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The History and Relationships of Northern Platypus (*Ornithorhynchus anatinus*) Populations

A Molecular Approach

by
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A Thesis submitted to
James Cook University
School of Marine and Tropical Biology
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in the discipline of Zoology and Tropical Ecology

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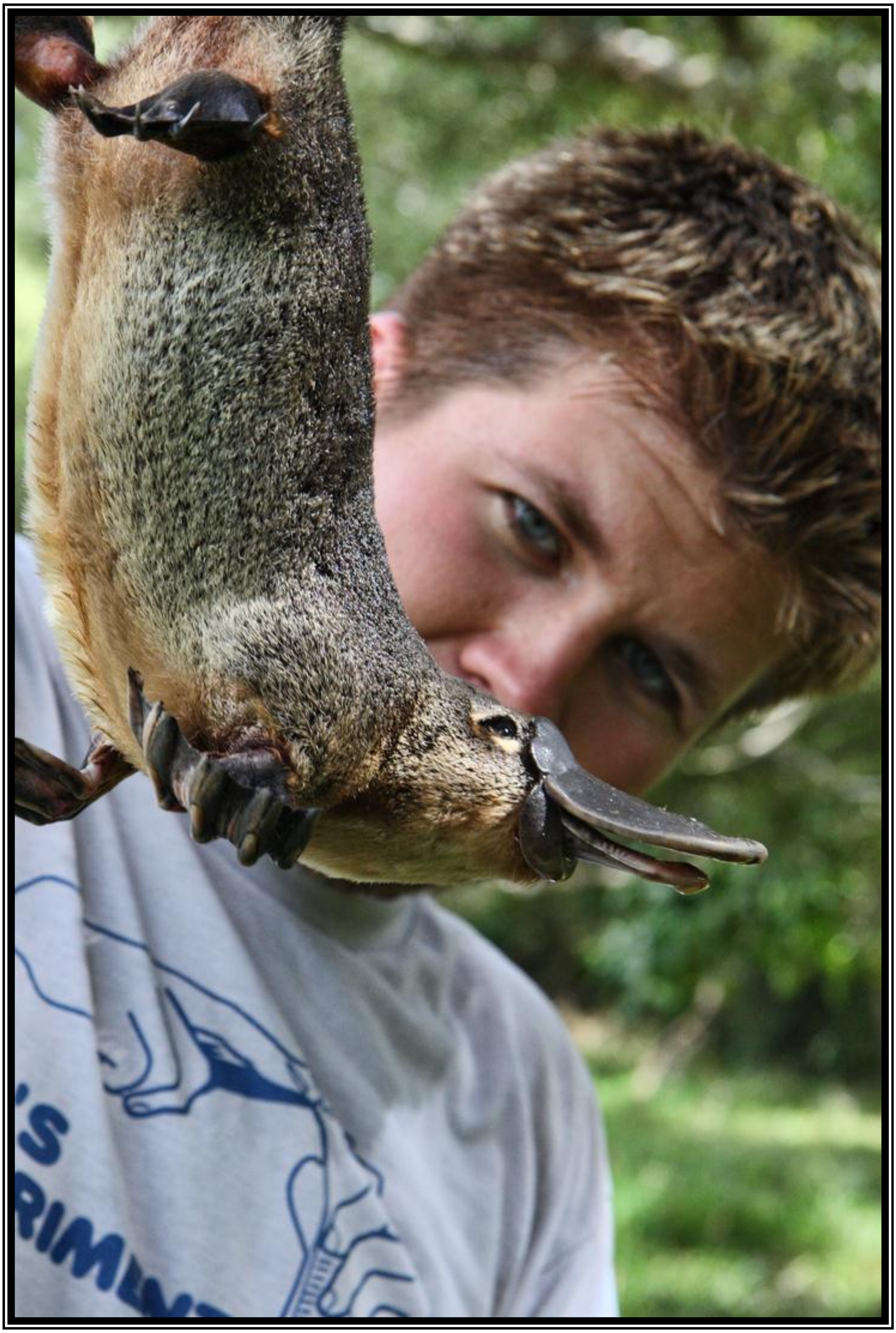

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Frontispiece: *Ornithorhynchus anatinus*

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I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

I also declare that all research procedures reported in the thesis complied with the guidelines of and was approved by the Animal Ethics Committee of James Cook University under Ethics Approval No. A1222. A copy of the original ethics approval and other regulatory permits can be found in appendix 1.

6/10/2010

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Stephen H Kolomyjec Date

STATEMENT OF CO-AUTHORSHIP

The research that went in to the production of this thesis has resulted in several publications and as such has collaboratively involved other individuals in various capacities. While the bulk of the work is the author's, it is important to recognise the input of the co-authors. The publications produced as part of this thesis are as follows:

Kolomyjec SH, Parsons JG, VanDerWal J, and Johnson CN. (in prep) Climate and the platypus (*Ornithorhynchus anatinus*). *Journal of Mammalogy*.

Kolomyjec SH, Grant TR, and Blair, D. 2008. Ten polymorphic microsatellite DNA markers for the platypus, *Ornithorhynchus anatinus*. *Molecular Ecology Resources*, **8**, 1133 – 1135.

Kolomyjec SH, Chong JYT, Blair D, Gongora J, Grant TR, Johnson CN, and Moran C. 2009. Population genetics of the platypus (*Ornithorhynchus anatinus*): a fine scale look at adjacent river systems. *Australian Journal of Zoology*, **57**, 225-334.

Kolomyjec SH, Grant TR, Johnson CN, and Blair D. (in review). Restriction of gene flow in the platypus (Ornithorhynchus anatinus) due to a large dam. *Australian Mammalogy*.

Kolomyjec SH, Grant TR, Johnson CN, and Blair D. (In prep) Regional population structuring and conservation units in the platypus (*Ornithorhynchus anatinus*). *Journal of Mammalogy*.

The people and institutions listed below have contributed to the research and publication that have lead to this thesis:

David Blair (James Cook University) and **Christopher N Johnson** (James Cook University) as the author's PhD supervisors have assisted with all aspects of the PhD. This includes: initial project planning, field design, grant writing and funding, and the preparation of publishable manuscripts.

Tom R Grant (University of New South Wales) provided invaluable training on the capture and handling of platypuses in the wild, personally collected samples for analysis and assistance in the preparation of publishable manuscripts.

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We, the undersigned, agree with the above statement of contribution and co-authorship for each of the above published (or submitted) peer-reviewed manuscripts contained within this thesis:

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David Blair	Mike Kingsford
(Candidate's Primary supervisor)	(Head of School)

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Abstract

The Aim of this study was to understand the distribution and genetic structure of platypus populations in Australia, and in particular to investigate the interactions of distribution and genetic structure. The research considered the entire distributional range of the platypus, but with a special focus on the scientifically neglected platypus populations of northern Queensland. Platypuses in north Queensland are smaller than their southern counterparts and have a more reddish colouration. There appears also to be a break in the distribution of the platypus between about Mackay and Townsville, which corresponds to the catchment of the Burdekin River and which geographically separates northern platypuses from southern populations. The relationship of northern and southern platypus populations of mainland Australia, together with the biogeographic significance to the platypus of the Burdekin break, was a binding thread throughout the study. However, before that relationship could be inferred there were several smaller gaps in the knowledge regarding the distribution of platypus that had to be filled. These gaps were represented by several intriguing questions: Where do platypuses occur and why is their distribution limited to those areas? And, how are local populations of platypuses structured and how do they relate to each other? With these key pieces of information it was possible to expand the scope of the study to a distribution-wide level.

Using distribution modelling software (MaxEnt), climate data and 4,315 occurrence records, I produced a climate-based distribution model to describe the current distribution of the platypus. The two most important climate factors determining environmental suitability for the platypus were precipitation during the driest quarter (which was positively associated with platypus occurrence) and maximum temperature (negatively associated), to the near exclusion of all other variables (53.8% and 41.2% contributions respectively). This distribution map supported the existence of a significant distribution break occurring in northern Queensland. Separate modelling of the northern and southern distributions revealed differences in the limiting factors in each part of the range. To the south, precipitation during the driest quarter and maximum temperature remained the two most important factors (76.2% and 18.9%

contribution respectively). However, in the north additional environmental factors were important. These were temperature seasonality, precipitation during wettest quarter, minimum temperature, and precipitation seasonality, with respective contributions to habitat suitability of 34.7%, 22.6%, 19.2%, 16.7% and 3.5%.

The initial species distribution model was projected onto palaeo-climate data representing the last glacial maximum (c. 22,000 years before present). This palaeo-model indicated that overall conditions were less favourable for the platypus at that time, and that the gap between the northern and southern portions of the distribution would have been even more pronounced, although there may have been connectivity between Tasmania and the mainland via the Bass land bridge. The platypus distribution was also projected forward to predict the effects of anthropogenic climate change. An aggregated mean across the complex models involved in this suggested a likely decline in range of approximately 15% by the year 2070 with best/worst case scenarios depicting an increase of 3.5% or a decrease of 65% respectively. The areas affected by these distributional changes were the marginal fringes surrounding the main areas of distribution.

After developing a reliable set of 12 microsatellite DNA markers for the study it was possible to investigate population structure and dynamics from a molecular perspective. At the finer scale of investigation (comparisons within and between adjacent river systems), I showed that despite individual sample sites within a river systems having some genetic differentiation, they generally exhibited a strong isolation-by-distance pattern within the system (e.g. Hawkesbury-Nepean system: $r = 0.7315$, $p = 0.02$). Moreover, significant differentiation between systems as suggested by pairwise F_{st}, AMOVA and Bayesian population clustering techniques indicates that the physical separation of river basins does limit gene flow and is responsible for local population structuring. The detection of several first generation migrants (13 of 120 samples) also provided a genetic indication that platypuses must move between river basins, which would require overland movement to occur more often than previously thought. I also showed that a large dam inhibited within-river gene flow and could lead to increased differentiation between populations: the construction of the Nepean Dam has lead to higher differentiation occurring within a single river (above vs. below dam pairwise F_{st} = 0.07681) then occurring between two rivers at three times the distance and requiring an overland crossing (Wingecarribee River vs. Nepean River pairwise F_{st} = 0.05978).

Genetic analysis across the entire platypus distribution revealed three evolutionarily significant units within the platypus distribution that are in strong consensus with the observations gathered from the distribution modelling. These represent the isolated Northern Region, the Southern Mainland Region, and Tasmania. Within these evolutionarily significant units six discrete population clusters were identified, which formed the basis of five proposed management units for the platypus (two clusters were combined due to the presence of active gene flow). Attempts to investigate population sub-clusters within these clusters were futile as genetic admixture between local river systems rendered their level of distinctiveness below that of discrete conservation units. Future conservation and management planners will have to keep in mind that not all platypuses are created equal; there are distinct groups that must be considered independently in order to maintain the genotypic and phenotypic features that currently exist across the species.

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Section 1 – Background

This section includes introductory material that establishes the theme, objectives and aims of the study.

Plate 1. Close-up of platypus
(Photo© S Kolomyjec)

Chapter 1 – General Introduction

Harry Burrell, the famous platypus biologist, once wrote “Every writer upon the platypus begins with an expression of wonder. Never was there such a disconcerting animal!” (Burrell 1927). I see no reason to break tradition; the platypus is truly a remarkable animal. As an icon of Australian fauna, it has fascinated scientists and non-scientists alike since its discovery by Europeans at the end of the 18th century (Moyal 2001). The platypus, however, is as elusive as it is remarkable and despite centuries of interest remains shrouded in mystery.

The platypus

The platypus (*Ornithorhynchus anatinus*) is a semi-aquatic mammal endemic to mainland Australia and Tasmania (Grant 1992; Grant 2007; Grant and Temple-Smith 1998). Possessing several features that are uncommon among the mammals, the platypus is unlike any other animal on Earth. It is one of only five extant species of egg-laying mammals in the Order Monotremata (Sub-Class: Prototheria) and the only one within the Family Ornithorhynchidae (Grant 2007). It is also one of very few mammals known to be venomous and the only one with a specialised apparatus for the injection of venom (hollow spurs on the hind ankles) (Grant 2007). In addition, the monotremes are the only group of mammals possessing teatless mammary gland (Grant 2007) and that use electroreception to detect prey, a trait most developed in the platypus (Bullock 1999), . The unique biology and phylogenetic position of the platypus gives the species intrinsic scientific value and warrants special consideration for biodiversity conservation (Crozier 1992).

Distribution

The platypus inhabits lentic and lotic waters along the eastern edge of Australia from the eastern edge of South Australia, as far north as Cooktown in north Queensland (Grant 2007). It can be found in almost every conceivable body of water within its distribution including: lakes, rivers, streams and a plethora of man made waterway, such as irrigation ditches and farm dams (Grant 1992; Grant and Temple-Smith 1998; Otley 2001). On occasion, platypuses are even found in the brackish waters of estuaries in Tasmania and the highly degraded waterways of dense urban metropolises (Grant 1998; Hird 1993; Serena *et al.* 1998).

On the mainland of Australia platypuses exist most prevalently in east-flowing watercourses and in the upper reaches of north and south-flowing waterways along the Great Dividing Range. Platypuses are also common throughout Tasmania and King Island (possibly Three Hummock Island and Bruny Island as well), and there is a stable, introduced population on Kangaroo Island (Grant 1992; Grant 2007; Grant and Temple-Smith 1998; Gust and Handasyde 1995; Serena 1994; Serena *et al*. 2001).

The northern limit of the platypus distribution has always been puzzling; the range continues north along the Great Dividing Range and then abruptly stops around Cooktown (Grant 2007). There are a few ideas as to why this might occur but the exact reason has been unclear until now.

The thermal stress of seasonally high temperatures may be the key variable to limiting platypus distribution. While dense insulating pelage and a complex counter-current arrangement of arteries veins and capillaries in its hind legs help the platypus to maintain its 32 degree body temperature in southern regions, where ambient air and water temperatures can fall below zero (Brice 2009), these features can make living in warmer climates more difficult (Grant 1992; Grant and Temple-Smith 1998). Platypuses are known to be highly intolerant of temperatures above 25 degrees, as they lack any means of evaporative cooling which makes it difficult to shed any excess heat (Grant and Temple-Smith 1998).

Western limits are probably determined by the intermittent nature of rivers draining onto the western plains of eastern Australia during droughts, coupled with their extreme flooding during wet periods. That type of condition probably decreases the suitability of soil and bank types for burrow making (Grant and Temple-Smith 1998). When the warmer water temperatures to the west are also considered, it quickly becomes obvious that that area is inhospitable to a platypus, hence their restriction to the upper, higher altitude, reaches of these waterways where conditions are much more favourable.

Understanding a species' distribution, and the limiting factors responsible for that distribution, is crucial for species conservation, management and ecology in general (Guisan and Zimmermann 2000). Current knowledge of distribution of the platypus is based on sightings (Grant 1992); little is known about the environmental determinants of that distribution.

Home ranges and dispersal

Reported home ranges for adult male platypuses vary from 350 m to 7 km depending on local stream dynamics and carrying capacity (Gardner and Serena 1995; Grant 1992; Grant and Temple-Smith 1998; Serena 1994; Serena *et al.* 1998; Serena *et al.* 2001). Male ranges do overlap in many areas for most the year; however, each has a distinct "core" home range that is smaller and much more exclusive (Gardner and Serena 1995, Serena 1994; Serena *et al.* 1998). During the breeding season male ranges contract to these core ranges which they defend fiercely from other males. The smaller females occupy smaller ranges with greater overlap (especially with juveniles) that occur within those of the males. Most male ranges overlap at least two female ranges, suggesting a polygynous breeding system (Grant *et al.* 1992; Grant and Temple-Smith 1998; Serena 1994). Sex-range correlations in *O. anatinus*, support the basic ecological observation that female distribution in the vertebrates tends to be resource-based while that of males tends to be mate-based (Gardner and Serena 1995). However, researchers readily admit that more behavioural work needs to be done on the female platypus (Gust and Handasyde 1995).

To further complicate the matter of studying platypus distribution, mark-recapture studies suggest that in any given area there are both resident and transient populations (Grant and Temple-Smith 1998). This is most likely due to the dispersal of sub-adults mediated by pressures placed on them by adults as the first (and hardest) winter approaches and transients may become residents when a local population vacancy appears (i.e. a resident adult dies)

(Gardner and Serena 1995; Grant and Temple-Smith 1998). The dispersal potential for the platypus is quite high and individuals have been shown to move as far as 48 km within a seven month period (Grant 2004).

The particular habitat used by any population or individual may also vary with season. During seasonal periods of increased stream flow, an increase in backwater and seasonal pond usage has been noted, with the implication that platypuses avoid water currents too rapid for them (Gust and Handasyde 1995; Serena *et al.* 1998).

