 2008) *Macquaria* (Dufty 1986; Faulks *et al.* 2009; Faulks *et al.* 2010) and *Melanotaenia* (Crowley *et al.* 1986). Furthermore, most populations of *T. tandanus* and *Tandanus* sp.1 on the NSW coast are genetically divergent from one another, which is most likely a result of the general lack of connectivity between rivers that drain into the ocean. Even during times of very low sea levels (500 m below current levels), only 20 – 40 km of continental shelf along coastal NSW is exposed, thus leading to little change to current drainage patterns (Unmack 2001). This pattern of restricted gene-flow among coastal catchments is not consistent with other species, suggesting that the particular life history of *Tandanus* may play an important role in a species' ability to overcome potential barriers to dispersal. For example, the euryhaline Pacific blue-eye (*Pseudomugil signifier*) is tolerant of high salinities, and displays little population structure from northern to southern NSW, and is thought to move between adjacent drainages when large freshwater flood plumes extend into

the ocean (Wong *et al.* 2004). There has been limited assessment of the salinity tolerance of *T. tandanus*, but Ryan *et al.* (1999) report that the species is quite tolerant to high salinities. However, the virtual lack of gene-flow among coastal populations suggests that the species does not regularly disperse among catchments in this way. Thus, where gene-flow is present, such as between the Macleay and Hastings populations, it is more likely to be due to dispersal during large flood events that may allow connectivity of small streams on the floodplain (Jerry and Woodland 1997).

Most of the un-stocked populations within the MDB displayed minimal genetic differentiation, even over very large distances. This is clearly due to the interconnected nature of rivers within the MDB, most of which ultimately coalesce in the Murray River. This is an interesting result given that *T. tandanus* are considered to be largely non-migratory. A tagging study found that up to 60% of fish do not move from the tagging area and only a single fish moved more than 10 km (Reynolds 1983). A more recent study of tagged fish (acoustic and radio tags) in two tributaries of the Clarence River also indicated limited movement of < 15 km in either an upstream or downstream direction (Gavin Butler, Industry and Investment NSW, pers. comm.). Very little is known about the movements of larval and juvenile fish, but juveniles are thought to move away from their natal site to colonise nearby habitat (Reynolds 1983). Thus, the movement of *T. tandanus* at all life stages is apparently low enough to promote small but significant F_{ST} differences among populations, but high enough to prevent strong genetic structure from developing even over thousands of river kilometres. Nevertheless, the presence of gene-flow does not necessarily preclude local adaptation (Conover *et al.* 2006), which needs to be taken into account for future management programs.

4.4.7. Management implications for future stocking programs

Previous work on mtDNA (Chapter 3) indicated that *Tandanus* sp. 1 and the undescribed species or subspecies from the NSW north coast should be designated as evolutionary significant units (ESU), and that each catchment within these ESU be further delineated into management units (MU) based on significant differences in mtDNA haplotypes. The microsatellite data presented here confirm the distinctiveness of *Tandanus* sp. 1 and the NSW north coast populations, and suggest that each coastal catchment be promoted to separate ESU status given the degree of microsatellite differentiation (Moritz 1994; Fraser and Bernatchez 2001). The only exception was the Hastings and Macleay populations, which were genetically indistinguishable and therefore comprised of a single ESU. Further support for the designation of these ESU could be obtained by conducting experiments for ecological exchangeability (the ability of fish from one population to survive in the habitat of another population), thus also fulfilling the ESU criteria of Crandall *et al*. (2000).

MtDNA data (Chapter 3) suggested that the MDB was comprised of a single ESU, but no further populations could be identified as MU. The microsatellite data presented here show there are genetic differences among some populations within the MDB (e.g., Berrygill Creek, Tahbilk Lagoon and Copeton Dam). However, these differences are likely a result of inbreeding and/or genetic drift following severe population fragmentation. The only naturally divergent populations within the MDB detected by this study were those isolated above major waterfalls (Tenterfield Falls and Macintyre Falls). Therefore, the MDB consists of a single ESU, which is further divided into three MU, one corresponding to the population above Tenterfield Falls, a second above Macintyre Falls, and a third comprising all other populations within the MDB.

To help minimise negative genetic effects that can occur from mixing divergent populations, we recommend that future stocking programs collect broodfish from the specific ESU and MU to be supplemented (Hindar *et al.* 1991; Hallerman 2003). Further, the number of broodfish should be maximised, and variance in family size minimised in order to promote high genetic diversity and N_e of the stocked populations (Allendorf 1993; Miller and Kapuscinski 2003; Theodorou and Couvet 2004; Rourke *et al.* 2009). Despite the presence of a single ESU in the MDB, we suggest that fish

not be stocked into areas large distances from the origin of the broodfish in order to avoid the breakdown of potential local adaptations. While it may be argued that the interbreeding of two MUs may be beneficial by alleviating the inbreeding depression that may have occurred in isolated populations (Madsen *et al.* 1999; Hallerman 2003), there is no way of predicting a priori the result of such interbreeding. The presence of local adaptations in the two above-waterfall MUs is equally as likely to result in outbreeding depression, potentially resulting in the loss of the MU (Hallerman 2003; Hughes *et al.* 2003). While stocking with hatchery-bred fish is a controversial issue (Meffe 1992; Waples 1999), it nevertheless will become increasingly important as fish species continue to decline and where there is little hope of natural recovery in areas where a species is locally extinct. By adopting a genetically responsible approach to stocking, potential genetic hazards associated with stocking can be minimised.

5. SYNTHESIS AND RECOMMENDATIONS

5.1. Identifying genetic populations

Genetic data are increasingly being utilised to prioritise populations for conservation by identifying those that possess a substantial amount of genetic diversity, and/or are genetically distinct or represent an undescribed species or subspecies (Rowland 1993; Briscoe and Tait 1995; Faulks *et al.* 2008). However, there is no single widely accepted method for identifying populations for conservation based on genetic data. Moritz (1994) suggested that a population that was reciprocally monophyletic at mitochondrial loci and significantly divergent at nuclear loci should be classified as a 'Evolutionary Significant Unit' (ESU) – a population or group of populations that are historically isolated. Such isolated populations are likely to be adapted to their local environment, giving them a fitness advantage over unrelated individuals from different populations. Moritz (1994) also recognises the 'Management Unit' (MU), a term applied to a population or group of populations that are not reciprocally monophyletic at mitochondrial loci, but still possess significant divergence at nuclear or mitochondrial loci. In short, a MU is more concerned with contemporary genetic structure and therefore short-term management issues (Moritz 1994).

Crandall *et al.* (2000) criticised the definition of Moritz (1994) because it was unlikely to detect ESUs in species that experience a high level of gene flow among populations, but still possess critical adaptive differences (Larsen *et al.* 2007). Instead, they advocated that an ESU be defined based on a combination of genetic exchangeability (genetic divergence) and ecological exchangeability (i.e., the ability for individuals from one population to successfully survive the same ecological niche of another population), over recent and historical timeframes. A review by Fraser and Bernatchez (2001) concluded that no single definition is applicable to all situations, given that the success of each approach will depend on the species and circumstances in question, and instead advocated a flexible approach. Here we apply the approach of Fraser and Bernatchez (2001) in the designation of ESUs and MUs for freshwater catfish of the genus *Tandanus* in south eastern Australia.

5.1.1. ESU and MU: MDB

Tandanus tandanus in the MDB are reciprocally monophyletic for mtDNA haplotypes (Chapter 3) and divergent from all natural coastal populations at nuclear loci (Chapter 4), thus it is appropriate to designate the entire MDB population as a single ESU distinct from coastal populations. Within the MDB, microsatellite analysis was able to distinguish significant differences in allele frequencies among nearly all MDB catchments, albeit at a low level. This indicates that gene flow is at least partially restricted among catchments, although it occurs at a high enough rate to ensure general homogenisation of genetic structure. The most divergent populations were those in stocked (Imperial Lake, Boral Dam, Centenary Reservoir, Amphitheatre Reservoir and the Wimmera River) and isolated or semi-isolated populations (Gwydir catchment upstream of Copeton Dam, above Tenterfield Falls, above Macintyre Falls, Berrygill Creek and Tahbilk Lagoon). There were only small differences between interconnected riverine populations (most comparisons $F_{ST} < 0.10$). We presume that prior to the collapse of *T. tandanus* populations over large areas of the MDB, the Berrygill Creek and Tahbilk Lagoon populations were likely to have been consistent with the general population of the MDB. Similarly, prior to the construction of Copeton Dam on the Gwydir River in 1976, the *T. tandanus* population in the upper Gwydir catchment was unlikely to have been divergent from the contiguous *T. tandanus* population across the rest of the MDB. The level of contemporary differentiation of these populations from riverine populations elsewhere in the MDB may be solely due to inbreeding and genetic drift since isolation. We therefore recommend

that these three populations be included in the same MU as the general MDB population. The only naturally isolated populations sampled in the present study were those above Tenterfield Falls and Macintyre Falls in the Border Rivers catchment. We suggest each of these isolated populations be designated a separate MU given that they are naturally isolated and are likely to have been so for an evolutionarily significant period of time sufficient to have lead to acquired characters unique to the respective populations. Neither MU should be stocked with fish from downstream populations. However, given that both populations are currently secure from many of the threatening processes affecting *T. tandanus* populations elsewhere in the MDB, there is no current need for stocking activities in these populations anyway.