Taxonomic history of the platypus

Currently there is only one species of platypus and no recognised subspecies (Grant 2007; Mahoney 1988). However, prior to 1988 there were four recognised subspecies based on geographic isolation and morphological differences (Iredale and Troughton 1934; Thomas 1923). These included:

Ornithorhynchus anatinus [Shaw, 1799]

1. *Ornithorhynchus anatinus anatinus* [Shaw, 1799] nominate subspecies, Coastal New South Wales, Victoria and South Australia. Synonymous with: *Platypus anatinus* [Shaw, 1799], *Ornithorhynchus paradoxus* [Blumenbach, 1800], *Ornithorincus fuscus* [Péron, 1807], *Ornithorincus rufus* [Péron, 1807], *Ornithorhynchus brevirostris* [Ogilby, 1832] and *Ornithorhynchus lævis* [Macgillivray, 1832].
2. *Ornithorhynchus anatinus triton* [Thomas, 1923] New South Wales, west of the dividing range. Characteristically larger than *Ornithorhynchus anatinus anatinus*.
3. *Ornithorhynchus anatinus phoxinus* [Thomas, 1923] North Queensland. Characteristically smaller than platypuses from any other region.
4. *Ornithorhynchus anatinus crispus* [Macgillivray, 1832] Tasmania.

In 1988 family Ornithorhynchidæ was reclassified as a single monotypic species with all previously accepted subspecies being labelled synonymous (Mahoney 1988). No justification for this decision is given in the associated literature, although there is mention of the discontinuous distribution of the platypus (Mahoney 1988). While this thesis alone is not enough to strike down the taxonomy put forward by Mahoney (1988), nor does it directly make any such attempt, it does offer substantial support to the Iredale and Troughton taxonomy (1934). It should be mentioned that there is evidence of large differences in body size / mass and pelage length / colour / density across the platypus distribution (Grant 2007) that also coincides with the regions that the Iredale and Troughton taxonomy (1934) identify as separate subspecies. Whether these are discrete differences or the result of clinal adaptations to differing climates across the distribution, has yet to be investigated.

Evolutionarily Significant Units and Management Units

Instead of a taxonomic approach, this thesis will focus most of the analysis and interpretation of data in terms of conservation units. The units used will be Evolutionarily Significant Units (ESU's) and Management Units (MU's). These terms share some similarity with taxonomic ideas, but there are subtle differences.

An ESU is generally analogous to a subspecies designation in that it refers to a population or group of populations that is reproductively isolated from other groups, genetically distinct and exhibits locally adaptive phenotypes (Palsbøll *et al.* 2006; Conner and Hartl 2004). Particular importance is placed on the genetic distinctiveness of the groups. In fact, the most often cited criterion for establishing ESU's was laid out by Moritz (1994) and states that "*ESU's should be reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci*".

MU's on the other hand can be considered those groups occurring within larger ESU's in which there is enough gene flow to prevent reciprocal monophyly but where gene flow is restricted enough to make each group genetically distinct (Moritz 1994; Palsbøll *et al.* 2006). The distinctiveness of each group must be enough to warrant separate conservation management.

Threats and challenges

The largest pressure on platypus populations comes from urban encroachment and habitat destruction in areas of poor land management (Grant 1992; Grant and Temple-Smith 1998). However, small populations of platypuses are known to exist within the highly degraded waterways around the major metropolitan areas of cities such as Sydney, Melbourne and Hobart (Grant 1992; Grant and Temple-Smith 1998; Serena 2000). One study even showed platypus activity increasing in anthropogenically eutrophied rivers (Kruuk 1993). While most aquatic vertebrates are negatively impacted by an increase of nitrates and phosphates, the invertebrate fauna can drastically increase. As these invertebrates are the primary food source of platypuses, platypus numbers then increase as well (Kruuk 1993). However, the study did not address any kind of long-term toxicity or bioaccumulation of nitrates and phosphates in platypuses.

Platypuses also show no compunction in using man-made structures. They have been known to use suitable drainage channels just as contentedly as natural streams. They will pass unhindered under busy bridges, so long as the flow rate of water does not markedly change (e.g. passing through a narrow culvert). Drowned platypuses have been recorded as the result of them entering underground piping, perhaps not surprising considering that most platypus burrow entrances are underwater (Serena *et al.* 1998). These are all factors that ecologically responsible industry must take into account.

The effects of climate change on the platypus are also unknown. Global climate change has already influenced the distribution of a number of species (Barry *et al.* 1995; Parmesan *et al.* 1999; Reif *et al.* 2008) and is predicted to alter the future distribution of many more (Beaumont and Hughes 2002; Williams *et al.* 2003). As the climate changes so will water availability. As a result, semi-aquatic mammals may be at a particular risk from the indirect effects of climate change, such as anthropogenic stream modifications, as humans attempt to adapt to the changing environment.

Beyond the platypus

Semi-aquatic mammals, such as the platypus, are a diverse but rare assemblage of mammals. Only about 124 out of approximately 5,500 mammal species (spanning ~70 of 1,200 mammalian genera) fall within this group (Veron *et al.* 2008). These include some of the most poorly understood and most endangered mammals on the planet (e.g. otters, tenrecs, water shrews *et cetera*; Veron *et al.* 2008). It is here that the platypus may be of global importance. While still shrouded in mystery the platypus is likely to be one of the most thoroughly studied of the semi-aquatic mammals and has the potential to act as a model species for basic conservation planning. When the varied linearization of populations along waterways, due to varying degrees of dependence on water, is taken into account, most semi-aquatic mammals occupy similar niches and habitats. So, patterns found in the platypus should be applicable to other semi-aquatic mammals.

Aims of the study

The general goal of this thesis is to explore the biogeography of the platypus. A population genetics approach was the primary method employed to investigate the patterns of gene flow, isolation and genetic identity of populations that influence the distribution of the platypus. Predictive distribution modelling based on climatic variables was also utilised to examine the limiting pressures on suitable platypus habitat. To facilitate this goal a series of detailed aims were produced as follow:

- To gain an understanding of the structure and connectivity of platypus populations.
- To understand historical factors that have influenced platypus distribution
- To sample and analyse data from as much of the platypus distribution as possible, with an emphasis on North Queensland
    - To produce a reliable set of microsatellite DNA markers for populations studies on the platypus
    - To model past, present, and future distributions

Scientific questions

The aims of this thesis can be restated in the form of questions that allow the formulation of hypotheses, which in turn form the focal point of each chapter within the thesis. The collective synthesis of these chapters offers an elucidation of the biogeography of the platypus and patterns that can be applied to other semi-aquatic freshwater mammals.

- Where do platypuses occur and why are these areas suitable habitat
- At what geographic scale are platypuses distinct enough to be considered separate populations and what degree of connectivity exists between them?
- Have the reputed distribution gaps in North Queensland lead to genetic isolation and divergence?
- What is the history of such distribution gaps, how long have they existed and why?

Thesis presentation

This thesis has been assembled using a publication-based style. The data chapters are represented by five manuscripts that have either been published or are in peer-review for publication. While each manuscript is, by its nature, a stand-alone piece of work, when placed together in the context of this thesis they provide the synthesis of ideas needed to address the aims and objectives of this study.

In this style of thesis there may be some repetition between chapters. However, each chapter, regardless of any overlap in samples used or methods employed, addresses a different aspect of platypus populations.

The only change made to the manuscripts from versions submitted to journals has been minor formatting changes in order to match style across the thesis. The style in use is Harvard as modified by the Australian Journal of Zoology. When currently unpublished data that is included elsewhere in this thesis is referenced within a chapter, the in text citation will indicate the appropriate chapter in small caps formatting (e.g. CHAPTER 1).

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Section 2 – History

This section will explore the history of platypus populations in Australia from a biogeographical context.

Plate 2. Release of platypus.
(Photo© S Kolomyjec)

Chapter 2 – Climate and the platypus

The following chapter has been reviewed by co-authors and prepared for forthcoming journal submission as:

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Climate and the platypus (*Ornithorhynchus anatinus*): past, present and future perspectives

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Abstract

Understanding the factors influencing the distribution of a species is necessary for informed management and conservation planning. The current distribution of the platypus is well known, but there has been no formal analysis of the environmental determinants of that distribution. Here we use a large data set of sighting records (n=4,315) to generate predictive climatic models that examine range-limiting environmental factors. We then project this distribution into the past to reconstruct the species' distribution at the Last Glacial Maximum (LGM) and also use future global climate projections to represent the distribution in 2030, 2050, and 2060. The factors contributing most to environmental suitability for the platypus were precipitation in the driest quarter of the year (53.8%) and the temperature during the hottest quarter (41.2%). Platypuses are limited by water availability, particularly as many fluvial systems in Australia are seasonally ephemeral. Temperature has been one the primary factors hypothesized in regards to the northern limit of the platypus distribution by researchers for decades. Our predictive models also support observations of regions lacking platypuses, which create distribution gaps in north Queensland. Projection onto the climate of the LGM suggests that the gaps were also present then. The gaps are on a scale that may warrant the future resurrection of regional sub-species nomenclature, particularly when combined with other observational data on northern Queensland platypuses and considering this historical evidence. Projection of the models into the future suggests that climate change may result in a reduction of habitat that is suitable for the platypus.

Introduction

Understanding a species' distribution and the limiting factors responsible for that distribution is a crucial part of species conservation, management and ecology in general (Guisan and Zimmermann 2000). Global climate change has already influenced the distribution of a number of species (Barry *et al.* 1995; Parmesan *et al.* 1999; Reif *et al.* 2008) and is predicted to alter the distributions of many more (Beaumont and Hughes 2002; Williams *et al.* 2003). While many factors can influence a species distribution, recent evidence suggests that climate models can be an excellent predictor of biological trends (Parmesan and Yohe 2003).

The platypus *Ornithorhynchus anatinus* is common in many parts of Australia, and while it is assigned the conservation status 'least concern' by the IUCN (Lunney *et al.* 2008) it is inherently vulnerable to threatening processes because of its dependence on freshwater habitat (Grant 2007; Veron *et al*. 2008). At both the global and national level, riverine systems, prime habitat for the platypus, are under constant threat of anthropogenic flow alteration and pollution (Goudie 2006; Kingsford 2000). In Fact, range reductions in the platypus have been well documented near human population centres (Grant 1998; Lintermans 1998; Lunney *et al.* 1998; Otley 2001; Rohweder and Baverstock 1999; Serena *et al.* 1998).

Despite its apparently secure status, the platypus should still be considered during management planning. Being the only amphibious, egg-laying mammal that forages using electroreception while having venomous spurs for defence, it is unique in many ways (Grant 2007). In addition to its perplexing biology, the special phylogenetic position of the platypus also warrants consideration for biodiversity conservation (Crozier 1992).

The platypus inhabits both lentic and lotic waters along the eastern edge of Australia from the eastern edge of South Australia up to about Cooktown in Queensland as the northern limit (Grant 2007). It is most prevalent in east-flowing water and the upper reaches of north and south-flowing waterways along the Great Dividing Range (Grant 2007). Platypuses are also common throughout Tasmania and King Island, and there is a stable, introduced population on Kangaroo Island (Grant 1992, 1998; Gust and Handasyde 1995; Serena 1994; Serena *et al.* 2001).

Within its distributional range the platypus may be common, albeit elusive. It has a confirmed presence in fifteen out of nineteen Tasmanian river systems, 26 out of 31 river systems in Victoria, all 22 east-flowing river systems and 13 out of 17 west-flowing systems in New South Wales, and 24 out of 71 river systems in Queensland (none in Cape York or rivers draining into the Gulf of Carpentaria) (Grant 1992). With the exception of a possible distribution break spanning approximately 250 km between the regional cities of Proserpine and Paluma in north Queensland that has been observed in platypus occurrence records and is supported by population genetic data (sightings data: the Queensland Platypus network; genetic data: CHAPTER 7), the platypus enjoys a relatively continuous distribution across its range (Grant 2007).

The reason that platypuses occur in some waterways but not others has remained unclear as the contribution of environmental factors to their distribution have been poorly understood. In light of potential distributional gaps, we need to explore the historical contribution of climate to this distribution and also the potential effects of future climate change. What causes the northern extent of the distribution to just stop in the midst of seemingly suitable habitat is a particularly vexing mystery (Grant 2007). The common hypothesis is that higher temperatures in the lower latitudes make the habitat ill suited for the platypus (Grant 1998; Grant and Temple-Smith 1998; Gust and Handasyde 1995; Serena 1994; Serena *et al.* 2001; Grant 1992).

Our aim was to use modern predictive modelling techniques to resolve this long-standing mystery and determine why platypuses are found where they are. In doing so we have endeavoured to answer several key questions:

1. What factors determine where platypuses are found?
2. Does temperature limit the range of platypus distribution to the north?
3. Does climate-based distribution modelling support the distribution gaps suggested by observations and population genetics analysis?
4. How has platypus distribution changed since the Last Glacial Maximum?
5. How will climate change affect the platypus distribution in the future?

Materials and Methods

Location data

Several sets of data containing sighting-based occurrence records were sourced from: the Queensland Platypus Care Network (now part of The Platypus Conservancy, http://www.platypus.asn.au), the Atlas of New South Wales Wildlife (Dept. of Environment and Conservation, http://wildlifeatlas.nationalparks.nsw.gov.au), the Victorian Department of Sustainability and Environment (http://www.dse.vic.gov.au) and the Tasmanian Natural Values Atlas (Dept. Primary Industries, Parks, Water and Environment, http://www.naturalvaluesatlas.dpiw.tas.gov.au).

After the raw data were acquired, all usable data combined into one data set. Records with no useable location data were removed, as were obviously spurious data points (such as those that mapped to the ocean), and clerical errors made during original data entry were corrected when possible. The scale of the data set made site by site verification impractical. The final data set contained 4,315 usable records (Figure 2.1).

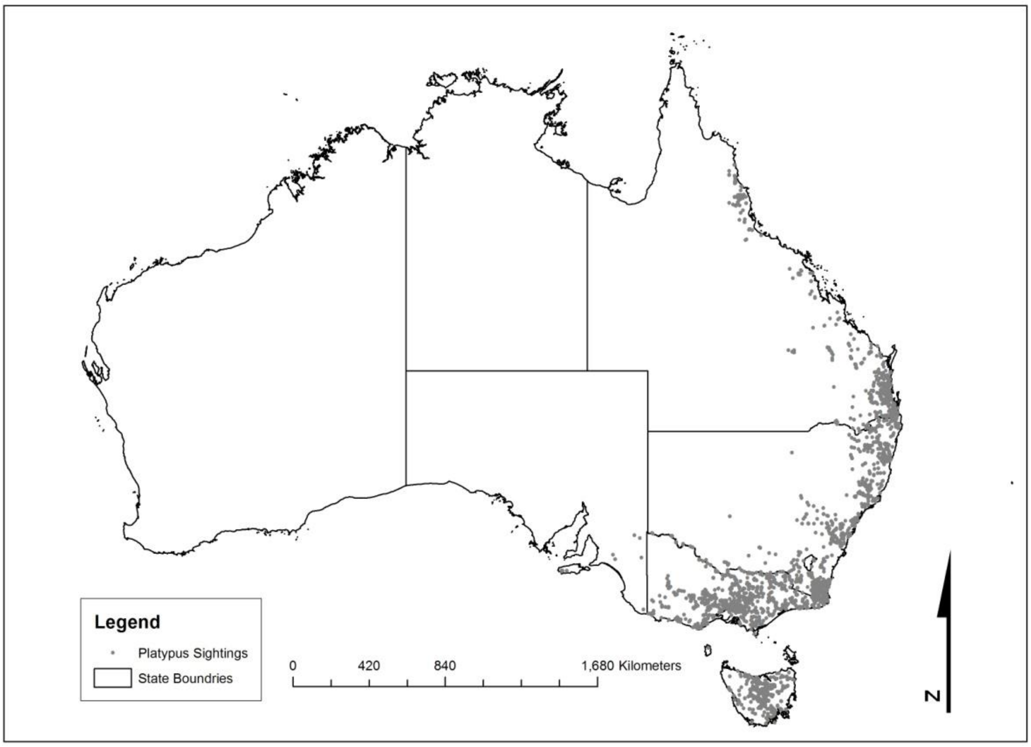

Figure 2.1 Sightings based occurrence records of the platypus across Australia. n = 4,315

Bioclimatic Modelling

Present

We utilized the maximum entropy algorithm employed by the software MAXENT (Phillips *et al.* 2006) to model the potential distribution of platypus across Australia. This algorithm represents an approach to species distribution modelling that has been shown to outperform other algorithms or techniques traditionally used in this type of study (Elith *et al.* 2006; Henandez *et al.* 2006). MAXENT has been optimized to use presence-only information (Phillips and Dudik 2008; Phillips *et al.* 2006) and has been shown to be insensitive to number of occurrences and can handle small number of occurrences (Henandez *et al.* 2006; Pearson *et al.* 2007). MAXENT produces spatial predictions of environmental suitability as a value between 0 (not suitable) and 1 (most suitable). Cells in the prediction that were outside the environmental conditions of the training data were also identified (termed as clamped predictions). Because there is no simple way to project the response of an organism outside the environmental conditions of the training data set, MAXENT assumes the response of the maximum or minimum value of the training data for projecting values beyond the conditions it was trained. Thus, MAXENT identifies such predictions onto novel environments as clamped and removes areas with a high degree of computational uncertainty from the final model.