5.1.2. ESUs: NSW coast

There is far more genetic differentiation in freshwater catfish among the eight catchment-specific native coastal populations. While reciprocal monophyly could not be demonstrated for any of the eight individual populations within either willung (*Tandanus* sp.1) or the north coast species/sub-species of freshwater catfish (*Tandanus tandanus*), possibly due to insufficient sampling, most coastal populations possessed unique mtDNA haplotypes. This, coupled with a high degree of nuclear genetic differentiation among populations indicates that there is highly restricted gene flow among most coastal catchments. Consequently, most of the coastal populations (catchments) should be recognised as separate ESUs (Tweed, Brunswick, Richmond, Clarence, Bellinger and Manning) (Fraser and Bernatchez 2001). The only exception is that the Macleay and Hastings catchments, which are not genetically differentiated and belong to the same ESU. It may be argued that these coastal populations may be more appropriately referred to as MU, given the lack of reciprocal monophyly (which may be demonstrated if more samples were sequenced). However, we argue that there is ample evidence for long-term isolation of each population, and as such they fulfil the flexible criteria of Frazer and Bernatchez (2001) for ESU.

5.2. Broodfish genetic zones

One of the critical aspects of stocking wild populations with hatchery-bred fish is ensuring that stocked fish are as similar as possible to the wild population. Specifically, the stocked fish must be genetically similar, have the same life history and have originated from a similar ecological environment (Miller and Kapuscinski 2003). In NSW, the genetic structure of stocked populations of freshwater fish has been considered in the preparation of the Hatchery Quality Assurance Scheme (HQAS) (NSW DPI 2008). The HQAS provides accreditation for NSW hatcheries that produce Murray cod, golden perch, silver perch and Australian bass for harvest or conservation stocking to ensure that wild populations are stocked appropriately. The program implements the essential and recommended criteria for all aspects of keeping these fish in captivity, including genetic considerations, as outlined in the Hatchery Quality Assurance Program (HQAP) (Rowland and Tully 2004). The HQAP specifies the number of broodfish for each species that must be collected from the population or genetic strain to be stocked to ensure they retain their genetic identity and genetic diversity. While *T. tandanus* are not included under the HQAS at this stage, future versions of the HQAS should include *T. tandanus* if a wide scale stocking program is implemented as recommended by the Recovery Plan (Clunie and Koehn 2001a).

5.2.1. Genetic zones: Murray-Darling Basin

Even though we identified only a single ESU within the MDB, the presence of minor genetic differentiation at neutral microsatellite loci can be indicative of the potential for local adaptations (Larsen *et al.* 2007; Larsen *et al.* 2008). Given the very large geographic distances among populations within the MDB, and the diversity of habitat types, this is likely to be the case for *T. tandanus* in the MDB. Accordingly, we recommend that the MDB be divided into multiple broodfish genetic zones in order to protect these potentially locally adapted populations from being

stocked with maladapted fish. The existing broodfish zones in the HQAS for golden perch splits the basin into three zones that roughly correspond to the northern (Darling and tributaries), central (Lachlan) and lower MDB (Murray and tributaries). We recommend that these same zones be adopted for *T. tandanus* management. We also recommend that the populations above Tenterfield Falls and Macintyre Falls not be stocked given their genetic divergence from other MDB populations and because they still support relatively abundant populations that are successfully recruiting (Fig. 5.1).

5.2.2. Genetic zones: NSW coast

The genetic analyses unequivocally showed that each coastal catchment is comprised of a distinctive ESU, with the exception of the Macleay and Hastings catchments, which were genetically indistinguishable. Consequently, we recommend that each ESU be recognised as a separate broodfish zone (Fig. 5.1), if stocking is ever considered for coastal catchments.

5.2.3. Sourcing broodfish for captive breeding programs

The presence of several *T. tandanus* populations of either translocated or stocked origin poses a dilemma for managers of future stocking programs. These populations represent a potential source of broodfish for future stocking programs within the MDB. However, genetic analysis has found that some of these populations possess very low genetic diversity and/or low N_e, indicating that they experienced a genetic bottleneck upon establishment from relatively few founders and most have undergone genetic differentiation from their original source populations (e.g., central and south coast *T. tandanus*).

Our microsatellite data suggests that the relatively abundant translocated populations in the Karuah, Hunter, Hawkesbury and Shoalhaven catchments were most probably established from fish translocated from the Namoi, Gwydir and lower Border Rivers catchments (Table 4.4). This is consistent with the report of Harris and Battaglene (1990) that the Hunter population was founded from *T. tandanus* sourced from the Namoi catchment. However, the Hawkesbury River population was reportedly established from Macquarie River *T. tandanus*. Regardless, none of the NSW central or south coast populations represent critical repositories of genetic diversity from the most endangered management unit in the southern Murray-Darling Basin: The southern and central MDB MUs. So the risks of utilising these populations as sources of broodfish or translocates probably outweigh the benefits.

Similarly, isolated remnant or translocated populations in the southern MU of the Murray-Darling Basin: Tahbilk Lagoon, Centenary Reservoir and Amphitheatre Reservoir, have relatively lower genetic diversity and have diverged significantly from remaining populations through founder effects and/or drift. These populations should not be used as the source of broodfish for stocking in the southern MDB MU. However, the translocated Wimmera catchment population, the remnant population in the Murray River and Washpen Creek, Cardross Lakes and the stocked Colombo Creek population all provide suitable sources of broodfish for a conservation stocking program in the Southern Murray-Darling Basin. The Victorian populations in lakes and dams mentioned above originated from fish sourced from the lower MDB (Clunie and Koehn 2001b). Consequently, they may retain alleles lost from the remaining natural populations. Therefore, they still serve a purpose as a potential source of broodfish for stocking programs in the southern MU, provided they are only ever crossed with fish from a remnant population. Crossing a broodfish from an impounded population with a broodfish from a more genetically diverse remnant population can bring about a genetic rescue, whereby genetic diversity of progeny can be increased, and inbreeding decreased (Madsen *et al.* 1999; Frankham *et al.* 2002; Tallmon *et al.* 2004). However, we emphasise that such populations must be crossed with wild broodfish from the same broodfish genetic zone to ensure

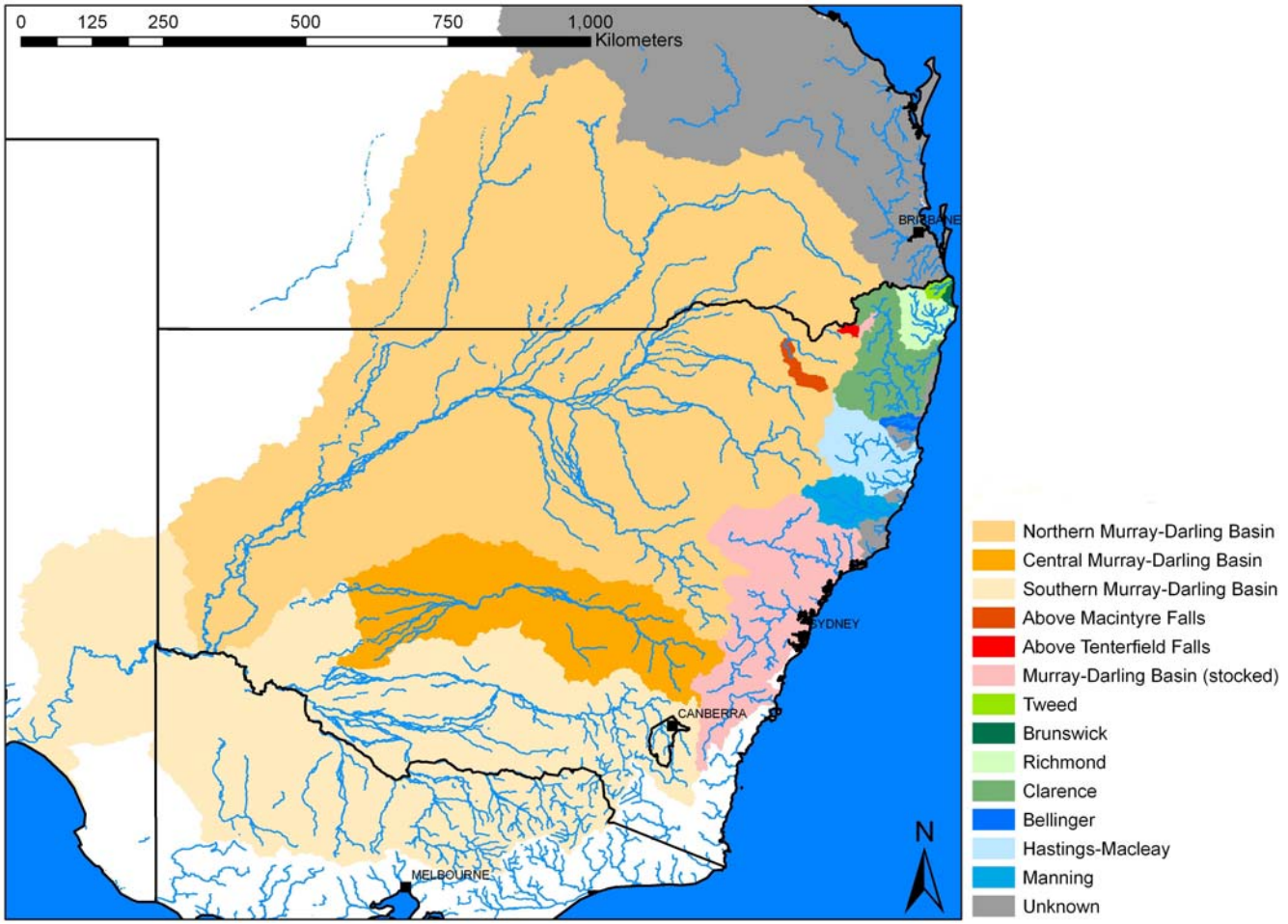

Figure 5.1. Proposed broodfish genetic zones in the MDB and coastal NSW catchments. Unknown indicates areas where broodfish genetic zones cannot be identified given microsatellite genetic data has not yet to be been collected.

that the stocked fish are as genetically and ecologically consistent as possible with the population to be stocked. To further improve the suitability of these stocked populations as a potential source of broodfish, a few individuals from remnant populations can be translocated into the stocked populations within the same broodfish genetic zone to achieve both the conservation of that population by increasing genetic diversity and reducing the gene frequency differences between remnant and stocked populations (Yamamoto *et al.* 2006), as well as creating an abundant source of broodfish for subsequent utilisation in a broader captive breeding – reintroduction program.

In the MDB, *T. tandanus* populations in large impoundments have been artificially isolated from those populations downstream, resulting in low but statistically significant changes in genetic structure, but no loss of genetic diversity. Differences between above and below dam populations can be ameliorated by translocating adult fish downstream of the dam into the dam (Yamamoto *et al.* 2006; Sato and Harada 2008). This process should be applied to all impounded *T. tandanus* populations to reduce genetic differentiation and potential inbreeding in above-dam populations brought about by their isolation. Alternatively, a combination of above-dam and below-dam broodfish may be collected, and their progeny be routinely released both above and below the dam to ensure that the current minor genetic differences between the two populations due to restriction of gene flow are broken down. Isolated populations above dams are typically not recommended as the sole source of broodfish for captive breeding or translocation programs owing to their potentially reduced genetic diversity and altered genetic structure, which may have a negative effect on the stocked population. However, the genetic evidence presented here shows no loss of genetic diversity and a very minor change in genetic structure to date, largely because the dams were constructed as recently as the late 20th century (1960 – 1976), a period in the order of < 10 *T. tandanus* generations. Consequently, a stocking program combining dam and river broodfish is likely to have a positive effect by removing genetic differences that exist purely as a result of the construction of the dam.

5.2.4. Future work

The current Recreational Freshwater Fishing Trust funded project also includes funding for a habitat mapping component to determine the habitat requirements for a self-sustaining freshwater catfish population. This component of the project is currently being analysed and written up as an additional report to be completed later this year. This second report will include additional details for gaining approval for a freshwater catfish stocking program in NSW.

6. REFERENCES

Allendorf, F.W. (1993). Delay of adaptation to captive breeding by equalizing family size. *Conservation Biology* **7**, 416–419.

Allendorf, F.W., Leary, R.F., Spruell, P. and Wenburg, J.K. (2001). The problem with hybrids: setting conservation guidelines. *Trends in Ecology and Evolution* **16**, 613–622.

Amos, W., Hoffman J. I., Frodsham A., Zhang L., Best S. and Hill A.V.S. (2007). Automated binning of microsatellite alleles: problems and solutions. *Molecular Ecology Notes* **7**, 10–14.

Anon (2007). The sea to Hume Dam: Restoring fish passage in the Murray River. Murray-Darling Basin Commission.

Avise, J.C. (2004). 'Molecular markers, natural history and evolution.' (Sinauer Associates, Inc.: Sunderland, Mass).

Bartley, D., Bagley, M., Gall, G. and Bentley, B. (1992). Use of linkage disequilibrium data to estimate effective size of hatchery and natural fish populations. *Conservation Biology* **6**, 365–375.

Bearlin, A.R. (2002). Identifying the weakest link: simulating adaptive management of the reintroduction of a threatened fish. *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 1709–1716.

Beheregaray, L.B., Möller, L.M., Schwartz, T.S., Chao, N.L. and Caccone, G. (2004). Microsatellite markers for the cardinal tetra *Paracheirodon axelrodi*, a commercially important fish from central Amazonia. *Molecular Ecology Notes* **4**, 330–332.

Bertozzi, T., Adams, M. and Walker, K.F. (2000). Species boundaries in carp gudgeons (Eleotrididae: *Hypseleotris*) from the River Murray, south Australia: evidence for multiple species and extensive hybridization. *Marine and Freshwater Research* **51**, 805–815.

Bishop, P. (1995). Drainage rearrangement by river capture, beheading and diversion. *Progress in Physical Geography* **19**, 449–473.

Briscoe, D.A. and Tait, N.N. (1995). Allozyme evidence for extensive and ancient radiations in Australian Onychophora. *Zoological Journal of the Linnean Society* **114**, 91–102.

Brock, P.M. (1998). The significance of the physical environment of the Macquarie Marshes. *Australian Geographer* **29**, 71–90.

Burridge, C.P., Craw D., Jack D.C., King T.M. and Waters J.M. (2008). Does fish ecology predict dispersal across a river drainage divide? *Evolution* **62**, 1484–1499.

Carlsson, J., Olsén, K.H., Nilsson, J., Øverli, Ø. and Stabell, O.B. (1999). Microsatellites reveal fine-scale genetic structure in stream-living brown trout. *Journal of Fish Biology* **55**, 1290–1303.

Clarke, K.R. and Gorley, R.N. (2006). 'PRIMER v6: User Manual/Tutorial.' (Primer-E: Plymouth).

Clement, M., Posada, D. and Crandall, K.A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**, 1657–1659.

Clunie, P. and Koehn, J. (2001a). Freshwater catfish: A recovery plan. Arthur Rylah Institute for Environmental Research. Department of Natural Resources and Environment, Heidelberg, VIC.

Clunie, P. and Koehn, J. (2001b). Freshwater catfish: A resource document. Arthur Rylah Institute for Environmental Research. Department of Natural Resources and Environment, Heidelberg, VIC.

Conover, D.O., Clarke, L.M., Munch, S.B. and Wagner, G.N. (2006). Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. *Journal of Fish Biology* **69**, 21–47.

Cornuet, J.M. and Luikart, G. (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**, 2001–2014.

Crandall, K.A., Bininda-Eamonds, O.R.P., Mace, G.M. and Wayne, R.K. (2000). Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution* **15**, 290–295.

Craw, D., Burridge, C., Anderson, L. and Waters, J.M. (2007). Late Quaternary river drainage and fish evolution, Southland, New Zealand. *Geomorphology* **84**, 98–110.

Crowley, L.E.L.M., Ivantsoff, W. and Allen, G.R. (1986). Taxonomic position of two crimson-spotted rainbowfish, *Melanotaenia duboulayi* and *Melanotaenia fluviatilis* (Pices: Melanotaeniidae), from eastern Australia, with special reference to their early life-history stages. *Australian Journal of Marine and Freshwater Research* **37**, 385–398.

DeWoody, J.A. and Avise, J.C. (2000). Microsatellite variation in marine, freshwater and anadromus fishes compared with other animals. *Journal of Fish Biology* **56**, 461–473.

Di Rienzo, A., Peterson A.C., Garza J.C., Valdes A.M., Slatkin M. and Freimer N.B. (1994). Mutational processes of simple-sequence repeat loci in human populations. *Proceedings of the National Academy of Sciences, USA* **91**, 3166–3170.

Donaldson, K.A. and Wilson, R.R. (1999). Amphi-Panamic Geminates of Snook (Percoidei: Centropomidae) Provide a Calibration of the Divergence Rate in the Mitochondrial DNA Control Region of Fishes. *Molecular Phylogenetics and Evolution* **13**, 208–213.

Drummond, A.J. and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214.