Eight environmental variables were used as predictors in the final model: annual mean temperature, temperature seasonality, maximum temperature of warmest period, minimum temperature of coldest period, temperature annual range, annual precipitation, precipitation seasonality, precipitation of wettest quarter and precipitation of driest quarter. Climate layers for current climate were estimated using the Anuclim 5.1 software (McMahon *et al.* 1995) and a nine second DEM (Version 2; Geoscience Australia, http://www.ga.gov.au/). Thresholds for the distribution map were set between 0.246 and 1. This indicated by the MAXENT output as the occurrence threshold with the maximum sensitivity and maximum specificity for the model.

Because the locality records suggest a distribution break between north Queensland and southern populations, which was also identified by initial modelling, distributions were then modelled separately for populations to the north and south of this break. This was done to view the tropical northern region of platypus distribution independently from the main distribution to the south in order to determine if the separate regions were under different climatic constraints. This modelling was carried out exactly as described above except the list of sightings was split into two at latitude 20 degrees South as this was approximately the midpoint in the 1.5 degree gap detected between Northern and Southern regions of sightings.

Past distribution

Climate models of the current distribution were then projected onto two global climate model reconstructions (CCSM and MIROC) for the Last Glacial Maximum (-21,000 BP) at 5km resolution, in order to predict the extent of suitable habitat available to *O. anatinus* at that time. These surfaces were downloaded from Worldclim-1 (www.worldclim.org). Further description of employed methodology and implementation can be found in VanDerWal *et al.* (2009).

Future

To model the effects of future climate change on the platypus the models were first trained to two climate baselines (Raupach *et al.*, 2008; 2009): the climate of 1975 (a 30-year summary of 1961-1990) or-of 1999 (an 18-year summary of 1991-2008). While the 1975 baseline represents the climate baseline commonly used to represent ‘recent’ climate conditions, the 1999 baseline serves as a distinct baseline that covers the time frame in which the majority of the sightings records were recorded. As this 1999 baseline was considered more likely to provide a more realistic current distribution (i.e. one that included the actual sighting locations and provided a better fit to the sightings data), it was used to project the models into the future.

The models were projected onto climates representing 30-year averages centered on 2030, 2050 and 2070 based on multiple global climate models (GCMs) and emission scenarios as provided by the IPPC 4th assessment (Pachauri and Reisinger 2007). These GCMs included BCCR, CSIRO mark 3.0 and 3.5, GISS AOM, INMCM, MIROC 3.2 high res and medium res, and NCAR CCSM 3.0. Emissions scenarios included SRES B1, SRES A2 and SRES A1B. This produced 25 individual scenarios for each of the 2030, 2050 and 2070 models. From the predicted distributions for each of the future climate periods, an aggregated mean and standard deviation of all scenarios was produced in order to provide the most likely outcome for each time frame.

Results

Present

The modelled distribution for platypuses (Figure 2.2) encompasses a long but often narrow range along the east and southeast coasts of the Australian mainland as well as Tasmania, Kangaroo Island and King Island where platypuses are known to occur. In addition the model also indicates that the current climate conditions on Flinders Island and southern Western Australia are potentially suitable for the platypus (Figure 2.2).

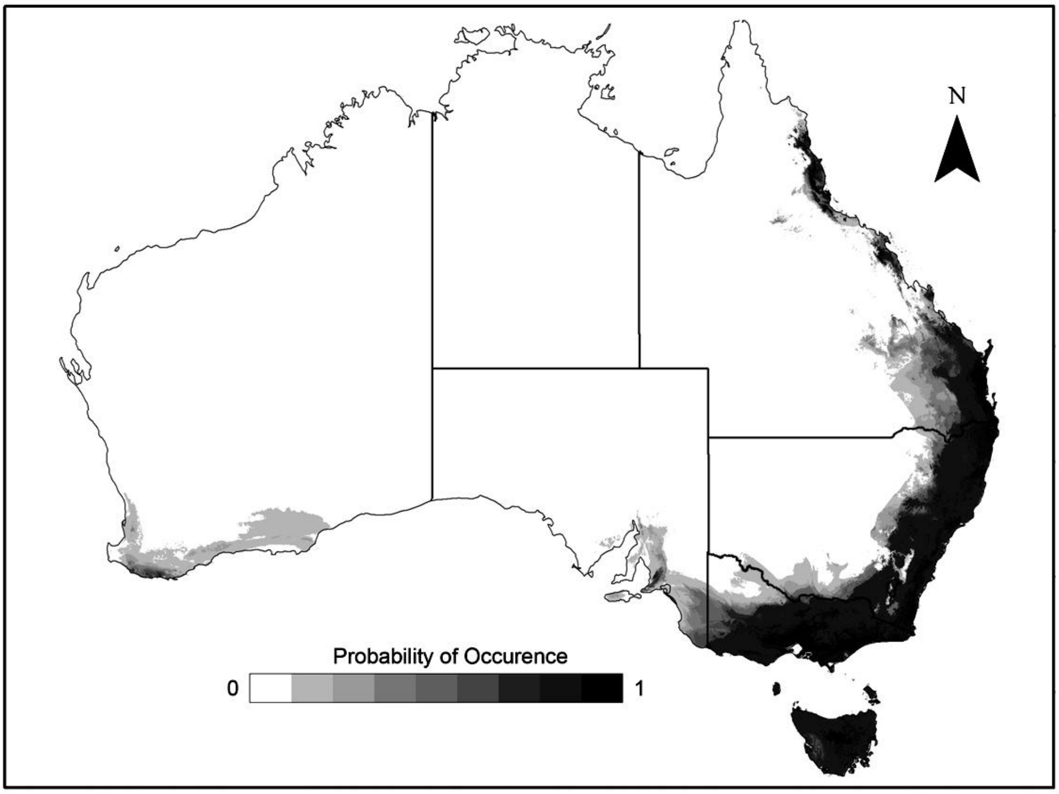

Figure 2.2 Present day distribution of the platypus. Shading indicates predicted habitat suitability.

The two climatic variables contributing most to the model (95.2% of factors responsible for platypus distribution) were precipitation during the driest quarter of the year (53.8%) and maximum temperature during the hottest quarter (41.4%) (Table 2.1).

Table 2.1 - Climatic constraints on platypus habitat across Australia.
Factors in **bold** represent 95% of environmental contribution to habitat suitability.

Climate Factor	% Contribution
Precipitation during driest quarter	**53.8**
Maximum temperature of warmest period	**41.2**
Temperature seasonality	1.7
Annual precipitation	1.7
Precipitation seasonality	0.9
Annual mean temperature	0.2
Minimum temperature of coldest period	0.2
Precipitation during wettest quarter	0.1

Modelled separately (Figure 2.3) the regions to the north and south of the distribution break in north Queensland predict little or no suitable habitat in the opposite region. However, both models support the suitability of Kangaroo Island where an introduced population does indeed flourish. The combined areas of predicted distribution for the independently modelled regions (Figure 2.3) closely approximates the distribution predicted by the initial model (Figure 2.2), indicating that platypuses within each region may have different habitat requirements.

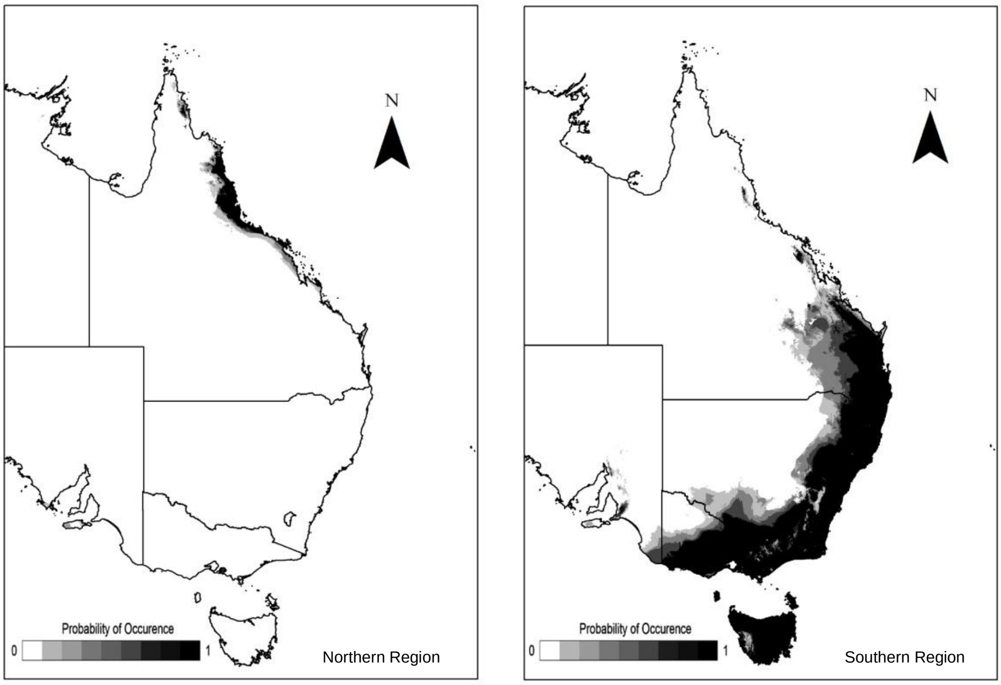

Figure 2.3 North and South regions modelled independently.

The climatic variables contributing to each region's independent model differed (Table 2.2). The variables accounting for 96.7% of the contribution to the northern model are maximum temperature during the hottest quarter (34.7%), temperature seasonality (22.6%), precipitation during the wettest quarter (19.2%), and minimum temperature during the coldest period (16.7%). Those contributing to 95.1% of the main region were precipitation during the driest quarter of the year (76.2%) and maximum temperature during the hottest quarter (18.9%).

Table 2.2 - Climatic constraints on platypus habitat based on region. Factors in **bold** represent 96.7% and 95.1% of environmental contribution to habitat suitability for the northern and southern regions respectively.

	Climate Factor	% Contribution
Northern Region	***Maximum temperature of warmest period***	**34.7**
	Temperature seasonality	**22.6**
	Precipitation during wettest quarter	**19.2**
	Minimum temperature of coldest period	**16.7**
	Precipitation seasonality	**3.5**
	Precipitation during driest quarter	2.1
	Annual mean temperature	1
	Annual precipitation	0.1
Southern Region	***Precipitation during driest quarter***	**76.2**
	Maximum temperature of warmest period	**18.9**
	Temperature seasonality	3.4
	Minimum temperature of coldest period	0.6
	Precipitation seasonality	0.5
	Annual mean temperature	0.2
	Annual precipitation	0.1
	Precipitation during wettest quarter	0.0

Last Glacial Maximum

As differences in predictions yielded by the two Last Glacial Maximum GCM models were minimal, we chose only to present the results from CCSM, as it provided the more conservative estimate of past distribution.

The modelled distribution at the LGM (Figure 2.4) depicts that overall conditions were less suitable for the platypus; however there was still an ample area of suitable climate space. The LGM model, like the present-day model, depicts a distribution break between what is now northern Queensland and the rest of Australia.

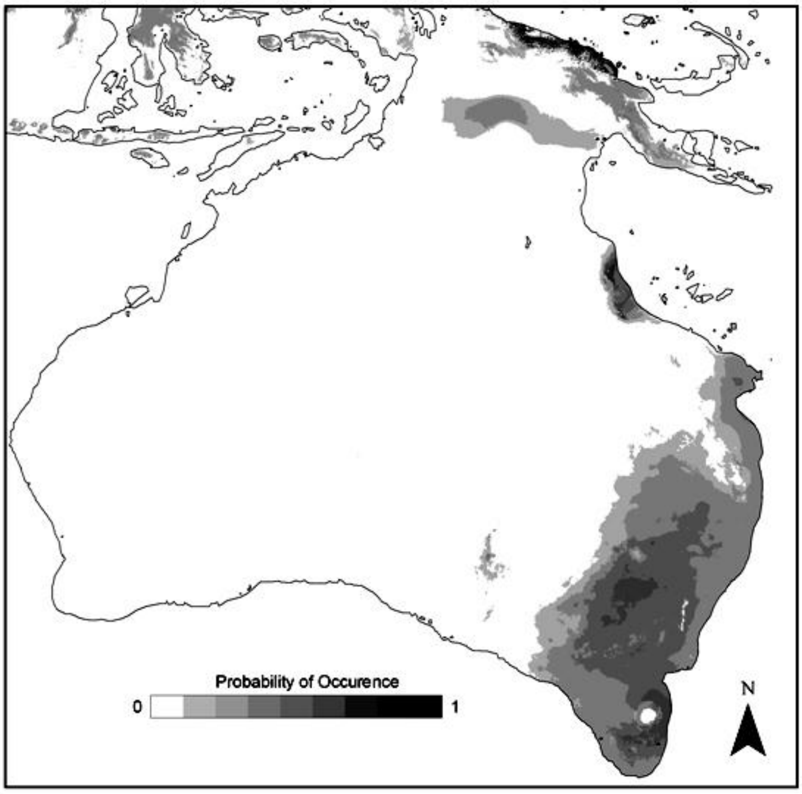

Figure 2.4 Distribution of the platypus during the Last Glacial Maximum. Based on predicted habitat suitability during the LGM, c. 21,000 BP.

Effects of future climate change

The aggregated results from modelling platypus distributions into the future depicts a minor overall reduction in the potential distribution of the platypus at each of the 30 year intervals surrounding 2030, 2050 and 2070 (Table 2.3). While the aggregated result is generally accepted as the most likely scenario, we have also included the best and worst case scenarios based on distribution area (Table 2.3).

Table 2.3 Predicted distributional changes due to climate change.

	Change from Present Distribution Area		
Projection Year	Mean (± Standard Deviation)	Best Case Scenario	Worst Case Scenario
2030	- 5.20% (± 5.20%)	+ 2.59%	-19.10%
2050	- 14.27% (± 21.25%)	+ 3.53%	-68.19%
2070	- 15.22% (± 19.76%)	+ 2.13%	-65.08%

Discussion

The use of bioclimatic modelling to produce a set of species distribution models has provided some interesting results. The output depicting current distribution (Figure 2.2) closely matches with known occurrence (Figure 2.1). That, in and of itself, is not particularly surprising as the output was based on known occurrences and current climate data. What is interesting is the indication that the isolation of north Queensland from the main platypus distribution is due to large areas that are climatically ill suited to the platypus. It also supports the suitability, although marginal, of the platypus on Kangaroo Island (South Australia), where an introduced population was established as the result of several translocations between the late 1920’s and early 1940’s (Grant 2007).

There are only two notable incongruities observed in this distribution map. They are Flinders Island (Tasmania) and the southern most aspect of Western Australia. No platypuses have ever been reported on Flinders Island (Tasmania) and no paleontological attests to them having ever lived in what is now Western Australia (pers. comm. Anne Musser). However, between 1940 and 1951 there were attempts to introduce the platypus to the region south of Perth (Western Australia) (Grant 2007). These introductions are believed to have been unsuccessful but there have been unsubstantiated sightings as recently as 2006 (Grant 2007).

The two environmental factors that are most important (95% contribution; Table 2.1), to the near exclusion of all others, in climatic suitability for the platypus are: the amount of precipitation during the driest quarter and the maximum temperature during the warmest quarter. The importance of these environmental factors is supported by previous research efforts into platypus physiology.

As a semi-aquatic mammal the platypus requires water year-round. While evidence indicates that platypuses are capable of moving substantial distances over land when necessary (Grant 2007; Kolomyjec 2009), they depend on water for feeding habitat (Grant 2007), predator avoidance (Rakick *et al.* 2001; Richards 1986; Seale 2008) and due to the physiological constraints imposed by evolutionary adaptation to an aquatic environment (Bethge *et al.* 2001; Fish *et al.* 1997, 2000, 2001).

The biological importance of maximum temperature to the platypus may be especially great in view of its low body temperature (approximately 32° C), amongst the lowest of any mammal (Grant 1983). Due to the very dense pelage of the platypus acting as insulation against cold water temperature, the only sites for rapid heat exchange are the bill and feet. Once the water and air temperatures exceed the platypus's body temperature, hyperthermia would rapidly set in while the animal attempts to forage (Grant 1992; Grant and Temple-Smith 1998). Increased time in underground burrows, which would maintain a degree of isothermal consistency throughout the year (Bethge *et al.* 2004), could, behaviourally, allow for a degree of amelioration of these effects. However, there are no studies that have looked at platypus burrow temperatures in northern Queensland, let alone the correlation between ambient temperature, burrow temperature and time spent resting. Even assuming some degree of behavioural adaptation to high ambient temperature, maximum temperature would still be likely to act as a sharp physiological threshold that determines habitat suitability for the platypus.

Given the large climatic differences between the tropical northern region of platypus distribution and the sub-tropical to alpine climates that encompass the main distribution, it is again no surprise that different environmental factors (Table 2.2) have different weight in the determination of suitable habitat for each of the two regions.

The southern region of platypus distribution, modelled independently, indicates that the same two environmental factors as the combined model are key (95.1% contribution, Table 2.2) to determining suitable habitat. However, the importance of precipitation during the driest quarter increases from the 53.8% contribution seen in the overall model to a 76.2% contribution in the model for the southern region alone. As parts of this region are more subject to droughts than the aptly named Wet Tropics, the minimum amount of water entering the region becomes increasingly important.

The environmental factors important in determining habitat suitability in the northern region, however, are very different. Here, 96.7% of environmental contribution (Table 2.2) is spread across five different variables: maximum temperature of the warmest period, temperature seasonality, precipitation during the wettest quarter, minimum temperature of the coldest period, and precipitation seasonality. While the complex interplay of these environmental factors is more difficult to interpret, some important links can be discerned.

Maximum temperature representing the highest contributing factor in this region supports our earlier discussion on the importance of temperature to the platypus. This also supports the long standing hypothesis that temperature is the key limiter to the northern extent of platypus distribution.

The importance of precipitation during the wettest quarter and precipitation seasonality instead of the driest is also logically consistent. Much of northern Queensland experiences a seasonal wet season, colloquially referred to as 'The Wet', characterised by extreme rainfall periods resulting in rapid and intense flooding throughout the Wet Tropics. Considering one of the author's field sites experienced an increase in water level of approximately eight meters at the peak of the wet season (pers. obs. S. Kolomyjec), sudden flooding has the potential wash away platypus burrows and the young that they may contain at that time of year (Grant 2007).

The inclusion of minimum temperature during the coldest period in this model may seem surprising but given the physical differences of platypuses in this region (e.g. much smaller size and shorter pelage; Grant 2007), they may have undergone some evolutionary adaptation to the warmer climate at the cost of some of the cold hardiness enjoyed by their southern counterparts. This is supported by the fact that taken separately, the two models indicate little to no suitability of habitat in the opposing region (Figure 2.3). There may have been ample time for this type of adaptation as the genetic data suggest these regions have been separated for a very long time (CHAPTER 7).

This regional isolation is further supported by the modelling of the distribution during the last glacial maximum. The LGM distribution model depicts the same gap between what is now northern Queensland and the rest of the distribution. During the LGM Australia was significantly drier as a whole (Nevill 2010). This is reflected by climatic suitability being much poorer at the LGM than it is at present. There was, however, substantial area that was still usable by the platypus and each region maintained a central area of higher suitability. It remains unknown when the last period of connectivity between the northern and southern regions was. It would most likely have been during a past inter-glacial wet period.

Modelling the platypus distribution under various climate change scenarios gives us a glimpse of the near future of the platypus. Using an aggregated model for each of the 2030, 2050 and 2070 data sets there is a predicted decline in the total area of suitable habitat of approximately 15% by the year 2070 (Table 2.3). This mean is generally considered a more realistic prediction than any single GCM outcome as individual model results ranged from an increase in distribution area of 3.5% to a decrease of 68.2%. Most of this change occurs between the 2030 and 2050 time periods after which the rate of decline greatly decreases by the 2070 projection, suggesting the impact of continued climate change reaches a plateau rather than having a continued linear effect on distribution.

While not a trivial decline, the change in available habitat over such a large overall distribution manifests itself as a contraction of the marginal areas at the edges of the current distributions, particularly on the western side of the Great Dividing Range. This contraction of available habitat is likely to represent a very broad-scale altitudinal shift as higher altitudes are more likely than lowland areas to retain temperature and precipitation levels that are suitable for the platypus. These kinds of shifts are well detailed in the literature (e.g.: Hickman *et al*. 2006; Lenoir *et al*. 2008; Parmesan 2006). Due to the highly generalised diet of the platypus (Herrin 2009; Grant 2007; McLaughlin-Troup 2010) it is likely to persist in any waterway that falls within its required environmental space. However, this persistence is likely to be highly influence by non-climatic, anthropogenic factors. The introduction of non-native invasive species could result in the platypus being out competed for food, or other changes to riverine habitat that may render it unsuitable for the platypus. As climate change alters the availability of freshwater on a global scale, the extraction of water from river systems for human use is also very likely to have an impact on the platypus.

The platypus is a hardy animal that has weathered several climate cycles in the past 5 million years (Grant 2007) and should survive this period of warming so long as critical parts of its distribution remain intact. These core regions can be clearly seen in the palaeo-modelling as those central regions in both the northern and southern distributions that remained most suitable during the extremes of an ice age. As long as these regions remain intact, humans remain vigilant in their stewardship of riverine habitats and aware of the platypus, there is no reason that future generations will not be able to enjoy the sight of platypus swimming in Australian waterways

Acknowledgements

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Section 3 – Relationships

This section explores the relationships within and between platypus populations at various scales of interpretation. A set of microsatellite DNA markers were developed to aid in this task. Implications for conservation and management are also discussed.

Plate 3. Adult female platypuses demonstrating extreme size difference at opposite extremes of the distribution: individual from northern Queensland on left and individual from Tasmania on right. (Photo© S Kolomyjec)

Chapter 3 – Microsatellite DNA Marker Development

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Ten polymorphic microsatellite DNA markers for the platypus *Ornithorhynchus anatinus.*

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Abstract

We identified and optimized ten microsatellite loci for the platypus *Ornithorhynchus anatinus* (Monotremata: Ornithorhynchidae) and screened 21 individuals from the southern tablelands area of New South Wales, Australia. Each polymorphic locus possessed between 2 and 12 alleles with observed heterozygosities between 0.118 and 0.950. The intent of this effort was to provide informative loci for studies on the population genetics of this species.

Primer Note

The platypus is a prototherian mammal endemic to the freshwaters of eastern Australia and Tasmania (Grant 1992; Grant and Temple-Smith 1998). Recapture data, combined with preliminary genetic investigation, suggest that platypus populations are made up of both resident animals and transient individuals that occupy waterways that have sufficient resources to maintain survival and reproduction (Akiyama 1998; Grant and Temple-Smith 1998). Early molecular work supported a genetic isolation-by-distance pattern with continuous gradation between distinct core populations on the mainland, but with the Tasmanian population being genetically distinct (Akiyama 1998; Akiyama *et al.* 1996). Updated and increasingly accurate molecular tools will allow the better understanding of platypus population structure and mating system. This understanding is critical to the conservation and management of the species.

The genome sequence from the platypus whole genome shotgun (WGS) project (GenBank AAPN00000000, genome project #12885) was scanned, chromosome by chromosome, for potential microsatellite-type motifs using SciRoKo (Kofler et al. 2007). The results were manually filtered based on motif characteristics such as: length, uniqueness of flanking regions, and physical location on the chromosome. Three loci were identified prior to the assembly of the genome (Table 3.1: Platy04, Platy08 and Platy19). This was accomplished by entering possible repeat motifs into BLASTN and searching the platypus genome project trace archive. Once found, trace files were assembled into contigs in BIOEDIT (Hall 1999). These three loci are not yet localized to a chromosome. Oligonucleotide primer pairs were designed, using PRIMER 3 (Rozen and Skaletsky 2000), to amplify 10 tri-, tetra-, penta- and hexanucleotide microsatellites identified during the searches outlined above. Fluorochrome labels (FAM, HEX or TET) were attached to the 5’ end of the forward primer during synthesis (Invitrogen). Details of primer sequences are given in Table 3.1.

DNA was extracted from toe-web biopsies using a proteinase K/salt precipitation method (Sunnucks and Hales 1996). Microsatellite loci were amplified for 21 individuals from the southern tablelands, New South Wales, Australia, using an Eppendorf Master Gradient thermal cycler. The polymerase chain reactions (PCRs) were optimized in such a way that each locus could be amplified individually at a single annealing temperature of 60.5ºC in conjunction with one of five varied reagent mixtures (Table 3.2). Thermal cycling parameters were: an initial hold at 94 ºC for 5 min, then 35 cycles at 94 ºC for 1 min, 60.5 ºC for 30 s and 72 ºC for 50 s, and a final extension at 72 ºC for 5 min. Aliquots of the PCR product were electrophoresed on 1.5% agarose gels pre-stained with ethidium bromide to verify successful amplification. PCR products were then purified via sodium acetate/ethanol precipitation prior to genotyping on a GE MegaBACE 1000. Allele sizes were calculated with MegaBACE Fragment Profiler (GE).

All 10 microsatellite loci were polymorphic (Table 3.1), with 2-12 (average, 7.2) alleles per locus and observed heterozygosities ranging from 0.118-0.950 (average, 0.718). Hardy-Weinberg and linkage tests were conducted using FSTAT (Goudet 2001) and GENALEX (Peakall and Smouse 2006). There was no significant linkage disequilibrium between loci tested following sequential Bonferroni correction (adjusted p-value for 0.1% nominal level = 0.000002). While *F*is values (Table 3.1) tended to be slightly negative, none was smaller than would be expected via random permutation (p-value 5%). This indicates that the population is in Hardy-Weinberg Equilibrium. These microsatellite markers will provide valuable tools for the study of *O. anatinus* populations.

Table 3.1 Microsatellite loci from *Ornithorhynchus anatinus*. Shown are: locus name, primer sequence, chromosome on which the locus is located where known, repeat motif, position of motif on chromosome, specific reagent mixture for individual PCR, observed size range (bp), number of alleles (k), observed heterozygosity (H_O), expected heterozygosity (H_E), and *F*is per population (one population). The annealing temperature (T_a °C) was 60.5°C for all PCR reactions. Individuals genotyped (N) = 21.

Locus	Primer sequences (5'-3')‡	Chromosome	Motif	Motif Start Position	Reagent Mixture	Size range	k	H_O	H_E	*F*is
OA.03.GTAG	F: fam-GCATTTAAAACAAGGCACTGG R: AGGCTGAAATCCATCTTTGC	3	$(GTAG)_{13}$	16574981	E	209-253	6	0.810	0.726	-0.091
OA.05.GTAGG	F: fam-TTCTCCTGGAGGAAGTGTGA R: GGTTTTGTGGGGGTTTTTGT	5	$(GTAGG)_{10}$	24104326	A	145-244	7	0.800	0.714	-0.008
OA.06.TAAC	F: tet-TCTTGGGCAAGTACCAACAA R: CTTAATCCCCATTTGGCAGA	6	$(TAAC)_7$	1861109	C	142-164	9	0.900	0.738	-0.196
OA.06.TATATC	F: tet-CACTCCCTTCTGCACACTCA R: CCACATGGAGCTCACAGACT	6	$(TATATC)_7$	8800932	C	149-188	7	0.900	0.783	-0.091
OA.07.GTTA	F: tet-GCCACCTGAAGTGGAATTAGA R: CTTGACTTCCCTTGCCTCTG	7	$(GTTA)_{12}$	22767154	E	143-169	9	0.857	0.799	-0.048
OA.11.CCA	F: tet-AACTCTCCCCCTCCATCACT R: GAAGGTGTCCTGCAAGTCCT	11	$(CCA)_6$	251083	C	154-157	2	0.150	0.139	-0.053
OA.12.TCCAT	F: tet-GGGTCGCTCCTGGATAGTTT R: TTCCACTCACTAGCCACTGC	12	$(TCCAT)_{11}$	6178749	E	134-159	8	0.750	0.771	0.107
Platy04	F: hex-GGGAAAGCGTGAAAGAGATG R: CACTCCTAATCCCCGTTTCA	-	$(TAG)_7$	-	A	216-219	2	0.118	0.208	0.465
Platy08	F: tet-GAAAGCAGGCAGAATGGAAC R: AGCTTCTGACAGGGCTGAAA	-	$(CAG)_{24}$	-	B	211-250	10	0.950	0.837	-0.109
Platy19	F: fam-GTGGACGGACACGCATGTAT R: CATGGGACAACCTGCTTACC	-	$(TAA)_{12}$	-	D	127-169	12	0.947	0.850	-0.021

‡Primers were 5' end labeled with the fluorochrome indicated.

Table 3.2 Details of reagent mixtures A-E concentrations for 15 µl PCR reactions.

Mixture	Primer 1 (pM)	Primer 2 (pM)	Template (ng)	$MgCl^{2+}$* (mM)	dNTP's (µM)	10x NH_4 Buffer*† (µl)	Polymerase* (units)
A	3	3	10	1.7	267	1.5	0.5
B	3	3	20	2.3	400	1.5	0.5
C	5	5	5	1.7	400	1.5	0.5
D	5	5	20	1.0	267	1.5	0.5
E	7	7	5	2.3	267	1.5	0.5

*included with Bio TAQ Red kit (BioLine)

†160mM $(NH_4)_2SO_4$, 670mM Tris-HCL (pH 8.8 at 25ºC, 0.1% Tween -20 (recipe from Bio TAQ Red product insert)

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Chapter 4 – Populations and River Systems

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Population genetics of the platypus (*Ornithorhynchus anatinus*): a fine scale look at adjacent river systems

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Abstract

Population genetics is a powerful tool to increase the understanding of animals that may otherwise be difficult to study, such as the platypus (*Ornithorhynchus anatinus)*. Focusing on two adjacent river systems in New South Wales, we used 12 polymorphic microsatellite loci to investigate the population dynamics of the platypus. We found that individual river systems acted as discrete population units. Evidence of migration, presumably overland, between systems explains how these units remain connected. This establishes an isolation-by-distance pattern that maintains species continuity across most of the mainland distribution. This improved understanding of population structure will be a valuable contribution to designing accurate management plans for the long-term conservation of this unique Australian animal.

Introduction

In a changing world where biodiversity management has become of vital importance, the study of population genetics is playing an increasingly important role, giving researchers and wildlife management planners insight into the nature and dynamics of animal populations that may be unachievable with conventional field techniques.

Defining distinct populations is a crucial step in the conservation of any animal, as it allows the assignment of management units (MUs) to best preserve crucial traits, both phenotypic and genotypic (Moritz 1995). Management units can be defined as groups of animals that are demographically independent (i.e. not reliant on external recruitment for maintenance of a stable gene pool) and are sub-units of larger Evolutionarily Significant Units (ESUs), which represent regions of divergence due to historical isolation (Moritz 1995; Moritz 1999; Palsbøll *et al.* 2006).

The platypus is a prototherian mammal endemic to the freshwaters of eastern mainland Australia and Tasmania (Grant 1992; Grant and Temple-Smith 1998). Platypuses are common in many parts of their distribution and are considered to be of "least concern" in terms of global conservation status as assessed by the IUCN (2008). However, their inherent vulnerability cannot be overlooked (Lunney *et al.* 2008). At both the global and national level, riverine systems are under constant threat of anthropogenic flow alteration and pollution (Goudie 2006; Kingsford 2000). At the local scale, both range reductions and expansions of platypuses have been reported (Grant 1998; Lintermans 1998; Lunney *et al.* 1998; Otley 2001; Rohweder and Baverstock 1999; Serena *et al.* 1998).

Even without any sign of imminent danger of extinction, the platypus should still be considered during management planning. Being the only amphibious, egg-laying mammal with venomous spurs (on the males) that forages using electroreception, it is unique (Grant 2007). In addition to its fascinating biology, the phylogenetic position of the platypus offers an unprecedented insight into mammalian evolution (Warren *et al*. 2008) and warrants special consideration for biodiversity conservation (Crozier 1992).

Little is known about the population dynamics of the platypus. Recapture data, combined with preliminary genetic investigation (Akiyama *et al*. 1996; Akiyama 1998; Gemmell *et al*. 1992; Gemmell 1994), suggest that platypus populations are made up of both resident animals and transient individuals that occupy waterways possessing sufficient resources to maintain survival and reproduction (Akiyama 1998; Grant and Temple-Smith 1998). Akiyama's research proposed that distinct core populations exist in an isolation-by-distance scenario with continuous gradation across mainland Australia; Tasmania was found to be genetically distinct (Akiyama 1998). However, no previous study has used modern genetic tools to look at migration between platypus populations in relation to factors such as geographic distance or putative geographic barriers to dispersal. However, detailed study of the platypus from a molecular biology point of view is now feasible due to the completion and publication of the platypus genome (Warren *et al.* 2008).

In this study, we use a recently published set of microsatellite DNA markers (Kolomyjec *et al.* 2008) as a tool to investigate population structuring and gene flow in platypus populations from two river catchments in the southern tablelands of New South Wales (NSW). For comparison, we also include data and analyses based on small samples from three river systems elsewhere in NSW. We then use those results to determine what constitutes a management unit for platypuses and discuss the wider management implications. Specifically, we examine the hypothesis that because platypuses are adapted to a largely aquatic life, rates of gene flow within catchments will be high; on the other hand, platypuses are believed to have a limited capacity for overland movement (Bethge *et al.* 2001; Fish *et al.* 1997; Fish *et al.* 2000; Fish *et al.* 2001; Grant 2007; Rakick *et al.* 2001; Richards 1986; Seale 2008), and this should mean that gene flow between catchments will be rare. In that case, genetic population structuring should be centred on major catchments, and these should represent distinct management units.