Dufty, S. (1986). Genetic and morphological divergence between populations of Macquarie perch (*Macquaria australasica*) east and west of the Great Dividing Range. Honours thesis, University of New South Wales.

England, P., Cornuet J.-M., Berthier P., Tallmon D. and Luikart G. (2006). Estimating effective population size from linkage disequilibrium: severe bias in small samples. *Conservation Genetics* **7**, 303–308.

Englbrecht, C.C., Schliewen, U. and Tautz, D. (2002). The impact of stocking on the genetic integrity of Arctic charr (*Salvelinus*) populations from the Alpine region. *Molecular Ecology* **11**, 1017–1027.

Evanno, G., Regnaut, S. and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**, 2611–2620.

Excoffier, L., Laval, G. and Schneider, S. (2005). Arlequin (version 3.0). An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**, 47–50.

Falush, D., Stephens, M. and Pritchard, J.K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164**, 1567–1587.

Faulks, L.K., Gilligan, D.M. and Beheregaray, L.B. (2008). Phylogeography of a threatened freshwater fish (*Mogurnda adspersa*) in eastern Australia: conservation implications. *Marine and Freshwater Research* **59**, 89–96.

Faulks, L.K., Gilligan, D.M. and Beheregaray, L.B. (2009). Evolution and maintenance of divergent lineages in an endangered freshwater fish, *Macquaria australasica*. *Conservation Genetics* **doi: 10.1007/s10592-009-9936-7**.

Faulks, L.K., Gilligan, D.M. and Beheregaray, L.B. (2010). Clarifying an ambiguous evolutionary history: range-wide phylogeography of an Australian freshwater fish, golden perch (*Macquaria ambigua*). *Journal of Biogeography*, in press.

Fischer, D. and Bachmann, K. (1998). Microsatellite enrichment in organisms with large genomes (*Allium cepa* L.). *BioTechniques* **24**, 796–802.

Frankham, R., Ballou, J.D. and Briscoe, D.A. (2002). 'Introduction to Conservation Genetics.' (Cambridge University Press: Cambridge, UK).

Franklin, I.R. (1980). Evolutionary change in small populations. In 'Conservation Biology: An evolutionary-Ecological Perspective'. (Eds M. E. Soule and B. A. Wilcox). (Sinauer: Sunderland, Massachusetts).

Franklin, I.R. and Frankham, R. (1998). How large must populations be to retain evolutionary potential? *Animal Conservation* **1**, 69–70.

Fraser, D.J. and Bernatchez, L. (2001). Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Molecular Ecology* **10**, 2741–2752.

Gilligan, D., Jess, L., McLean, G., Asmus, M., Wooden, I., Hartwell, D., McGregor, C., Stuart, I., Vey, A., Jefferies, M., Lewis, B. and Bell, K. (2010). Identifying and implementing targeted carp control options for the lower Lachlan catchment. Industry & Investment NSW – Fisheries Final Report Series No.118. Cronulla, NSW Australia. 126pp.

Goudet, J. (1995). FSTAT (vers. 1.2): a computer program to calculate F-statistics. *Journal of Heredity* **86**, 485–486.

Hallerman, E.M. (2003). Coadaptation and outbreeding depression. In 'Population genetics: principles and applications for fisheries scientists'. (Ed. E.M. Hallerman) pp. 239–259. (American Fisheries Society: Bethesda, Maryland).

Hammer, M.P., Adams, M., Unmack, P.J. and Walker, C.W. (2007). A rethink on *Retropinna*: conservation implications of new taxa and significant genetic sub-structure in Australian smelts (Pisces: Retropinnidae). *Marine and Freshwater Research* **58**, 327–341.

Harris, J.H. and Battaglene, S.C. (1990). The introduction and translocation of native freshwater fishes in south-eastern Australia. In 'Australian Society of Fish Biology Workshop. Proceedings No. 8'. (Ed. D.A. Pollard) pp. 136–142: Magnetic Island, Townsville).

Hasegawa, M., Kishino, K. and Yano, T. (1985). Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22**, 160–174.

Heggenes, J., Skaala, O., Borgstrom, R. and Igland, O.T. (2006). Minimal gene flow from introduced brown trout (*Salmo trutta* L.) after 30 years of stocking. *Journal of Applied Ichthyology* **22**, 119–124.

Hindar, K., Ryman, N. and Utter, F. (1991). Genetic effects of cultured fish on natural fish populations. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 945–957.

Hughes, J., Goudkamp, K., Hurwood, D., Hancock, M. and Bunn, S.E. (2003). Translocation Causes Extinction of a Local Population of the Freshwater Shrimp *Paratya australiensis*. *Conservation Biology* **17**, 1007–1012.

Imbrie, J., Hayes, J., Martinson, D.G., McIntyre, A., Mix, A.C., Morley, J.J., Pisias, N.G., Prell, W.L. and Shackleton N. J. (1984). The orbital theory of the Pleistocene climate: support from a revised chronology of the marine δ18O record. *In* 'Milankovitch and Climate, part 1'. (Eds A.L. Berger, J. Imbrie, J. Hayes, G. Kukla and B. Salzman): Riedel, Dordrecht).

Jarne, P. and Lagoda, P.J.L. (1996). Microsatellites, from molecules to populations and back. *Trends in Ecology & Evolution* **11**, 424–429.

Jerry, D.R. (1997). Population genetic structure of the catadromous Australian bass from throughout its range. *Journal of Fish Biology* **51**, 909–920.

Jerry, D.R. (2008). Phylogeography of the freshwater catfish *Tandanus tandanus* (Plotosidae): a model species to understand evolution of the eastern Australian fish fauna. *Marine & Freshwater Research* **59**, 351–360.

Jerry, D.R. and Woodland, D.J. (1997). Electrophoretic evidence for the presence of the undescribed 'Bellinger' catfish (*Tandanus* sp.) (Teleostei: Plotosidae) in four New South Wales mid-northern coastal rivers. *Marine & Freshwater Research* **48**, 235–240.

Josefsson, M. and Andersson, B. (2001). The environmental consequences of alien species in the Swedish lakes Mälaren, Hjälmaren, Vänern and Vättern. *AMBIO* **30**, 514–521.

Kawecki, T.J. and Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters* **7**, 1225–1241.

Keenan, C.P., Watts, R.J. and Serafini, L.G. (1995). Population genetics of golden perch (*Macquaria ambigua*), silver perch (*Bidyanus bidyanus*) and eel-tailed catfish (*Tandanus tandanus*) within the Murray-Darling Basin. Final Report of NRMS Project Number M262, Murray-Darling Basin Commission, Canberra.

Kershaw, P., Moss, P. and Van Der Kaars, S. (2003). Causes and consequences of long-term climatic variability on the Australian continent. *Freshwater Biology* **48**, 1274–1283.

Kimura, M. and Crow, J.F. (1963). The measurement of effective population number. *Evolution* **17**.

Kingsford, R.T., Brandis, K., Thomas, R.F., Crighton, P., Knowles, E. and Gale, E. (2004). Classifying landform at broad spatial scales: the distribution and conservation of wetlands in New South Wales, Australia. *Marine and Freshwater Research* **55**, 17–31.

Knight, J.T. (2009). Conservation implications of distinct genetic structuring in the endangered freshwater fish *Nannoperca oxleyana* (Percichthyidae). *Marine and Freshwater Research* **60**, 34–44.

Koskinen, M.T., Sundell, P., Piironen, J. and Primmer, C.R. (2002). Genetic assessment of spatiotemporal evolutionary relationships and stocking effects in grayling (*Thymallus thymallus*, Salmonidae). *Ecology Letters* **5**, 193–205.

Larsen, P.F., Hansen, M.M., Nielsen, E.E., Jensen, L.F. and Loeschcke, V. (2005). Stocking impact and temporal stability of genetic composition in a brackish northern pike population (*Esox lucius* L.), assessed using microsatellite DNA analysis of historical and contemporary samples. *Heredity* **95**, 136–143.

Larsen, P.F., Nielsen, E.E., Williams, T.D., Hemmer-Hansen, J., Chipman, J.K., Kruhøffer, M., Grønkjær, P., George, S.G., Dyrskjøts, L. and Loeschcke, V. (2007). Adaptive differences in gene expression in European flounder (*Platichthys flesus*). *Molecular Ecology* **16**, 4674–4683.

Larsen, P.F., Nielsen, E.E., Williams, T.D. and Loeschcke, V. (2008). Intraspecific variation in expression of candidate genes for osmoregulation, heme biosynthesis and stress resistance suggests local adaptation in European flounder (*Platichthys flesus*). *Heredity* **101**, 247–259.

Lee, W.-J., Huntting Howell, W. and Kocher, T.D. (1995). Structure and evolution of teleost mitochondrial control regions. *Journal of Molecular Evolution* **41**, 54–66.