Methods

Study Area

Two adjacent river systems (catchments), the Shoalhaven River and the Hawkesbury-Nepean system, were selected as the main study region (Figure 4.1). These catchments were selected because of the large number of samples available (collected during other field studies) (Grant 2004; Grant 2006) and their geographical contiguity, which allowed us to test the effects of land barriers on gene flow over relatively short geographical distances. In addition, samples from three more distant river systems were also analysed so that variation over greater distances could be assessed. The additional sample sites were at Laura Creek (Gwydir river system), Tenterfield Creek (Border river system), and the Cotter river (part of the Murrumbidgee River in ACT). These three additional river systems, however, lack the sample sizes needed for detailed analysis but are useful in adding context to the main analysis.

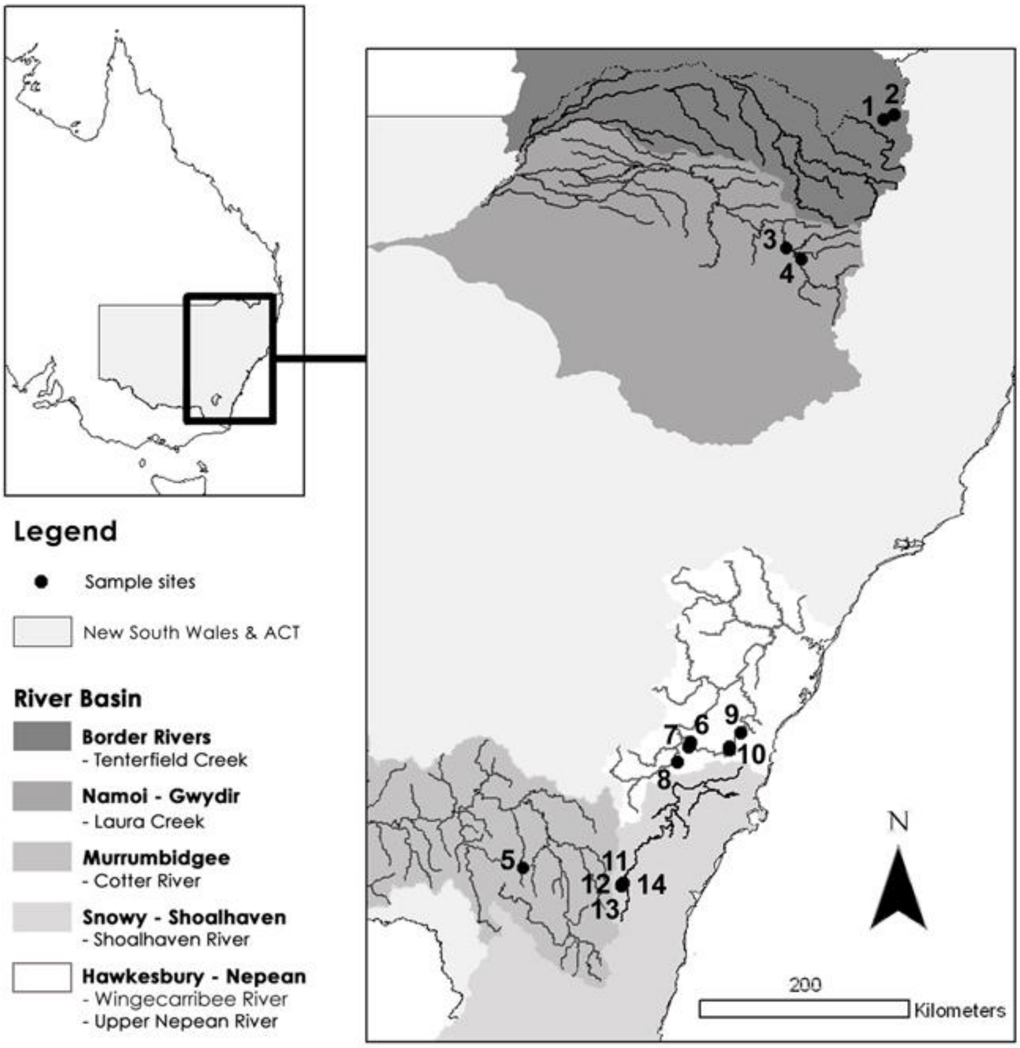

Figure 4.1 Location of sample sites used in this study. River systems and their basins are displayed to provide geographical context. Study sites: **1,** 1 specimen (-29.0250° 151.8667° datum = WGS84); **2,** 1 specimen (-28.9852° 151.9504°); **3,** 2 specimens (-30.1373° 151.0250°); **4,** 3 specimens (-30.2627° 151.4194°); **5,** 3 specimens (-35.3461° 148.8897°); **6,** 15 specimens (-34.4118° 150.1951°); **7,** 7 specimens (-34.4371° 150.1900°); 8, 20 specimens (-34.4488° 150.1800°); **9,** 9 specimens (-34.4555° 150.5308°); **10,** 8 specimens (-34.4769° 150.5341°); **11,** 15 specimens (-35.6270° 149.6129°); **12,** 18 specimens (-35.6394° 149.6073°); **13,** 9 specimens (-35.6560° 149.5988°); **14,** 19 specimens (-35.6411° 149.6107°).

Sampling and Genotyping

DNA was extracted from toe-web biopsies (2 x 2 mm specimens stored in 70% ethanol) using a proteinase K/salt precipitation method (Sunnucks and Hales 1996) and the QIAmp DNA mini kit (QIAGEN). Ten published microsatellite loci were amplified and scored according to standard technique (Kolomyjec *et al.* 2008). An additional two, previously unpublished, loci were used (Table 4.1). These two loci were originally identified directly from the platypus genome (GenBank AAPN00000000, genome project no. 12885) during the previous effort of Kolomyjec *et al* (2008) but were untested at that time. Successful DNA isolation and genotyping yielded usable data for 130 individual platypuses: 61 from the Shoalhaven system (4 sample sites), 59 from the Hawkesbury-Nepean system (5 sample sites), 5 from the Gwydir river system (2 sample sites), 3 from the Cotter River (1 sample site) and 2 from the Border Rivers system (2 sample sites). The very low genotyping error rates (mean allelic drop out = 0.4% and mean false allele = 1.6%) estimated with Pedant v1.0 for this set of markers suggests a highly robust nature (Johnson and Haydon, 2007a; 2007b).

Table 4.1 Additional microsatellite loci from *Ornithorhynchus anatinus.*
Shown are: locus name, primer sequence, chromosome on which the locus is located, repeat motif, position of motif on chromosome, observed size range (bp). Reagent mixture for 15 µL PCR reaction: 5 pM primer (forward and reverse), 5 ng template DNA, 1.7 mM MgCl^{2+}, 400 µM dNTPs, 1.5 µL 10x NH$_4$ buffer and 0.5 units Bio*Taq* Red polymerase (BioLine). The annealing temperature (T_a °C) was 60.5 °C for all PCRs.

Locus	Primer Sequence (5’ - 3’)*	Chromosome	Motif	Motif start Position (bp)	Size range (bp)
OA.01.TAGTA	F: FAM-CCACCATACTCTCCCAAATCC R: CACAATCATGATGGAAATAGGC	1	$(TAGTA)_{11}$	27,826,096	201 - 231
OA.03.GAATA	F: FAM-ACCAGGGGCTTAGAGTGGAT R: GGCTACTGCTCTGATCTGGTG	3	$(GAATA)_{11}$	59,124,431	139 - 184

*Forward primers were 5’ end labeled with fluorochrome indicated.

Genetic diversity and data quality

Exact probabilities for Hardy-Weinberg (HWE) proportions were calculated in Genepop v3.4 (option1: dememorisation = 1,000; batches = 100; iterations = 1,000) while Cervus v3.0.3 was used to estimate null allele frequencies (Kalinowski *et al*. 2007). Genotypic linkage disequilibrium and F$_{st}$ values among river systems were calculated in Arlequin v2.000 (1,000 permutations) (Raymond and Rousset 1995; Schneider *et al.* 2000). Private allele frequencies and numbers of shared alleles per locus were examined in Genalex by producing allele frequency matrices in order to add context to the F$_{st}$ values (Peakall and Smouse 2006).

Population structure and gene flow

We used a hierarchal series of analysis of molecular variance (AMOVA) in Genalex to estimate the partitioning of variation between the two focal river systems, with and without individual sample sites taken into consideration, and within each of the two focal river systems

(Peakall and Smouse 2006). For a three-level AMOVA test, the results are divided into three scores. The among river system variance indicates the genetic distinctiveness of the separate river systems (roughly analogous to F_{st} and indicative of structure and limits of gene flow). The among sample sites variance effectively provides an average of the variance within each river system. The within sample site variance is expected to be high in most normal sexually reproducing species, and has little importance in the context of this study (Excoffier *et al.* 1992). A two-level AMOVA is similar except that it has one less level of hierarchy and simplifies the direct comparison across a single level of hierarchy.

Population assignment tests and migrant detection were performed using the allele frequency based and Bayesian methods in GENECLASS2 (likelihood ratio: L_{home}/L_{max}, Criterion: Paetkau et al. 1995, missing allele frequency: 0.00001, threshold: 0.05) (Piry *et al.* 2004). Mis-assignments with high probabilities (≥95%) indicated genotypes that are unlikely to result from the random combination of alleles within the sampled population and are thus interpreted as migration events. The validity of this method for population assignment has been tested in studies on animals with known origins and in simulation studies using computer-generated datasets (Berry *et al.* 2004; Paetkau *et al.* 2004; Seddon *et al.* 2006).

Using the Bayesian clustering method implemented in STRUCTURE V2.2 (admixture model with 100,000 burn-in period followed by 100,000 Markov chain Monte Carlo repetitions), we tested if animals from the different river systems grouped into distinct population clusters (Pritchard *et al.* 2000). STRUCTURE V2.2 is a useful tool for this as it can operate without *a priori* assumptions about sample origins. By running the program multiple times with different estimates of population number (K), the user can determine the probable number of population clusters present and compare this to the geographically based population designations.

We also tested for isolation-by-distance across New South Wales and within both the Hawkesbury-Nepean and the Shoalhaven rivers using ISOLATION BY DISTANCE WEB SERVICE V3.16 (IBDWS), (10,000 randomisations, analysed with genetic distance F_{st}) (Jensen *et al.* 2005).

Results

Genetic diversity and data quality

The grand mean of all populations sampled deviated significantly from Hardy-Weinberg equilibrium due to homozygote excess at several loci across populations (Table 4.2). Null alleles may be a possible cause of this deviation as Cervus v3.0.3 did find possible evidence of null alleles, particularly in the Hawkesbury-Nepean population (Table 4.2). To investigate the influence of these null alleles on the outcome and patterns detected, all analyses carried out on the data set were repeated excluding loci with certain levels of estimated null alleles. This was done once excluding any locus with a null allele rate greater than 0.2 and again excluding any locus with a null allele rate greater than 0.1. No change in patterns was detected in these repeated analyses.

Table 4.2 Detailed population genetics data. Presented by locus, by population and locus, by population and grand mean. (N = no. of successfully genotyped samples, N_a = no. of alleles, H_o = observed heterozygosity, H_e = expected heterozygosity, F_{is} = fixation index, HWE p-value = probability of Hardy-Weinberg (significant results in **bold**), null allele freq. = calculated rate of null alleles. Monomorphic indicates no allelic variation detected at a particular locus within a population. ND = "not done", an indicator that a particular subset of data was unable to be calculated by the software.)

Population	Locus	N	Na	Ho	He	Fis	HWE p-value	null allele freq.
By Locus								
	OA.01.TGTA	123	12	0.764	0.808	-0.161	**0.010**	0.023
	OA.03.GAATA	125	15	0.912	0.933	-0.209	0.330	0.010
	OA.03.GTAG	122	7	0.811	0.797	-0.253	0.924	-0.013
	OA.05.GTAGG	125	8	0.736	0.740	-0.209	0.680	0.004
	OA.06.TAAC	115	11	0.443	0.735	0.465	**0.000**	0.270
	OA.06.TATATC	123	9	0.618	0.788	0.128	**0.003**	0.114
	OA.07.GTTA	128	10	0.656	0.737	0.009	0.062	0.062
	OA.11.CCA	126	9	0.230	0.365	0.573	**0.000**	0.262
	OA.12.TCCAT	103	7	0.563	0.748	0.242	**0.000**	0.139
	Platy04	119	4	0.185	0.242	0.112	**0.000**	0.120
	Platy08	123	12	0.764	0.845	0.009	**0.026**	0.047
	Platy19	102	15	0.706	0.863	-0.016	**0.000**	0.101
By Locus and Populations								
Cotter, ACT	OA.01.TGTA	3	4	1.000	0.722	-0.385	0.609	ND
	OA.03.GAATA	2	3	1.000	0.625	-0.600	0.572	ND
	OA.03.GTAG	3	3	1.000	0.611	-0.636	0.506	ND
	OA.05.GTAGG	3	4	1.000	0.667	-0.500	0.809	ND
	OA.06.TAAC	3	1			Monomorphic		
	OA.06.TATATC	3	2	0.333	0.278	-0.200	0.729	ND
	OA.07.GTTA	3	2	0.667	0.444	-0.500	0.386	ND
	OA.11.CCA	3	1			Monomorphic		
	OA.12.TCCAT	3	3	0.333	0.500	0.333	0.112	ND
	Platy04	3	1			Monomorphic		
	Platy08	3	2	0.000	0.444	1.000	0.083	ND

	Platy19	3	4	0.667	0.667	0.000	0.387	ND
Gwydir	OA.01.TGTA	5	7	0.800	0.780	-0.026	0.444	ND
	OA.03.GAATA	5	6	0.800	0.760	-0.053	0.451	ND
	OA.03.GTAG	5	4	0.800	0.720	-0.111	0.411	ND
	OA.05.GTAGG	4	4	0.750	0.719	-0.043	0.530	ND
	OA.06.TAAC	5	5	0.200	0.740	0.730	**0.029**	ND
	OA.06.TATATC	5	5	0.600	0.720	0.167	0.744	ND
	OA.07.GTTA	5	4	0.600	0.640	0.063	0.477	ND
	OA.11.CCA	5	2	0.000	0.480	1.000	**0.025**	ND
	OA.12.TCCAT	5	3	0.400	0.580	0.310	0.484	ND
	Platy04	4	2	0.250	0.219	-0.143	0.775	ND
	Platy08	5	6	1.000	0.800	-0.250	0.451	ND
	Platy19	3	3	0.333	0.611	0.455	0.343	ND
Hawkesbury - Nepean	OA.01.TGTA	55	8	0.800	0.808	0.010	**0.041**	0.003
	OA.03.GAATA	58	12	0.948	0.844	-0.123	**0.048**	-0.033
	OA.03.GTAG	55	6	0.873	0.740	-0.179	0.414	-0.064
	OA.05.GTAGG	59	7	0.746	0.745	-0.001	0.841	0.004
	OA.06.TAAC	53	8	0.585	0.809	0.277	**0.000**	0.169
	OA.06.TATATC	58	8	0.724	0.819	0.116	**0.000**	0.062
	OA.07.GTTA	59	7	0.559	0.641	0.127	0.449	0.077
	OA.11.CCA	57	7	0.386	0.512	0.247	**0.001**	0.173
	OA.12.TCCAT	47	7	0.617	0.781	0.210	0.089	0.119
	Platy04	52	3	0.250	0.339	0.262	**0.000**	0.138
	Platy08	54	10	0.815	0.753	-0.082	**0.000**	-0.039
	Platy19	48	15	0.771	0.882	0.126	0.132	0.066
Shoalhaven	OA.01.TGTA	58	8	0.707	0.773	0.085	0.742	0.039
	OA.03.GAATA	58	12	0.879	0.848	-0.037	0.091	0.035
	OA.03.GTAG	57	5	0.754	0.687	-0.098	0.903	0.029
	OA.05.GTAGG	57	5	0.702	0.717	0.021	0.423	0.015
	OA.06.TAAC	53	9	0.358	0.587	0.389	**0.000**	0.283
	OA.06.TATATC	55	7	0.527	0.636	0.171	**0.000**	0.091
	OA.07.GTTA	59	6	0.763	0.767	0.006	**0.026**	0.001
	OA.11.CCA	59	7	0.119	0.190	0.377	**0.000**	0.255
	OA.12.TCCAT	46	4	0.543	0.673	0.193	**0.001**	0.098
	Platy04	59	4	0.136	0.158	0.144	**0.000**	0.069
	Platy08	59	10	0.729	0.829	0.121	0.072	0.060
	Platy19	47	11	0.660	0.716	0.079	0.367	0.044
Border	OA.01.TGTA	2	3	1.000	0.625	-0.600	0.572	ND
	OA.03.GAATA	2	4	1.000	0.750	-0.333	0.423	ND
	OA.03.GTAG	2	2	0.500	0.375	-0.333	0.637	ND
	OA.05.GTAGG	2	3	1.000	0.625	-0.600	0.572	ND
	OA.06.TAAC	1	1			Monomorphic		
	OA.06.TATATC	2	3	0.500	0.625	0.200	0.261	ND
	OA.07.GTTA	2	3	0.500	0.625	0.200	0.261	ND
	OA.11.CCA	2	1			Monomorphic		
	OA.12.TCCAT	2	3	0.500	0.625	0.200	0.261	ND
	Platy04	1	1			Monomorphic		
	Platy08	2	4	1.000	0.750	-0.333	0.423	ND
	Platy19	1	2	1.000	0.500	-1.000	0.317	ND
Population means								
Cotter (ACT)		2.917	3	0.500	0.413	-0.165	0.332	ND
Gwydir		4.667	4	0.544	0.647	0.175	**0.002**	ND
Hawkesbury-Nepean		54.583	8	0.673	0.723	0.082	**0.000**	0.056
Shoalhaven		55.583	7	0.573	0.632	0.121	**0.000**	0.085

Border		1.750	3	0.583	0.458	-0.289	0.215	ND
Grand Mean								
Total	Mean	23.900	5	0.575	0.575	0.008	**0.000**	0.095

No globally significant linkage disequilibrium was detected between loci after sequential Bonferroni correction (Rice 1989) (Table 4.3). Expected heterozygosity (H_e) ranged from 0.413 to 0.723 across the sampled populations (mean = 0.575) (Table 4.2).

Table 4.3 Genotypic Linkage disequilibrium p-values. Adjusted critical p-value equivalent to 0.05 is 0.0008 after sequential Bonferroni correction.

	OA.01.TGTA	OA.03.GAATA	OA.03.GTAG	OA.05.GTAGG	OA.06.TAAC	OA.06.TATATC	OA.07.GTTA	OA.11.CCA	OA.12.TCCAT	Platy04	Platy08
OA.03.GAATA	0.300										
OA.03.GTAG	0.055	0.645									
OA.05.GTAGG	0.086	0.321	0.194								
OA.06.TAAC	0.455	0.727	0.430	0.885							
OA.06.TATATC	0.117	0.475	0.027	0.350	0.127						
OA.07.GTTA	0.316	0.011	0.399	0.801	0.028	0.016					
OA.11.CCA	0.611	0.777	0.904	0.219	0.002	0.005	0.605				
OA.12.TCCAT	0.390	0.801	0.158	0.132	0.550	0.012	0.058	0.510			
PLATY04	0.805	0.061	0.080	0.205	0.989	0.156	0.920	0.239	0.355		
PLATY08	0.668	0.526	0.036	0.027	0.791	0.814	0.010	0.996	0.163	0.730	
PLATY19	0.455	0.622	0.153	0.268	0.038	0.465	0.003	0.412	0.509	0.362	0.628

Pairwise F_{st} values between different river systems ranged from 0.018 to 0.152 (Table 4.4). The F_{st} calculated for comparison only between the Hawkesbury-Nepean and Shoalhaven systems was 0.05375 ($p < 0.001$). The number of private alleles in either the Hawkesbury-Nepean or the Shoalhaven river system was low at most loci while many alleles were identified as shared between systems indicating that the systems are unlikely to be highly isolated (Table 4.5).

Table 4.4 Pairwise F_{st} values at microsatellite loci among sample sites. F_{st} values significant at the $\alpha = 0.05$ level are bold

	River system				
River System	A*	B*	C	D	E*
A - Cotter (ACT)*	0.00000				
B – Gwydir*	0.09168	0.00000			
C - Hawkesbury-Nepean	**0.09168**	0.01809	0.00000		
D - Shoalhaven	**0.07827**	**0.06829**	**0.05138**	0.00000	
E – Border*	0.15173	0.03710	0.03259	**0.09744**	0.00000

*very low sample sizes, values added for context only

Table 4.5 Private and shared alleles.Frequency of private alleles per locus, the number of shared alleles and the total number of alleles present across both river systems.

	Private Allele Frequency			
	Hawkesbury Nepean	Shoalhaven	Shared Alleles	Total Alleles
OA.01.TGTA	0.028	0.034	6	10
OA.03.GAATA	0.045	0.155	8	13
OA.03.GTAG	0.028	0.009	4	7
OA.05.GTAGG	0.035	0.009	4	8
OA.06.TAAC	0.029	0.028	7	10
OA.06.TATATC	0.018	0.000	7	8
OA.07.GTTA	0.044	0.178	5	8
OA.11.CCA	0.027	0.025	5	9
OA.12.TCCAT	0.111	0.000	4	7
Platy04	0.000	0.017	3	4
Platy08	0.019	0.042	8	12
Platy19	0.087	0.000	11	15

Population structure and gene flow

The results of the AMOVA tests (Table 4.6) indicate that more molecular variance occurs between the adjacent river systems than between sample sites in the same river. Most of the within-system variation for the Hawkesbury-Nepean system occurs between the two sub-catchments with some additional variance between individual sample sites (Table 4.6). The Shoalhaven river on the other hand exhibited very little variance between individual sample sites and produced a non-significant result. The failure to detect significant molecular variance in the Shoalhaven river system may be due to the close proximity of the individual sample sites (Figure 4.1).

Table 4.6 Analysis of Molecular Variance. Results of two and three level AMOVA, p-values representing significant amounts of between site variance are in **bold.**

	Molecular Variance			
	Among River Systems	Among Sample Sites	Within Sample Sites	p-value
Hawkesbury-Nepean (5 sample sites) Vs. Shoalhaven (4 sample sites)	6%	3%	91%	**0.000**
Hawkesbury-Nepean Vs. Shoalhaven (no subdivision)	7%	-	93%	**0.001**
Hawkesbury-Nepean (2 subcatchments*, 5 sample sites)	8%	3%	89%	**0.001**
Hawkesbury-Nepean (2 subcatchments)	10%	-	90%	**0.001**
Hawkesbury-Nepean (5 sample sites)	-	7%	93%	**0.000**
Shoalhaven (4 sample sites)	-	1%	99%	0.425

*Subcatchment A = sample sites 6, 7, and 8;
Subcatchment B = sample sites 9 and 10; Figure 5.1.

Population assignment (Figure 4.2) and first-generation migrant tests performed on the Hawkesbury-Nepean and Shoalhaven river systems suggest that 13 individuals (11% of the total sample of 120 individuals) were first-generation migrants. Eight of the detected migrants (62%) were female while five (38%) were male. This female-bias in detected migrants was not significant ($\chi^2 = 0.405$, $p > 0.05$).

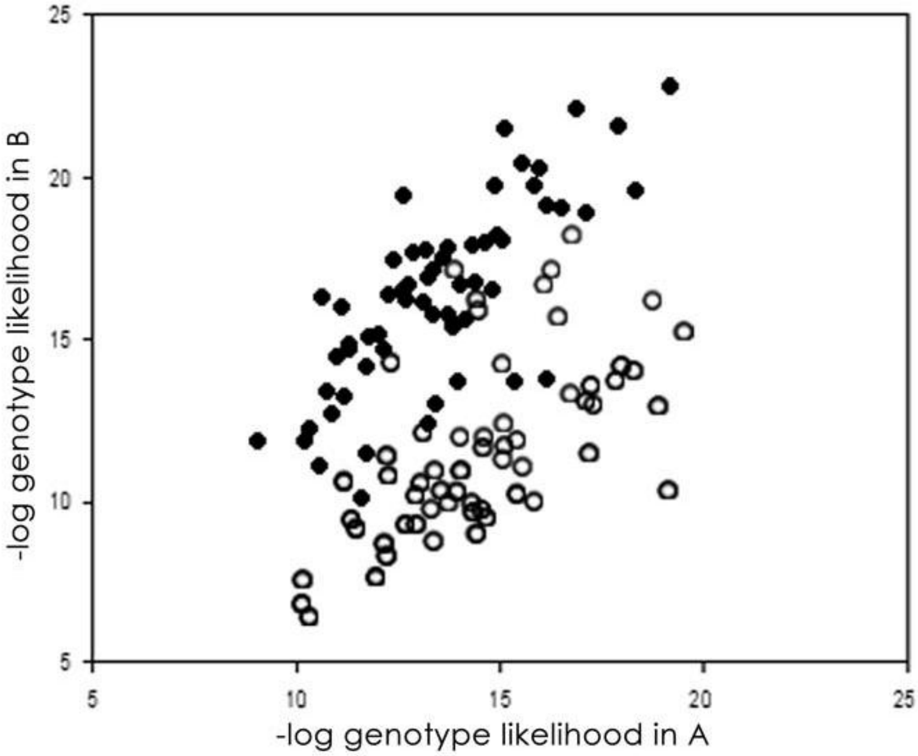

Figure 4.2 Assignment plot of genotype likelihoods values between the Hawkesbury-Nepean and Shoalhaven river systems. Each river system forms a distinct group with a narrow area of overlap, suggesting that the systems form distinct populations with some gene flow between them. Closed circles represent Hawkesbury-Nepean individuals and open circles represent individuals from the Shoalhaven river system.

The most appropriate number of population clusters (K) based on the likelihood values as indicated by STRUCTURE was three (Figure 4.3). One cluster (SH-1) clearly represents the individuals from the Shoalhaven system while the other two interspersed clusters (HN-1 and HN-2) form the Hawkesbury-Nepean. These two clusters within the Hawkesbury-Nepean do not conform to the subcatchments or any other known population division. Examination of the STRUCTURE plots also reveals individuals of mixed ancestry that represent the descendants of past migrants (Figure 4.3, B).

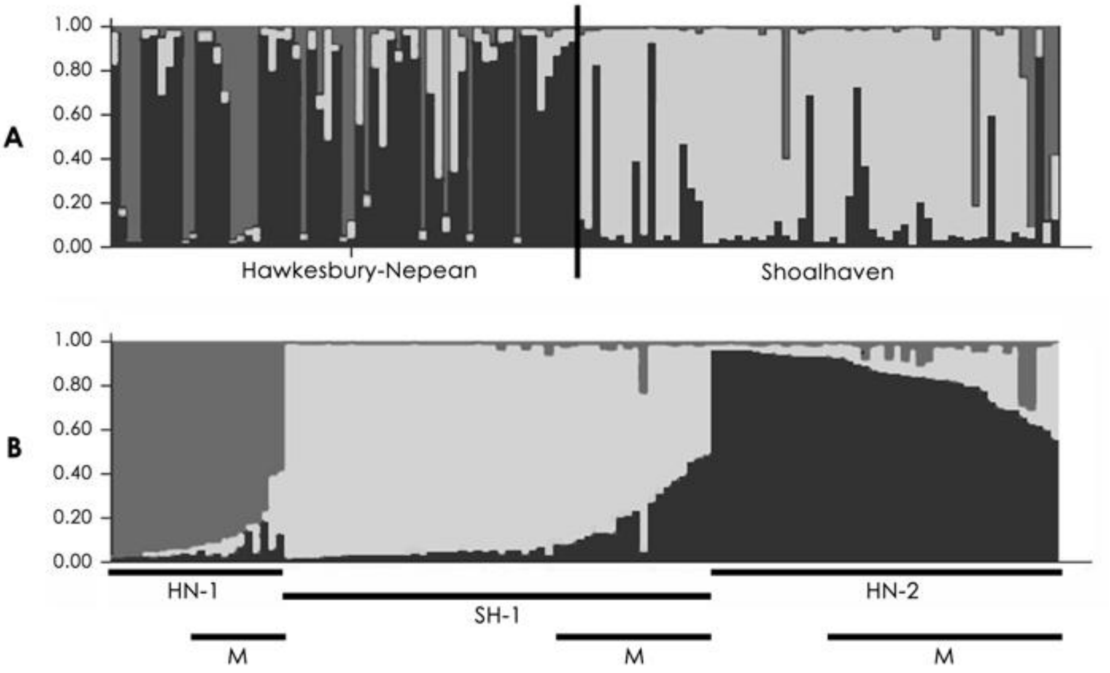

Figure 4.3 STRUCTURE results. **A -** Three population clusters (K) distributed across the two river systems. Each vertical bar represents an individual. The amount of each colour represents the proportion of each population cluster responsible for that individual's genotype. **B -** The results from **A** sorted by population cluster demonstrating the individual clusters and the individuals with mixed ancestry (M).

We found significant evidence of correlation between geographic and genetic distances supporting an isolation-by-distance scenario between the Hawkesbury-Nepean and the Shoalhaven river systems ($r^2 = 0.3739$, $p = 0.002$) and within the Hawkesbury-Nepean systems ($r^2 = 0.5351$, $p = 0.02$). No significant pattern of isolation-by-distance was detected within the Shoalhaven river system ($r^2 = 0.0040$, $p = 0.590$), but this may be because distance between sample sites was very small (Figure 4.1).

Discussion

We investigated gene flow and genetic structuring between platypuses in two adjacent river systems using 12 polymorphic microsatellite loci. Homozygous excess at several loci led to the rejection of HWE across all populations sampled. One of the most common causes for deviation from HWE is the presence of null alleles occurring due to natural (mutating microsatellite flanking regions) or technical (PCR amplification problems) reasons (Callen *et al.* 1993; Dakin and Avise 2004). Null alleles were detected but shown not to influence the interpretation of any patterns detected during analysis. The suggestion of null alleles and the deviation from HWE might be the result of a Wahlund-effect resulting from population sub-structuring as indicated by the STRUCTURE analysis (Neves *et al.* 2008; Stow *et al.* 2006). If a Wahlund-effect were present then it would affect the observed heterozygosity, which in turn may lead to the software proposing more null alleles than are actually present (Karlsson and Mork 2005). This could also occur due to sampling multiple generations simultaneously. The sampling of several generations within a few field seasons is a distinct possibility as the platypus is a fairly long lived animal, living up to 20 years in captivity and at least 21 years in the wild (Grant 2007).

The low, but highly significant pairwise F_{st} value (0.05374, $p < 0.001$) between the Hawkesbury-Nepean and Shoalhaven river systems indicates two things. The high significance indicates the river systems are genetically distinct enough to cause population structuring at that level. However, in light of the large number of shared alleles, the low F_{st} value may indicate that while distinct the two systems are not highly divergent, and that there is still gene flow between them (Bossart and Prowell 1998; Slatkin 1987).

The populations sampled exhibit an isolation-by-distance pattern connected by the movement of migrants between river systems. In the absence of streams connecting the river systems, we infer that migrants must have moved overland. If land crossings were an impossible feat for the platypus then genetic drift would rapid remove any isolation-by-distance pattern. This happens as factors other than traversable geographic distance contribute significantly to the observed genetic distances (Hardy and Vekemans 1999). This agrees with the general conclusion of Akiyama (1998) that, across the mainland distribution, platypuses were continuously graded between distinct core populations. The microsatellites used in our study allowed a finer scale investigation, permitting identification of the individual river systems as the significantly distinct populations units. The continuous gradation occurring is presumably the result of overland migration between systems lacking hydrological connectivity. The exchange of individuals between river systems in turn produces the gradient of similarity between the systems based on proximity previously observed (Akiyama 1998).

Such a stepping-stone pattern of gene flow explains the level of genetic continuity of the platypus at the distribution-wide scale. Only in the presence of long-standing, impassable barriers, such as between mainland Australia and Tasmania, and between north and south Queensland, is large-scale genetic divergence observed (Akiyama 1998; CHAPTER 7). The AMOVA tests further support this by showing an increased proportion of variance at higher levels of division. The level of variance demonstrated between subcatchments indicates that population structuring is based on local geography in addition to linear distances.

The number of migrants detected ($n_m = 13$, $n = 120$) between the Hawkesbury-Nepean and Shoalhaven river systems is interesting. At their closest, in the Goulburn-Marulan region, several branches of these two river systems are approximately 1.5 km apart during normal conditions. These distances would, however, be increased during dry or drought conditions, decreasing the chance of inter-catchment movement, especially in streams affected by the reduced rainfall conditions predicted for much of the current distribution of the platypus as a result of climate change.

Overland dispersal by platypuses was previously believed to be extremely limited due to the species' poor tolerance to high ambient temperatures (Grant and Dawson 1978a; Grant and Dawson 1978b), only being known to feed in the water (Grant 2007), the higher metabolic cost and slower overland movement (Bethge *et al.* 2001; Fish *et al.* 1997; Fish *et al.* 2000; Fish *et al.* 2001; Grant 2007) and higher predation risks (Grant 2007; Rakick *et al.* 2001; Richards 1986; Seale 2008). However, particularly during drought conditions, platypuses can be found some distance from water with some of these being identified as juveniles (Grant 2007). Burrell (1927) also recorded an experiment where a platypus was moved a mile (1.6 km) from its point of capture and immediately made its way overland back to the stream.