Lippé, C., Dumont, P. and Bernatchez, L. (2006). High genetic diversity and no inbreeding in the endangered copper redhorse, *Moxostoma hubbsi* (Castostomidae, Pisces): the positive sides of a long generation time. *Molecular Ecology* **15**, 1769–1780.

Luttikhuizen, P.C., Drent, J., Van Delden, W. and Piersma, T. (2003). Spatially structured genetic variation in a broadcast spawning bivalve: quantitative vs. molecular traits. *Journal of Evolutionary Biology* **16**, 260–272.

Madeira, M.J., Gómez-Moliner, B.J. and Machordom Barbé, A. (2005). Genetic introgression on freshwater fish populations caused by restocking programmes. *Biological Invasions* **7**, 117–125.

Madsen, T., Shine, R., Olsson, M. and Wittzell, H. (1999). Restoration of an inbred adder population. *Nature* **402**, 34–35.

Maruyama, T. and Fuerst, P.A. (1985). Population bottlenecks and nonequilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. *Genetics* **111**, 675–689.

McGlashan, D.J. and Hughes, J.M. (2001). Genetic evidence for historical continuity between populations of the Australian freshwater fish *Craterocephalus stercusmuscarum* (Atherinidae) east and west of the Great Dividing Range. *Journal of Fish Biology* **59 (Supplement A)**, 55–67.

Meffe, G.K. (1992). Techno-arrogance and halfway technologies: Salmon hatcheries on the Pacific coast of North America. *Conservation Biology* **6**, 350–354.

Miller, A.D., Waggy, G., Ryan, S.G. and Austin, C.M. (2004). Mitochondrial 12S rRNA sequences support the existence of a third species of freshwater blackfish (Percicthyidae: *Gadopsis*) from south-eastern Australia. *Memoirs of Museum Victoria* **6**, 121–127.

Miller, L.M. and Kapuscinski, A.R. (2003). Genetic guidelines for hatchery supplementation programs. In 'Population Genetics Principles and Applications for Fisheries Scientists'. (Ed. E. M. Hallerman) pp. 329–355. (American Fisheries Society: Bethesda).

Moore, A., Ingram, B.A., Friend, S., King, Ho, H., Robinson, N., McCormack, R., Coughran, J. and Hayes, B. (2010). Management of genetic resources for fish and crustaceans in the Murray-Darling Basin. Bureau of Rural Sciences, Canberra.

Moritz, C. (1994). Defining 'Evolutionarily Significant Units' for conservation. *Trends in Ecology and Evolution* **9**, 373–375.

Musyl, M.K. (1990). Meristic, morphometric and electrophoretic studies of two native species of freshwater fishes, *Macquaria ambigua* (Percichthyidae) and *Tandanus tandanus* (Plotosidae) in southeastern Australia. PhD thesis, University of New England.

Musyl, M.K. and Keenan, C.P. (1996). Evidence for Cryptic Speciation in Australian Freshwater Eel-Tailed Catfish, *Tandanus tandanus* (Teleostei: Plotosidae). *Copeia* **3**, 526–534.

Nicol, S.J., Lieschke, J.A., Lyon, J.P. and Koehn, J.D. (2004). Observations on the distribution and abundance of common carp and native fish, and their response to a habitat restoration trial in the Murray River, Australia. *New Zealand Journal of Marine and Freshwater Research* **38**, 541–552.

Nosil, P., Vines, T.H. and Funk, D.J. (2005). Perspective: Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution* **59**, 705–719.

O'Brien, P.E. and Burne, R.V. (1994). The Great Cumbung Swamp-terminis of the low-gradient Lachlan River, Eastern Australia. *ASGO Journal of Australian Geology and Geophysics* **15**, 223–233.

Ohta, T. and Kimura, M. (1973). A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genetical Research* **22**, 201–204.

Ollier, C.D. (1982a). Geomorphology and tectonics of the Dorrigo Plateau, N.S.W. *Journal of the Geological Society of Australia* **29**, 431–435.

Ollier, C.D. (1982b). The Great Escarpment in eastern Australia: tectonic and geomorphic significance. *Journal of the Geological Society of Australia* **29**, 13–23.

Ollier, C.D. and Haworth, R.J. (1994). Geomorphology of the Clarence-Moreton basin. Geology and petroleum potential of the Clarence-Moreton Basin, New South Wales and Queensland, Bulletin 241, Australian Geological Survey Organisation, Department of Primary Industries and Energy.

Palstra, F.P. and Ruzzante, D.E. (2008). Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? *Molecular Ecology* **17**, 3428–3447.

Peel, D., Ovenden, J.R. and Peel, S.L. (2004). NeEstimator: software for estimating effective population size, (Version 1.3). Department of Primary Industries and Fisheries, Queensland Government, Queensland.

Piry, S., Alapetite A., Cornuet J.-M., Paetkau D., Baudouin L. and Estoup A. (2004). GeneClass2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity* **95**, 536–539.

Piry, S., Luikart, G. and Cornuet, J.-M. (1999). BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity* **90**, 502–503.

Pollard, D.A., Davis, T.L.O. and Llewellyn, L.C. (1996). Eel-tailed catfishes. In 'Freshwater fishes of south east Australia (New South Wales, Victoria and Tasmania)'. (Ed. R. McDowall) pp. 109–113. (Reed: Sydney).

Posada, D. and Crandall. K.A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

Pritchard, J.K., Stephens, M. and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Rambaut, A. (2006). FigTree v1.2.3. Available from: http://tree.bio.ed.ac.uk/software/figtree.

Rambaut, A. and Drummond, A.J. (2007). Tracer v1.4.1. Available from: http://beast.bio.ed.ac.uk/Tracer.

Rannala, B. and Mountain, J.L. (1997). Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences, USA* **94**, 9197–9201.

Raymond, M. and Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248–249.

Reynolds, L.F. (1983). Migration patterns of five fish species in the Murray-Darling River System. *Australian Journal of Marine and Freshwater Research* **34**, 857–871.

Rice, W.F. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Rogers, A.R. and Harpending, H. (1992). Population growth makes waves in the Distribution of pairwise genetic differences. *Molecular Biology and Evolution* **9**, 552–569.

Rourke, M.L. (2007). Population genetic structure of Murray cod (*Maccullochella peelii peelii*) and impacts of stocking in the Murray-Darling Basin. PhD thesis, Monash University.

Rourke, M.L., McPartlan, H.C., Ingram, B.A. and Taylor, A.C. (2009). Polygamy and low effective population size in a captive Murray cod (*Maccullochella peelii peelii*) population: genetic implications for wild restocking programs. *Marine and Freshwater Research* **60**, 873–883.

Rowland, S.J. (1985). Aspects of the biology and artificial breeding of the Murray cod *Maccullochella peeli*, and the eastern freshwater cod, *M. Ikei* sp. nov. (Pisces: Percichthyidae). PhD thesis, Macquarie University.

Rowland, S.J. (1993). *Maccullochella ikei*, an Endangered Species of Freshwater Cod (Pisces: Percichthyiday) from the Clarence River System, NSW and M. *peelii mariensis*, a New Subspecies from the Mary River System, Qld. *Records of the Australian Museum* **45**, 121–145.

Rowland, S.J. and Tully, P. (2004). Hatchery quality assurance program for Murray cod (*Maccullochella peelii peelii*) golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*). New South Wales Department of Primary Industries, Sydney.

Rozas, L.P. and Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 1451–1452.

Rozen, S. and Skaletsky, H.J. (2000). Primer3 on the WWW for general users and for biologist programmers. In 'Bioinformatics Methods and Protocols: Methods in Molecular Biology'. (Eds S. Krawetz and S. Misener). (Humana Press, Totowa, NJ).

Ryan, T., Gasior, R. and Steegstra, D. (1999). Habitat degradation associated with saline stratification. A report for the Murray Darling Basin Commission, Natural Resource Management Strategy Project V238. Department of Natural Resources and Environment, Victoria.

Saint-Laurent, R., Legault, M. and Bernatchez, L. (2003). Divergent selection maintains adaptive differentiation despite high gene flow between sympatric rainbow smelt ecotypes (*Osmerus mordax* Mitchill). *Molecular Ecology* **12**, 315–330.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). 'Molecular cloning. A laboratory manual.' (Cold Harbour Spring Laboratory).

Sato, T. and Harada, Y. (2008). Loss of genetic variation and effective population size of Kirikuchi charr: implications for the management of small, isolated salmonid populations. *Animal Conservation* **11**, 153–159.

Swofford, D.L. (2003). PAUP*. Phylogenetic Analysis Using Parsimony (*And Other Methods). Version 4. Sunderland, MA.

Tajima, F. (1989a). The effect of change in population size on DNA polymorphism. *Genetics* **123**, 597–601.

Tajima, F. (1989b). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**, 585–595.

Tallmon, D.A., Luikart, G. and Waples, R.S. (2004). The alluring simplicity and complex reality of genetic rescue. *Trends in Ecology & Evolution* **19**, 489–496.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596–1599.

Thacker, C.E., Unmack, P.J., Matsui, L., Duong, P. and Huang, E. (2008). Phylogeography of *Philypnodon* species (Teleostei: Eleotridae) across eastern Australia: comparative evolution of a sister pair. *Biological Journal of the Linnean Society* **95**, 175–192.

Thacker, C.E., Unmack, P.J., Matsui, L. and Rifenbark, N. (2007). Comparative phylogeography of five sympatric *Hypseleotris species* (Teleostei: Eleotridae) in south-eastern Australia reveals a complex pattern of drainage basin exchanges with little congruence across species. *Journal of Biogeography* **34**, 1518–1533.

Theodorou, K. and Couvet, D. (2004). Introduction of captive breeders to the wild: Harmful or beneficial? *Conservation Genetics* **5**, 1–12.

Trueman, W.T. (2006). Methods for the hatchery production of the freshwater jewfish or eel tailed catfish *Tandanus tandanus*. Native Fish Australia Technical Report #1.

Unmack, P.J. (2001). Biogeography of Australian freshwater fishes. *Journal of Biogeography* **28**, 1053–1089.

Utter, F. (2004). Population genetics, conservation and evolution in salmonids and other widely cultured fishes: some perspectives over six decades. *Reviews in Fish Biology and Fisheries* **14**, 125–144.

Vähä, J.-P. and Primmer, C.R. (2006). Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. *Molecular Ecology* **15**, 63–72.

Waples, R.S. (1999). Dispelling Some Myths about Hatcheries. *Fisheries* **24**, 12–21.

Waples, R.S. and Do, C. (2008). LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources* **8**, 753–756.

Wellman, P. (1979). On the Cainozoic uplift of the southeastern Australian highland. *Journal of the Geological Society of Australia* **26**, 1–9.

Wong, B.B.M., Keogh, J.S. and McGlashan, D.J. (2004). Current and historical patterns of drainage connectivity in eastern Australia inferred from population genetic structuring in a widespread freshwater fish *Pseudomugil signifer* (Pseudomugilidae). *Molecular Ecology* **13**, 391–401.

Yamamoto, S., Maekawa K., Tamate T., Koizumi I., Hasegawa K. and Kubota H. (2006). Genetic evaluation of translocation in artificially isolated populations of white-spotted charr (Salvelinus leucomaenis). *Fisheries Research* **78**, 352–358.

Zenger, K.R., Richardson, B.J. and Vachot-Griffin, A.M. (2003). A rapid population expansion retains genetic diversity within European rabbits in Australia. *Molecular Ecology* **12**, 789–794.

Zhivotovsky, L.A. and Feldman, M.W. (1995). Microsatellite variability and genetic distances. *Proceedings of the National Academy of Sciences, USA* **92**, 11549–11552.

Other titles in this series:

ISSN 1440-3544 (NSW Fisheries Final Report Series)

No. 1 Andrew, N.L., Graham, K.J., Hodgson, K.E. and Gordon, G.N.G., 1998. Changes after 20 years in relative abundance and size composition of commercial fishes caught during fishery independent surveys on SEF trawl grounds.

No. 2 Virgona, J.L., Deguara, K.L., Sullings, D.J., Halliday, I. and Kelly, K., 1998. Assessment of the stocks of sea mullet in New South Wales and Queensland waters.

No. 3 Stewart, J., Ferrell, D.J. and Andrew, N.L., 1998. Ageing Yellowtail (*Trachurus novaezelandiae*) and Blue Mackerel (*Scomber australasicus*) in New South Wales.

No. 4 Pethebridge, R., Lugg, A. and Harris, J., 1998. Obstructions to fish passage in New South Wales South Coast streams. 70pp.

No. 5 Kennelly, S.J. and Broadhurst, M.K., 1998. Development of by-catch reducing prawn-trawls and fishing practices in NSW's prawn-trawl fisheries (and incorporating an assessment of the effect of increasing mesh size in fish trawl gear). 18pp + appendices.

No. 6 Allan, G.L. and Rowland, S.J., 1998. Fish meal replacement in aquaculture feeds for silver perch. 237pp + appendices.

No. 7 Allan, G.L., 1998. Fish meal replacement in aquaculture feeds: subprogram administration. 54pp + appendices.

No. 8 Heasman, M.P., O'Connor, W.A. and O'Connor, S.J., 1998. Enhancement and farming of scallops in NSW using hatchery produced seedstock. 146pp.

No. 9 Nell, J.A., McMahon, G.A. and Hand, R.E., 1998. Tetraploidy induction in Sydney rock oysters. 25pp.

No. 10 Nell, J.A. and Maguire, G.B., 1998. Commercialisation of triploid Sydney rock and Pacific oysters. Part 1: Sydney rock oysters. 122pp.

No. 11 Watford, F.A. and Williams, R.J., 1998. Inventory of estuarine vegetation in Botany Bay, with special reference to changes in the distribution of seagrass. 51pp.

No. 12 Andrew, N.L., Worthington D.G., Brett, P.A. and Bentley N., 1998. Interactions between the abalone fishery and sea urchins in New South Wales.

No. 13 Jackson, K.L. and Ogburn, D.M., 1999. Review of depuration and its role in shellfish quality assurance. 77pp.

No. 14 Fielder, D.S., Bardsley, W.J. and Allan, G.L., 1999. Enhancement of Mulloway (*Argyrosomus japonicus*) in intermittently opening lagoons. 50pp + appendices.

No. 15 Otway, N.M. and Macbeth, W.G., 1999. The physical effects of hauling on seagrass beds. 86pp.

No. 16 Gibbs, P., McVea, T. and Louden, B., 1999. Utilisation of restored wetlands by fish and invertebrates. 142pp.

No. 17 Ogburn, D. and Ruello, N., 1999. Waterproof labelling and identification systems suitable for shellfish and other seafood and aquaculture products. Whose oyster is that? 50pp.

No. 18 Gray, C.A., Pease, B.C., Stringfellow, S.L., Raines, L.P. and Walford, T.R., 2000. Sampling estuarine fish species for stock assessment. Includes appendices by D.J. Ferrell, B.C. Pease, T.R. Walford, G.N.G. Gordon, C.A. Gray and G.W. Liggins. 194pp.

No. 19 Otway, N.M. and Parker, P.C., 2000. The biology, ecology, distribution, abundance and identification of marine protected areas for the conservation of threatened Grey Nurse Sharks in south east Australian waters. 101pp.

No. 20 Allan, G.L. and Rowland, S.J., 2000. Consumer sensory evaluation of silver perch cultured in ponds on meat meal based diets. 21pp + appendices.

No. 21 Kennelly, S.J. and Scandol, J. P., 2000. Relative abundances of spanner crabs and the development of a population model for managing the NSW spanner crab fishery. 43pp + appendices.

No. 22 Williams, R.J., Watford, F.A. and Balashov, V., 2000. Kooragang Wetland Rehabilitation Project: History of changes to estuarine wetlands of the lower Hunter River. 82pp.

No. 23 Survey Development Working Group, 2000. Development of the National Recreational and Indigenous Fishing Survey. Final Report to Fisheries Research and Development Corporation. (Volume 1 – 36pp + Volume 2 – attachments).

No.24 Rowling, K.R and Raines, L.P., 2000. Description of the biology and an assessment of the fishery of Silver Trevally *Pseudocaranx dentex* off New South Wales. 69pp.

No. 25 Allan, G.L., Jantrarotai, W., Rowland, S., Kosuturak, P. and Booth, M., 2000. Replacing fishmeal in aquaculture diets. 13pp.

No. 26 Gehrke, P.C., Gilligan, D.M. and Barwick, M., 2001. Fish communities and migration in the Shoalhaven River – Before construction of a fishway. 126pp.

No. 27 Rowling, K.R. and Makin, D.L., 2001. Monitoring of the fishery for Gemfish *Rexea solandri*, 1996 to 2000. 44pp.

No. 28 Otway, N.M., 1999. Identification of candidate sites for declaration of aquatic reserves for the conservation of rocky intertidal communities in the Hawkesbury Shelf and Batemans Shelf Bioregions. 88pp.

No. 29 Heasman, M.P., Goard, L., Diemar, J. and Callinan, R., 2000. Improved Early Survival of Molluscs: Sydney Rock Oyster (*Saccostrea glomerata*). 63pp.

No. 30 Allan, G.L., Dignam, A and Fielder, S., 2001. Developing Commercial Inland Saline Aquaculture in Australia: Part 1. R&D Plan.

No. 31 Allan, G.L., Banens, B. and Fielder, S., 2001. Developing Commercial Inland Saline Aquaculture in Australia: Part 2. Resource Inventory and Assessment. 33pp.

No. 32 Bruce, A., Growns, I. and Gehrke, P., 2001. Woronora River Macquarie Perch Survey. 116pp.