Our study clearly implies that over-land dispersal between catchments can occur and that it plays an important role in determining the population structure of the platypus.

Over-land dispersal also explains how the analyses performed in STRUCTURE detected three population clusters instead of two, as was expected if population clustering was determined solely by catchments (Figure 4.3). One population cluster (SH-1) represented samples collected from the Shoalhaven river system. The presence of the other two clusters (HN-1 and HN-2) is not surprising considering the AMOVA detected a significant amount of genetic variance between the sub-catchments in the Hawkesbury-Nepean river system. However, the population clusters as detected do not correlate to the individuals taken from each sub-catchment. Instead, their existence can logically be reconciled if bimodal dispersal via water and land based movements is taken into account[1]. The population clusters may represent sub-populations generated by the physical characteristics of the sub-catchments that limit water based routes of dispersal while proximity could allow for dispersal over land. Bimodal dispersal is known to affect the Bayesian clustering techniques utilised by STRUCTURE and can, if frequent enough, prevent the program from ever resolving the predicted number of populations (k) (Chaput-Bardy *et al.* 2008).

The results of our study supported our hypothesis. While able to disperse both by along rivers and across land, adaptations for a semi-aquatic life lead to greater gene flow along watercourses so that major catchments form the basis of regional population structuring, although further sub-structuring is possible dependant on local geography. Despite higher than anticipated inter-catchment migration, in-river dispersal was still more frequent. Both routes of gene flow play an important role in shaping platypus populations.

Conclusion

The platypus poses an interesting conservation challenge, with individual management units (MUs) varying from the catchment to sub-catchment levels. This means that to preserve the genetic diversity and evolutionary health within each MU, entire catchments or sub-catchments (depending on local geography) must be preserved in a condition usable by the platypus (Moritz 1995; Moritz 1999). To optimally preserve the local evolutionarily significant unit (ESU), which includes at the very least all of the sampled regions of New South Wales and may extend to include most of the mainland distribution of the platypus excluding two additional ESUs known to exist in North and Central Queensland (CHAPTER 7), the condition

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[1] Other potential sources of gene flow include stream capture, head water separation, and major flooding events. However, the former two means operate on a more geological time frame and the later is unlikely due to this region not having a major flood event within an applicable time frame around this study.

and topography (both natural and anthropogenic) of the land between catchments should also be considered.

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Chapter 5 – Anthropogenic Barriers

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Restriction of gene flow in the platypus (*Ornithorhynchus anatinus*) due to a large dam.

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Abstract

As a preliminary attempt to understand the impact of anthropogenic modification of stream flow on gene flow in the platypus we analysed platypus DNA samples from several locations in two adjacent tributary streams in part of a major river system to test the effects of a large, long-established dam on gene flow along one of these rivers. Analysis of microsatellite markers showed greater differentiation across the dam within one of the rivers than between the two rivers ($F_{st} = 0.07681$ and 0.05978 respectively). This suggests that the dam is a barrier to gene flow. Not only does this observation provide information for the conservation and management of platypus populations, but it is also the first assessment of the effects of a large water storage dam on the population structure of any semi-aquatic mammal and as such we hope it will spark other similar investigations.

Introduction

Habitat fragmentation is a major threat to biodiversity. In terrestrial environments, fragmentation is often a consequence of the clearing of land for agricultural or urban use, producing a patchwork environment that inhibits the normal movement of species across their original ranges (Saunders *et al.* 1991). The same process can occur in riverine systems when dams are built, or water is diverted away from its normal course.

The impact of riverine habitat fragmentation on population dynamics has been well documented for several species of fish. Dams can prevent normal patterns of movement and migration, reducing gene flow, accelerating genetic drift and leading to divergence of local populations (Heggenes and Røed 2006; Meldgaard *et al.* 2003; Neraas and Spruell 2001; Reid *et al.* 2008; Yamamoto *et al.* 2004).

Little information is available on the effects of such environmental alteration on populations of semi-aquatic mammals, a phylogenetically diverse group with representatives found on every continent. They have varying dependence on water. Our lack of knowledge may be in part due to the difficulty of capturing and observing these species. Semi-aquatic mammals occur at low densities and must be captured in a manner that prevents drowning. They retain varying abilities to leave the water to navigate around barriers. Their anatomical features (e.g. size and mobility on land), physiological constraints (e.g. water and temperature regulation) and the risk of predation limit the ability and ease of land-based movement.

The platypus is a semi-aquatic, prototherian mammal endemic to the freshwaters of eastern mainland Australia and Tasmania (Grant 1992; Grant 2007; Grant and Temple-Smith 1998). Platypuses, though elusive, are common in many parts of their distribution and have been assigned a conservation status of “least concern” by the IUCN (Lunney *et al.* 2008). However, their inherent vulnerability cannot be overlooked as riverine systems are under constant threat of anthropogenic alteration of flow and general degradation at both local and national levels (Goudie 2006; Kingsford 2000).

The Nepean Dam is a large dam (82m tall, 216m long and completed in 1935) in the upper Hawkesbury-Nepean River system in New South Wales Australia (Sydney Catchment

Authority). Platypuses are known from all the major streams and many tributaries of the Hawkesbury-Nepean system (Grant 1998; *Atlas of NSW Wildlife Database*. http://wildlifeatlas.nationalparks.nsw.gov.au), and prior to the development of the various stages of the water supply for the greater metropolitan area of Sydney and the Illarrawa region, gene flow presumably could have occurred throughout this large river system. However, since the completion of the Cordeaux, Avon, Nepean and Warragamba dams in 1926, '27, '35 and '60 respectively, it might be expected that these large dams and the deep water storages impounded by them will have affected population connectivity.

This dam was selected for this study as there were known platypus populations both above and below the dam, with accessible sites for sampling. An adjacent river (the Wingecarribee) also provided sampling sites along a reach of river with no intervening dam. This provided an opportunity to examine the effects of the dam on gene flow in the local populations. Specifically, we examined the hypothesis that the Nepean Dam restricts gene flow in platypuses by testing for genetic differentiation between populations upstream and downstream of the dam, in comparison to genetic differentiation along the Wingecarribee, and between the Nepean and Wingecarribee Rivers.

Methods

Sampling and genotyping

Platypuses were captured from the upper Nepean River at two sites above and one site below the Nepean Dam (-34.335082, 150.617715; Figure 5.1) using unweighted, constantly monitored gill nets according to standard methods (Grant and Carrick 1974). After capture individual animals were marked with implanted passive integrated transponder tags (microchips) (Grant and Whittington 1991) to prevent re-sampling of the same individuals. We included three sample sites from the neighbouring Wingecarribee River in the same catchment (Fig. 1). Unlike the samples from the upper Nepean River, those in the Wingecarribee were not separated by any large dams. The close proximity of the two rivers' headwaters creates several possible corridors for platypus dispersal between the rivers (Figure 5.1).

DNA was extracted from toe-web biopsies (2 x 2 mm specimens stored in 70% ethanol) using a proteinase K/salt precipitation method (Sunnucks and Hales 1996). Twelve published microsatellite loci were amplified and scored according to standard techniques (Kolomyjec *et al.* 2009; Kolomyjec *et al.* 2008). Successful DNA isolation and genotyping yielded usable data for 63 individual animals; 7 from below the Nepean Dam, 11 individuals from above the Nepean Dam and 42 individuals from the Wingecarribee River (Grant 2006; Kolomyjec *et al.* 2009).

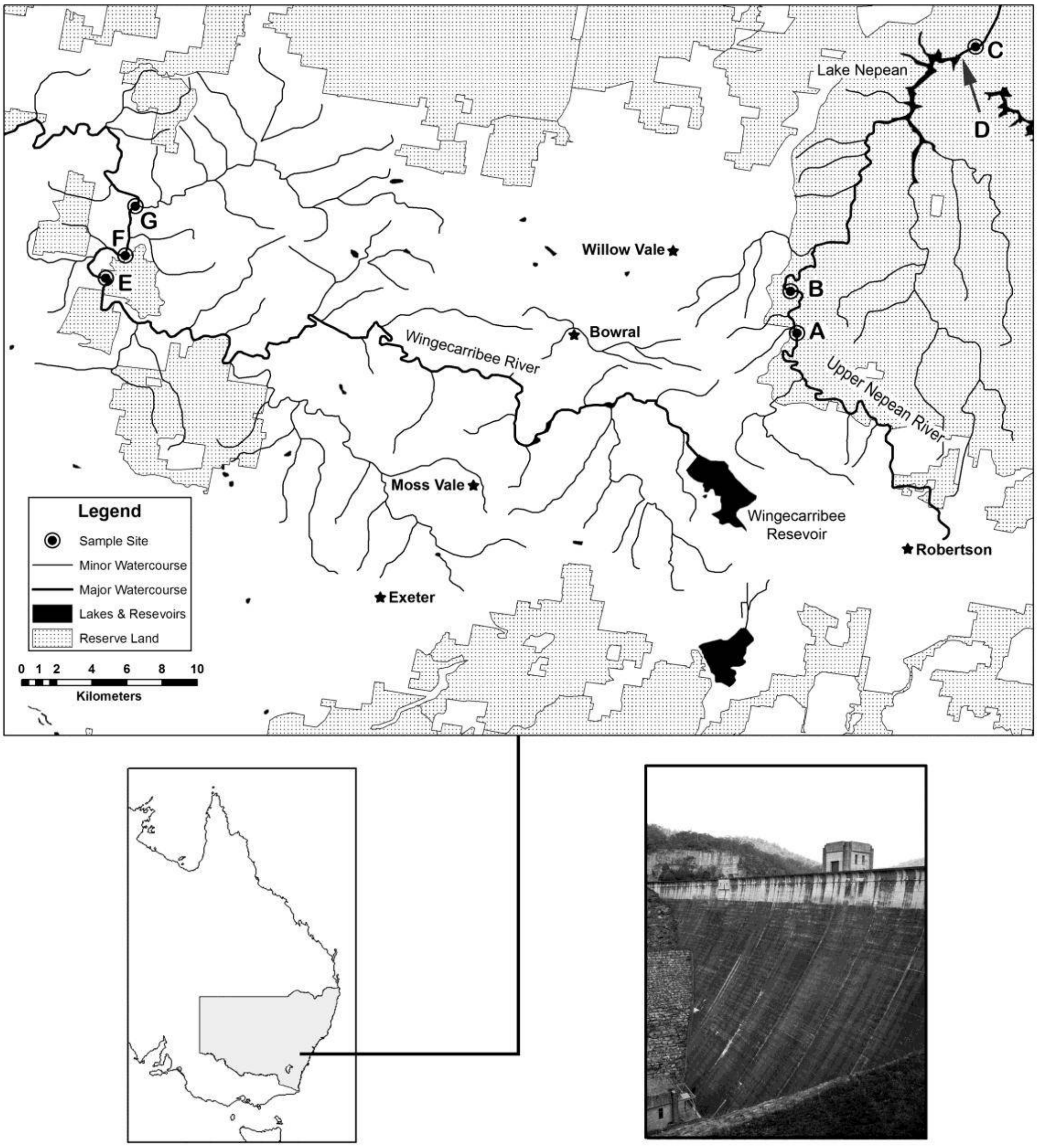

Figure 5.1 - Location and detailed view of the Nepean Dam and sample sites. Sites: A - McGuire's Crossing, 6 individuals; B - Chalker's Crossing, 5 individuals; C - Below the dam, 7 individuals; D - Nepean Dam wall (see inset photograph); E - Wingecarribee Site 1, 15 individuals; F - Wingecarribee Site 2, 7 individuals; G - Wingecarribee Site 3, 20 individuals.

Population differentiation

Population differentiation was examined using population pairwise F_{st} as calculated in ARLEQUIN (Schneider *et al.* 2000). Hardy-Weinberg Equilibrium (HWE) and basic allelic information was also calculated in ARLEQUIN (Schneider *et al.* 2000).

Isolation-by-distance (IBD) was tested using simple Mantel tests in GENALEX v6.3 (Peakall and Smouse 2006) to infer the correlation (if any) between genetic and geographic distance (10,000 permutations; N x N - the pairwise genetic distance between individuals and geographically shortest traversable route).

Results

Patterns of allelic diversity

All loci were polymorphic with three to ten alleles per locus (mean = 6.1) and observed heterozygosity (H_o) ranging from 0.133 to 0.833 (mean = 0.555). The population and grand means were all in Hardy-Weinberg equilibrium, although 11 out of 60 possible loci-population combinations did show deviation from Hardy-Weinberg equilibrium. These deviations most likely results from a Wahlund effect due to multi-generation sampling as detailed in a previous study (Kolomyjec *et al.* 2009).

Population differentiation

The pairwise F_{st} results (Table 5.1) demonstrate that the within-river F_{st} for both the upper Nepean and Wingecarribee rivers is low and the between-river F_{st} is much higher, as is expected. However, the F_{st} across the dam is greater than that between the two adjacent rivers.

A pattern of isolation-by-distance (Table 5.1) is consistent with the genetic differences between the Wingecarribee and upper Nepean Rivers ($r = 0.233$; $p < 0.010$) and between the three Wingecarribee sample sites ($r = 0.127$; $p < 0.01$). There was no significant correlation between genetic and geographic distances across the Nepean Dam ($r = 0.166$, $p = 0.090$).

Table 5.1. Population differentiation. Significant values are in **bold** $_{(\alpha = 0.05)}$.

		Isolation by Distance		
	Fst	R	p-value	Distance (km)
Wingecarribee River *(sites: E, F, & G)*	**0.01750**	**0.127**	< **0.010**	4 - 7
Nepean River *(Above Dam; sites: A & B)*	0.00420	-	-	3.5
Between Wingecarribee and Nepean Rivers *(sites: A & B vs. E, F, & G)*	**0.05978**	**0.233**	< **0.010**	65
Across Nepean Dam *(sites: A & B vs. C)*	**0.07681**	0.166	0.090	25

Discussion

The platypus can very easily disperse within a river and will even cross land between adjacent systems on occasion (Kolomyjec *et al.* 2009). Within a river, transient and dispersing platypuses have been shown to move as far as 48 km within a 7 month period (Grant 2004) and established males have been recorded moving as much as 7 km within a single night (Serena *et al.* 1998). Thus, the dispersal potential for the platypus is high with most population structuring likely to be the result of distance, established home ranges (350 m to 7 km depending on sex and local stream carrying capacity; Gardner and Serena 1995; Grant 1992; Grant and Temple-Smith 1998; Serena 1994; Serena *et al.* 1998; Serena *et al.* 2001), and barriers to movements such as large waterfalls, gorges, and large overland distance between waterways (Kolomyjec *et al.* 2009).

The greater differentiation found between the Wingecarribee and upper Nepean sites relative to the within-river differentiation is probably due to the reduced frequency of genetic exchange occurring over land compared to that within a river, coupled with the effects of distance. Gene flow between the rivers must be occurring at a sufficiently high rate to maintain an isolation-by-distance pattern, yet still be quite rare as suggested by the relatively large F_{st} values observed (Table 5.1), showing differentiation without complete isolation. Conversely, the low within-river F_{st} supports the frequent exchange of genetic information between connected sample locations. The lack of significance of F_{st} and IBD between the two above-dam sites in the Nepean River likely indicates that the two sites are genetically contiguous, with no significant restriction to gene flow.

The fact that the observed differentiation between the above and below dam samples from within the upper Nepean River is greater than that found between the upper Nepean and Wingecarribee rivers, even though it spans only a little over a third the distance, does not follow the established pattern of differentiation. In fact, the observed F_{st} of 0.07681 at a distance of ~25 km is even larger than that found between rivers located in separate catchments during a previous study. During that study, an F_{st} of 0.05138 was observed between the Shoalhaven and Wingecarribee Rivers (Kolomyjec *et al.* 2009) which represent two completely separate river systems (the Shoalhaven and Hawkesbury-Nepean systems respectively). The sample sites in question were approximately 142 km (as the crow flies) apart (Kolomyjec *et al.* 2009).

This, along with the lack of correlation between genetic and geographic distance (IBD), suggests that a barrier exists between these sample locations. There are no observed natural barriers between these locations; the only candidate is the Nepean Dam. This suggests that the Nepean Dam may pose a greater barrier to normal gene flow in platypuses than the dry land separating neighbouring rivers.

Unfortunately, the generation length of the platypus is unknown and it is not possible to calculate the rates of genetic drift that would be expected to occur in the absence of migration. The low rate of capture in the upper Nepean River suggests a low population density which could produce a relatively high rate of genetic drift and explain the large differentiation observed. Lack of historical samples, however, makes it impossible to measure any change in differentiation between populations across the dam since construction. We can only be certain that there is greater differentiation occurring across the dam than occurs elsewhere.