No. 33 Morris, S.A., Pollard, D.A., Gehrke, P.C. and Pogonoski, J.J., 2001. Threatened and Potentially Threatened Freshwater Fishes of Coastal New South Wales and the Murray-Darling Basin. 177pp.

No. 34 Heasman, M.P., Sushames, T.M., Diemar, J.A., O'Connor, W.A. and Foulkes, L.A., 2001. Production of Micro-algal Concentrates for Aquaculture Part 2: Development and Evaluation of Harvesting, Preservation, Storage and Feeding Technology. 150pp + appendices.

No. 35 Stewart, J. and Ferrell, D.J., 2001. Mesh selectivity in the NSW demersal trap fishery. 86pp.

No. 36 Stewart, J., Ferrell, D.J., van der Walt, B., Johnson, D. and Lowry, M., 2001. Assessment of length and age composition of commercial kingfish landings. 49pp.

No. 37 Gray, C.A. and Kennelly, S.J., 2001. Development of discard-reducing gears and practices in the estuarine prawn and fish haul fisheries of NSW. 151pp.

No. 38 Murphy, J.J., Lowry, M.B., Henry, G.W. and Chapman, D., 2002. The Gamefish Tournament Monitoring Program – 1993 to 2000. 93pp.

No. 39 Kennelly, S.J. and McVea, T.A. (Ed), 2002. Scientific reports on the recovery of the Richmond and Macleay Rivers following fish kills in February and March 2001. 325pp.

No. 40 Pollard, D.A. and Pethebridge, R.L., 2002. Report on Port of Botany Bay Introduced Marine Pest Species Survey. 69pp.

No. 41 Pollard, D.A. and Pethebridge, R.L., 2002. Report on Port Kembla Introduced Marine Pest Species Survey. 72pp.

No. 42 O'Connor, W.A, Lawler, N.F. and Heasman, M.P., 2003. Trial farming the akoya pearl oyster, *Pinctada imbricata*, in Port Stephens, NSW. 170pp.

No. 43 Fielder, D.S. and Allan, G.L., 2003. Improving fingerling production and evaluating inland saline water culture of snapper, *Pagrus auratus*. 62pp.

No. 44 Astles, K.L., Winstanley, R.K., Harris, J.H. and Gehrke, P.C., 2003. Experimental study of the effects of cold water pollution on native fish. 55pp.

No. 45 Gilligan, D.M., Harris, J.H. and Mallen-Cooper, M., 2003. Monitoring changes in the Crawford River fish community following replacement of an effective fishway with a vertical-slot fishway design: Results of an eight year monitoring program. 80pp.

No. 46 Pollard, D.A. and Rankin, B.K., 2003. Port of Eden Introduced Marine Pest Species Survey. 67pp.

No. 47 Otway, N.M., Burke, A.L., Morrison, NS. and Parker, P.C., 2003. Monitoring and identification of NSW Critical Habitat Sites for conservation of Grey Nurse Sharks. 62pp.

No. 48 Henry, G.W. and Lyle, J.M. (Ed), 2003. The National Recreational and Indigenous Fishing Survey. 188 pp.

No. 49 Nell, J.A., 2003. Selective breeding for disease resistance and fast growth in Sydney rock oysters. 44pp. (Also available – a CD-Rom published in March 2004 containing a collection of selected manuscripts published over the last decade in peer-reviewed journals).

No. 50 Gilligan, D. and Schiller, S., 2003. Downstream transport of larval and juvenile fish. 66pp.

No. 51 Liggins, G.W., Scandol, J.P. and Kennelly, S.J., 2003. Recruitment of Population Dynamacist. 44pp.

No. 52 Steffe, A.S. and Chapman, J.P., 2003. A survey of daytime recreational fishing during the annual period, March 1999 to February 2000, in Lake Macquarie, New South Wales. 124pp.

No. 53 Barker, D. and Otway, N., 2003. Environmental assessment of zinc coated wire mesh sea cages in Botany Bay NSW. 36pp.

No. 54 Growns, I., Astles, A. and Gehrke, P., 2003. Spatial and temporal variation in composition of riverine fish communities. 24pp.

No. 55 Gray, C. A., Johnson, D.D., Young, D.J. and Broadhurst, M. K., 2003. Bycatch assessment of the Estuarine Commercial Gill Net Fishery in NSW. 58pp.

No. 56 Worthington, D.G. and Blount, C., 2003. Research to develop and manage the sea urchin fisheries of NSW and eastern Victoria. 182pp.

No. 57 Baumgartner, L.J., 2003. Fish passage through a Deelder lock on the Murrumbidgee River, Australia. 34pp.

No. 58 Allan, G.L., Booth, M.A., David A.J. Stone, D.A.J. and Anderson, A.J., 2004. Aquaculture Diet Development Subprogram: Ingredient Evaluation. 171pp.

No. 59 Smith, D.M., Allan, G.L. and Booth, M.A., 2004. Aquaculture Diet Development Subprogram: Nutrient Requirements of Aquaculture Species. 220pp.

No. 60 Barlow, C.G., Allan, G.L., Williams, K.C., Rowland, S.J. and Smith, D.M., 2004. Aquaculture Diet Development Subprogram: Diet Validation and Feeding Strategies. 197pp.

No. 61 Heasman, M.H., 2004. Sydney Rock Oyster Hatchery Workshop 8 – 9 August 2002, Port Stephens, NSW. 115pp.

No. 62 Heasman, M., Chick, R., Savva, N., Worthington, D., Brand, C., Gibson, P. and Diemar, J., 2004. Enhancement of populations of abalone in NSW using hatchery-produced seed. 269pp.

No. 63 Otway, N.M. and Burke, A.L., 2004. Mark-recapture population estimate and movements of Grey Nurse Sharks. 53pp.

No. 64 Creese, R.G., Davis, A.R. and Glasby, T.M., 2004. Eradicating and preventing the spread of the invasive alga *Caulerpa taxifolia* in NSW. 110pp.

No. 65 Baumgartner, L.J., 2004. The effects of Balranald Weir on spatial and temporal distributions of lower Murrumbidgee River fish assemblages. 30pp.

No. 66 Heasman, M., Diggles, B.K., Hurwood, D., Mather, P., Pirozzi, I. and Dworjanyn, S., 2004. Paving the way for continued rapid development of the flat (angasi) oyster (*Ostrea angasi*) farming in New South Wales. 40pp.

ISSN 1449-9967 (NSW Department of Primary Industries – Fisheries Final Report Series)

No. 67 Kroon, F.J., Bruce, A.M., Housefield, G.P. and Creese, R.G., 2004. Coastal floodplain management in eastern Australia: barriers to fish and invertebrate recruitment in acid sulphate soil catchments. 212pp.

No. 68 Walsh, S., Copeland, C. and Westlake, M., 2004. Major fish kills in the northern rivers of NSW in 2001: Causes, Impacts & Responses. 55pp.

No. 69 Pease, B.C. (Ed), 2004. Description of the biology and an assessment of the fishery for adult longfinned eels in NSW. 168pp.

No. 70 West, G., Williams, R.J. and Laird, R., 2004. Distribution of estuarine vegetation in the Parramatta River and Sydney Harbour, 2000. 37pp.

No. 71 Broadhurst, M.K., Macbeth, W.G. and Wooden, M.E.L., 2005. Reducing the discarding of small prawns in NSW's commercial and recreational prawn fisheries. 202pp.

No. 72. Graham, K.J., Lowry, M.B. and Walford, T.R., 2005. Carp in NSW: Assessment of distribution, fishery and fishing methods. 88pp.

No. 73 Stewart, J., Hughes, J.M., Gray, C.A. and Walsh, C., 2005. Life history, reproductive biology, habitat use and fishery status of eastern sea garfish (*Hyporhamphus australis*) and river garfish (*H. regularis ardelio*) in NSW waters. 180pp.

No. 74 Growns, I. and Gehrke, P., 2005. Integrated Monitoring of Environmental Flows: Assessment of predictive modelling for river flows and fish. 33pp.

No. 75 Gilligan, D., 2005. Fish communities of the Murrumbidgee catchment: Status and trends. 138pp.

No. 76 Ferrell, D.J., 2005. Biological information for appropriate management of endemic fish species at Lord Howe Island. 18 pp.

No. 77 Gilligan, D., Gehrke, P. and Schiller, C., 2005. Testing methods and ecological consequences of large-scale removal of common carp. 46pp.

No. 78 Boys, C.A., Esslemont, G. and Thoms, M.C., 2005. Fish habitat and protection in the Barwon-Darling and Paroo Rivers. 118pp.

No. 79 Steffe, A.S., Murphy, J.J., Chapman, D.J. and Gray, C.C., 2005. An assessment of changes in the daytime recreational fishery of Lake Macquarie following the establishment of a 'Recreational Fishing Haven'. 103pp.

No. 80 Gannassin, C. and Gibbs, P., 2005. Broad-Scale Interactions Between Fishing and Mammals, Reptiles and Birds in NSW Marine Waters. 171pp.

No. 81 Steffe, A.S., Murphy, J.J., Chapman, D.J., Barrett, G.P. and Gray, C.A., 2005. An assessment of changes in the daytime, boat-based, recreational fishery of the Tuross Lake estuary following the establishment of a 'Recreational Fishing Haven'. 70pp.

No. 82 Silberschnieder, V. and Gray, C.A., 2005. Arresting the decline of the commercial and recreational fisheries for mulloway (*Argyrosomus japonicus*). 71pp.

No. 83 Gilligan, D., 2005. Fish communities of the Lower Murray-Darling catchment: Status and trends. 106pp.

No. 84 Baumgartner, L.J., Reynoldson, N., Cameron, L. and Stanger, J., 2006. Assessment of a Dual-frequency Identification Sonar (DIDSON) for application in fish migration studies. 33pp.

No. 85 Park, T., 2006. FishCare Volunteer Program Angling Survey: Summary of data collected and recommendations. 41pp.

No. 86 Baumgartner, T., 2006. A preliminary assessment of fish passage through a Denil fishway on the Edward River, Australia. 23pp.

No. 87 Stewart, J., 2007. Observer study in the Estuary General sea garfish haul net fishery in NSW. 23pp.

No. 88 Faragher, R.A., Pogonoski, J.J., Cameron, L., Baumgartner, L. and van der Walt, B., 2007. Assessment of a stocking program: Findings and recommendations for the Snowy Lakes Trout Strategy. 46pp.

No. 89 Gilligan, D., Rolls, R., Merrick, J., Lintermans, M., Duncan, P. and Kohen, J., 2007. Scoping knowledge requirements for Murray crayfish (*Euastacus armatus*). Final report to the Murray Darling Basin Commission for Project No. 05/1066 NSW 103pp.

No. 90 Kelleway, J., Williams. R.J. and Allen, C.B., 2007. An assessment of the saltmarsh of the Parramatta River and Sydney Harbour. 100pp.

No. 91 Williams, R.J. and Thiebaud, I., 2007. An analysis of changes to aquatic habitats and adjacent land-use in the downstream portion of the Hawkesbury Nepean River over the past sixty years. 97pp.

No. 92 Baumgartner, L., Reynoldson, N., Cameron, L. and Stanger, J. The effects of selected irrigation practices on fish of the Murray-Darling Basin. 90pp.

No. 93 Rowland, S.J., Landos, M., Callinan, R.B., Allan, G.L., Read, P., Mifsud, C., Nixon, M., Boyd, P. and Tally, P., 2007. Development of a health management strategy for the Silver Perch Aquaculture Industry. 219pp.

No. 94 Park, T., 2007. NSW Gamefish Tournament Monitoring – Angling Research Monitoring Program. Final report to the NSW Recreational Fishing Trust. 142pp.

No. 95 Heasman, M.P., Liu, W., Goodsell, P.J., Hurwood D.A. and Allan, G.L., 2007. Development and delivery of technology for production, enhancement and aquaculture of blacklip abalone (*Haliotis rubra*) in New South Wales. 226pp.

No. 96 Ganassin, C. and Gibbs, P.J., 2007. A review of seagrass planting as a means of habitat compensation following loss of seagrass meadow. 41pp.

No. 97 Stewart, J. and Hughes, J., 2008. Determining appropriate harvest size at harvest for species shared by the commercial trap and recreational fisheries in New South Wales. 282pp.

No. 98 West, G. and Williams, R.J., 2008. A preliminary assessment of the historical, current and future cover of seagrass in the estuary of the Parramatta River. 61pp.

No. 99 Williams, D.L. and Scandol, J.P., 2008. Review of NSW recreational fishing tournament-based monitoring methods and datasets. 83pp.

No. 100 Allan, G.L., Heasman, H. and Bennison, S., 2008. Development of industrial-scale inland saline aquaculture: Coordination and communication of R&D in Australia. 245pp.

No. 101 Gray, C.A and Barnes, L.M., 2008. Reproduction and growth of dusky flathead (*Platycephalus fuscus*) in NSW estuaries. 26pp.

No. 102 Graham, K.J., 2008. The Sydney inshore trawl-whiting fishery: codend selectivity and fishery characteristics. 153pp.

No. 103 Macbeth, W.G., Johnson, D.D. and Gray, C.A., 2008. Assessment of a 35-mm square-mesh codend and composite square-mesh panel configuration in the ocean prawn-trawl fishery of northern New South Wales. 104pp.

No. 104 O'Connor, W.A., Dove, M. and Finn, B., 2008. Sydney rock oysters: Overcoming constraints to commercial scale hatchery and nursery production. 119pp.

No. 105 Glasby, T.M. and Lobb, K., 2008. Assessing the likelihoods of marine pest introductions in Sydney estuaries: A transport vector approach. 84pp.

No. 106 Rotherham, D., Gray, C.A., Underwood, A.J., Chapman, M.G. and Johnson, D.D., 2008. Developing fishery-independent surveys for the adaptive management of NSW's estuarine fisheries. 135pp.

No. 107 Broadhurst, M., 2008. Maximising the survival of bycatch discarded from commercial estuarine fishing gears in NSW. 192pp.

No. 108 Gilligan, D., McLean, A. and Lugg, A., 2009. Murray Wetlands and Water Recovery Initiatives: Rapid assessment of fisheries values of wetlands prioritised for water recovery. 69pp.

No. 109 Williams, R.J. and Thiebaud, I., 2009. Occurrence of freshwater macrophytes in the catchments of the Parramatta River, Lane Cove River and Middle Harbour Creek, 2007 – 2008. 75pp.

No. 110 Gilligan, D., Vey, A. and Asmus, M., 2009. Identifying drought refuges in the Wakool system and assessing status of fish populations and water quality before, during and after the provision of environmental, stock and domestic flows. 56pp.

ISSN 1837-2112 (Industry & Investment NSW – Fisheries Final Report Series)

No. 111 Gray, C.A., Scandol. J.P., Steffe, A.S. and Ferrell, D.J., 2009. Australian Society for Fish Biology Annual Conference & Workshop 2008: Assessing Recreational Fisheries; Current and Future Challenges. 54pp.

No. 112 Otway, N.M. Storrie, M.T., Louden, B.M. and Gilligan, J.J., 2009. Documentation of depth-related migratory movements, localised movements at critical habitat sites and the effects of scuba diving for the east coast grey nurse shark population. 90pp.

No. 113 Creese, R.G., Glasby, T.M., West, G. and Gallen, C., 2009. Mapping the habitats of NSW estuaries. 95pp.

No. 114 Macbeth, W.G., Geraghty, P.T., Peddemors, V.M. and Gray, C.A., 2009. Observer-based study of targeted commercial fishing for large shark species in waters off northern New South Wales. 82pp.

No. 115 Scandol, J.P., Ives, M.C. and Lockett, M.M., 2009. Development of national guidelines to improve the application of risk-based methods in the scope, implementation and interpretation of stock assessments for data-poor species. 186pp.

No. 116 Baumgartner, L., Bettanin, M., McPherson, J., Jones, M., Zampatti, B. and Kathleen Beyer., 2009. Assessment of an infrared fish counter (Vaki Riverwatcher) to quantify fish migrations in the Murray-Darling Basin. 47pp.

No. 117 Astles, K., West, G., and Creese, R.G., 2010. Estuarine habitat mapping and geomorphic characterisation of the Lower Hawkesbury river and Pittwater estuaries. 229pp.

No. 118 Gilligan, D., Jess, L., McLean, G., Asmus, M., Wooden, I., Hartwell, D., McGregor, C., Stuart, I., Vey, A., Jefferies, M., Lewis, B. and Bell, K., 2010. Identifying and implementing targeted carp control options for the Lower Lachlan Catchment. 126pp.

No. 119 Montgomery, S.S., Walsh, C.T., Kesby, C.L and Johnson, D.D., 2010. Studies on the growth and mortality of school prawns. 90pp.

No. 120 Liggins, G.W. and Upston, J., 2010. Investigating and managing the *Perkinsus*-related mortality of blacklip abalone in NSW. 182pp.

No. 121 Knight, J., 2010. The feasibility of excluding alien redfin perch from Macquarie perch habitat in the Hawkesbury-Nepean Catchment. 53pp.

No. 122 Ghosn, D., Steffe, A., Murphy, J., 2010. An assessment of the effort and catch of shore and boat-based recreational fishers in the Sydney Harbour estuary over the 2007/08 summer period. 60pp.

No. 123 Rourke, M. and Gilligan, D., 2010. Population genetic structure of freshwater catfish (*Tandanus tandanus*) in the Murray-Darling Basin and coastal catchments of New South Wales: Implications for future re-stocking programs. 73pp.