Capture rates of platypuses were low at all sites on the upper Nepean River, including three additional downstream sites, where no platypuses were caught during two earlier studies (Grant 2006; Grant, unpublished data). Additionally, the upper Nepean River is incised into steep sandstone gorges making access very limited. It is not possible to increase sample sizes or sampling locations below the dam and so we have kept our analysis simple to maintain robustness. We have focused on F_{st} as the measure of differentiation, both to make the interpretation straightforward and because sample size only becomes particularly important if examining small differences in F_{st} values (Kalinowski 2005). As we were only concerned with broad-scale F_{st} differentiation, we consider our sample sizes sufficient to provide an accurate interpretation of our data (Kalinowski 2005).

The conservation implications of these findings are particularly important for water-dependent species such as the platypus. However, all semi-aquatic mammals have special requirements that should be considered when planning water extraction and storage projects. Their dependence on water makes them vulnerable to anthropogenic modifications of natural stream dynamics. Populations that are isolated by barriers will eventually begin to diverge due to genetic drift. If the populations isolated by the construction of dams are small, as is the case with the platypus in the upper Nepean River (Grant 2006), the subsequent reduction of genetic diversity could in turn reduce fecundity, survival, competitive ability, and increase the rate of developmental abnormalities (Quattro and Vrijenhoek 1989; Vrijenhoek 1994).

While the long-term consequences of such genetic changes in platypus populations are unknown, the loss of genetic diversity is likely to reduce the ability of the platypus to adapt to environmental changes. Inability to adapt, and the restricted possibility of outside recruitment (Kolomyjec *et al.* 2009), could lead to the collapse of local populations fragmented by one or more large dams in a stream or catchment. The construction of large dams in Australia and world-wide has had adverse effects on riverine species and their environments. For example, disruption to breeding migrations of indigenous fish species in Australia has resulted in the extinction of self-sustaining populations of some species above large dams and losses of local populations of others in streams fragmented by the construction of dams (Boulton and Brock 1999; NSW Department of Primary Industries 2005; Young 2001). As a consequence of these impacts, other methods of water extraction and storage have been considered and implemented in Australia (Boulton and Brock 1999). This study suggests that the long-term impact of fragmentation by large dams could also be significant for the conservation of the platypus.

As this is the first attempt to look at the effects of a dam on the gene flow of a mammalian species, further studies will be needed to fully elucidate our initial finding. Of particular use would be larger studies that examine gene flow across several different barrier types with less disparity in the distance between sample sites at each study locale.

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Chapter 6 – Relationship of Populations at the Distribution Scale

The following chapter has been reviewed by co-authors and prepared for forthcoming journal submission as:

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Regional population structuring and conservation units in the platypus (*Ornithorhynchus anatinus*)

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Abstract

We examined broad scale genetic differences between populations of platypuses (*Ornithorhynchus anatinus*) across their distributional range in order to identify the occurrence or absence of population structuring and interconnectivity. Using a Bayesian approach we identified three large scale groupings that correspond closely to geographically distinct regions of the species' distribution. Six additional clusters were then identified within those three regional groups. Most of the six clusters were isolated with gene flow only detected between the two occurring in New South Wales. Pairwise F_{st} values for the regions and clusters identified ranged from 0.065 to 0.368 and 0.037 to 0.479 respectively. The differentiation between clusters was not due to isolation-by distance effects. We propose a series of evolutionarily significant units (ESUs) and possible management units (MUs) that should facilitate further study in relation to conservation of the platypus.

Introduction

The platypus *(Ornithorhynchus anatinus)* is the last extant species in the family Ornithorhynchidae and one of only five living species of egg laying monotremes left on earth (Grant 2007). Endemic to Australia, the semi-aquatic platypus can be found in inland waters of eastern Australia from the island of Tasmania in the south to around Cook Town, in north Queensland (Grant 1992; Grant and Temple-Smith 1998). While the IUCN lists the conservation status of the platypus as ‘of least concern’ (Lunney *et al.* 2008), its dependency on water bodies places it at risk of sudden declines due to the constant threat of anthropogenic habitat modification of stream, lake and wetland systems (Goudie 2006; Kingsford 2000). This threat is demonstrated by reports of population fragmentation and declines in agricultural and urban areas (Lintermans 1998; Lunney *et al.* 1998; Otley 2001; Rohweder and Baverstock 1999; Serena *et al.* 1998).

Currently the platypus is recognised as a single species with no subspecific categorisation (Mahoney 1988). Morphological and behavioural variation has been reported between parts of the species’ current distribution (Figure 6.1) (Connolly and Obendorf 1998; Grant 1992; Grant 2007; Munks *et al.* 1998) but studies to date have not included the investigation of these differences in an evolutionary context. Modern molecular biology techniques and analyses now provide greater opportunity for such investigations. The earliest attempt at a distribution scale analysis of platypus genetics, utilising microsatellite DNA markers and mitochondrial DNA haplotypes, was reported in a PhD thesis by Akiyama (1998). While his study showed regional genetic differences among platypus populations, especially between Tasmania and mainland Australia, there has been little follow up until recently.

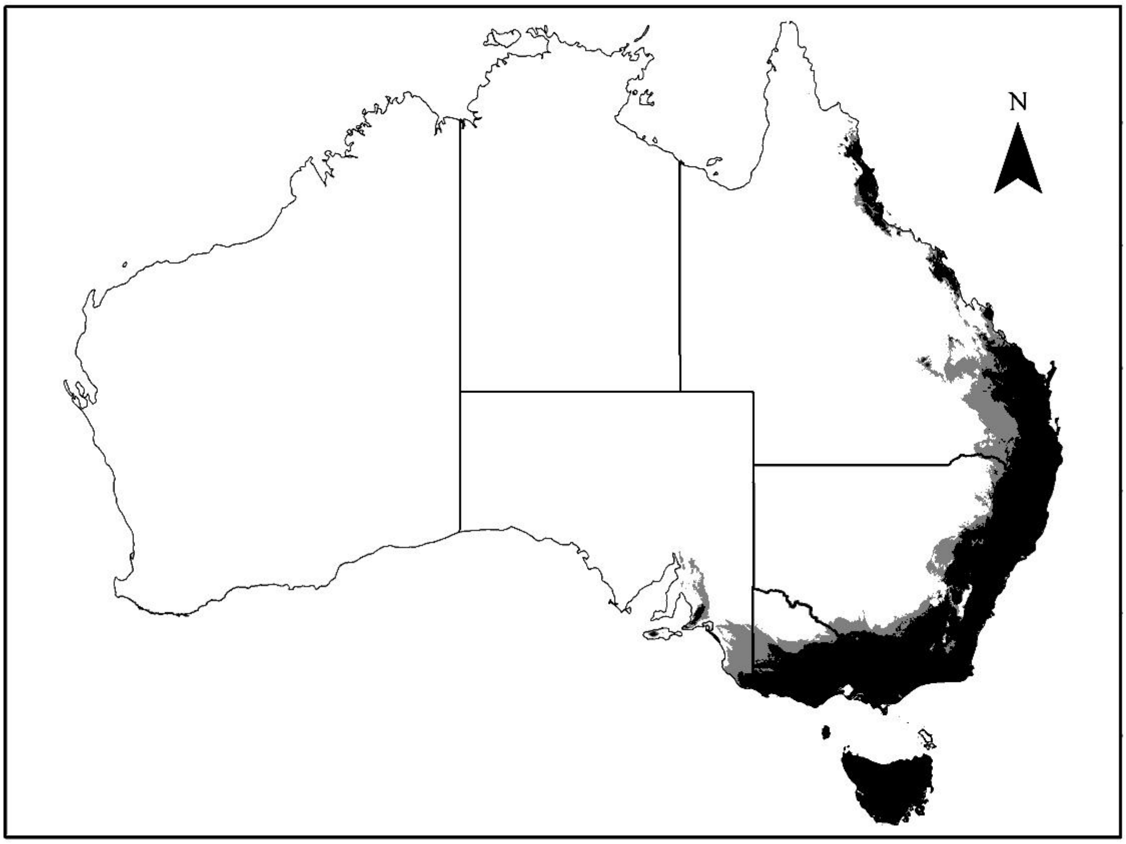


Figure 6.1 Species distribution model for *O. anatinus*. Black areas indicate highly suitable habitat while grey areas are marginally suitable. Based on Kolomyjec *et al.* (Chapter 2).

Two current, independent research efforts have used microsatellite DNA markers to demonstrate that limitations to gene flow at the river system level play an important role in establishing local population differentiation (Furlan *et al.* 2009; Kolomyjec *et al.* 2009). Furlan *et al.* (2009) were also able to use microsatellite DNA markers to show a high degree of genetic differentiation between Tasmania and Victoria (mainland Australia).

The successful identification of genetically distinct regional populations is vital to the conservation and management of any species, as the identification of separate management units (MU's) can protect diverse phenotypic and genotypic traits that broad-scale conservation efforts may not address (Moritz 1995). Management units are independent groups of animals that share enough gene flow to prevent reciprocal monophyly while being reproductively isolated to a degree at which each group is genetically distinct enough to warrant separate management (Moritz 1994; Palsbøll *et al.* 2006). MU's are often subunits of larger evolutionarily significant units (ESUs). ESU's represent groups of animals exhibiting any combination of genetic, geographic or phenotypic divergence resulting from historical isolation. The genetic divergence is at a great enough level to display reciprocal monophyly of mitochondrial haplotypes (Moritz 1995; Moritz 1999; Palsbøll *et al.* 2006).

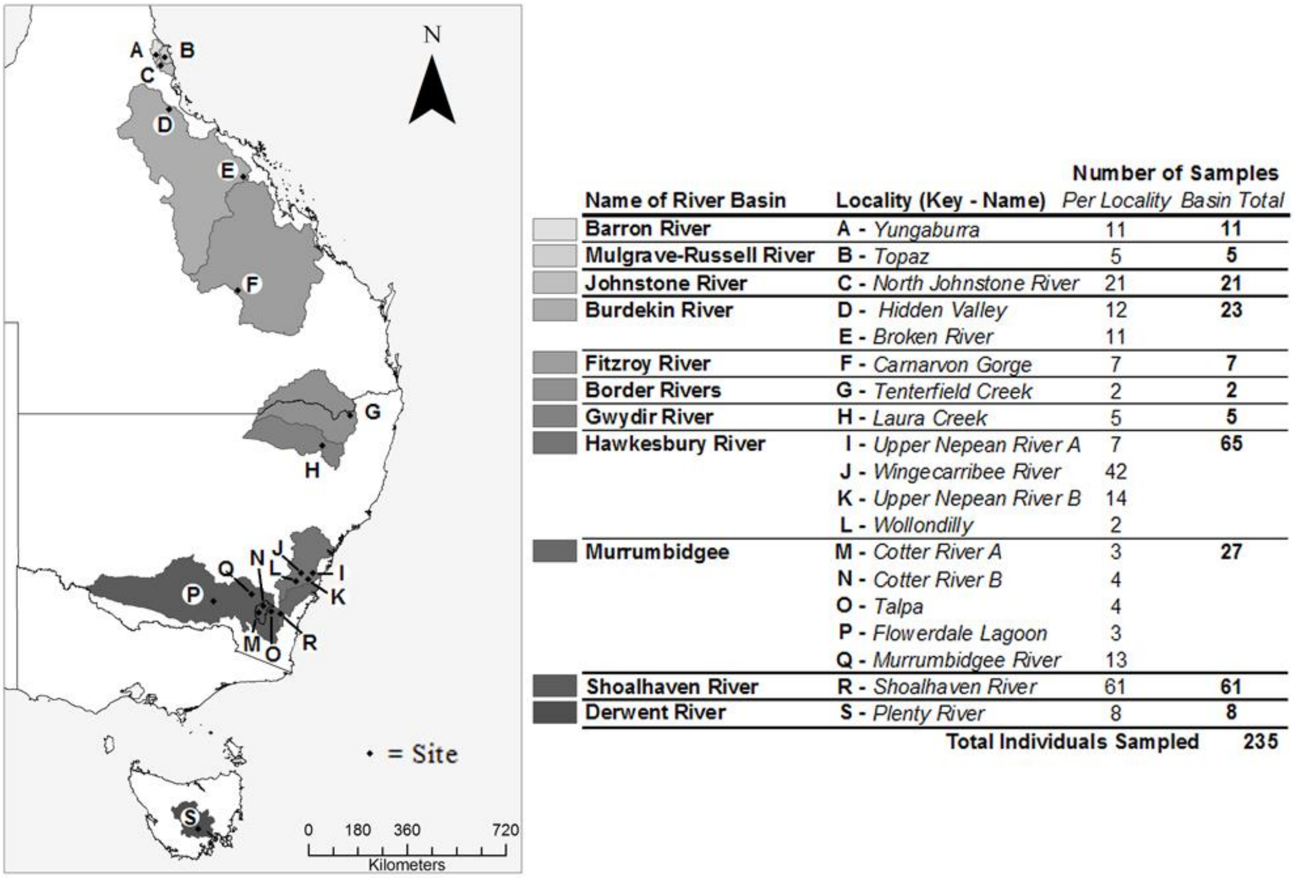
In this study, we used microsatellite DNA markers (Kolomyjec *et al.* 2008) as a tool to investigate regional population structure in the platypus across its current distribution in order to better understand its evolutionary history, how populations are interconnected across large distances, and to define meaningful conservation units.

Methods

Sampling and genotyping

Using unweighted, constantly monitored gill nets, 227 platypuses were captured across mainland Australia (Figure 6.2) according to standard methods (Grant and Carrick 1974). After capture, toe-web biopsies were taken and prior to release the individual animals were implanted with passive integrated transponder tags (microchips) (Grant and Whittington 1991) for identification and to prevent accidental re-sampling.

DNA was extracted from the toe-web biopsies (2 x 2 mm specimens stored in 70% ethanol) using a proteinase K/salt precipitation method (Sunnucks and Hales 1996). Twelve published microsatellite loci were amplified and scored according to standard techniques (Kolomyjec *et al.* 2009; Kolomyjec *et al.* 2008). Successful DNA isolation and genotyping yielded usable data for 227 mainland animals. This represents individuals from 13 river basins (Figure 6.2), in Queensland, New South Wales and the Australian Capital Territory. Unfortunately, material from Victoria was not available.



			Number of Samples	
	Name of River Basin	**Locality (Key - Name)**	*Per Locality*	*Basin Total*
▨	**Barron River**	**A** - *Yungaburra*	11	**11**
▨	**Mulgrave-Russell River**	**B** - *Topaz*	5	**5**
▨	**Johnstone River**	**C** - *North Johnstone River*	21	**21**
▨	**Burdekin River**	**D** - *Hidden Valley*	12	**23**
		E - *Broken River*	11	
▨	**Fitzroy River**	**F** - *Carnarvon Gorge*	7	**7**
▨	**Border Rivers**	**G** - *Tenterfield Creek*	2	**2**
▨	**Gwydir River**	**H** - *Laura Creek*	5	**5**
▨	**Hawkesbury River**	**I** - *Upper Nepean River A*	7	**65**
		J - *Wingecarribee River*	42	
		K - *Upper Nepean River B*	14	
		L - *Wollondilly*	2	
▨	**Murrumbidgee**	**M** - *Cotter River A*	3	**27**
		N - *Cotter River B*	4	
		O - *Talpa*	4	
		P - *Flowerdale Lagoon*	3	
		Q - *Murrumbidgee River*	13	
▨	**Shoalhaven River**	**R** - *Shoalhaven River*	61	**61**
▨	**Derwent River**	**S** - *Plenty River*	8	**8**
			Total Individuals Sampled	**235**

Figure 6.2 Sampled river basins and localities.

From Tasmania, a total of 19 buccal swab (ISOHELIX brand) samples were obtained. This was a previously un-trialled technique for collecting DNA from the platypus and had a poor rate of success. This was probably due to that fact that the platypus has cheek pouches for the temporary storage of food (Grant 2007) and as a result most samples were highly contaminated with partially masticated food. This coupled with the generally low yield of DNA obtained from buccal swabs (Mitsouras and Faulhaber 2009) relative to tissue biopsy resulted in usable data from only 8 Tasmanian platypuses (Figure 6.2). These samples brought the total number of individuals analysed in the study to 235.

Population clustering and differentiation

We utilised the Bayesian clustering methods implemented in the program STRUCTURE VER. 2.3 (Pritchard *et al.* 2000) to establish population clusters based on *a priori* genotype data. Each run in STRUCTURE involved 20 replicates, using an admixture and independent allele frequency model with a burn-in period of 20,000 followed by 20,000 Markov Chain Monte Carlo repetitions, for each estimate of the number of population clusters present (K). The range of values modelled for K was chosen as one to the number of populations sampled plus five, in order to ensure the likely value of K is obtained. Pritchard and Wren (2003) warn that in some circumstances there are potential computational difficulties in estimating K and that resulting biological interpretation of those estimates may be difficult. To avoid this we used the methods detailed by Evanno *et al.* (2005) to calculate ΔK which takes both the log-likelihood and the variance between replicates into account and allows a more reliable predictor of K.

The original data were then divided, based on the detected population clusters, and returned to STRUCTURE to repeat the process in order to assess the possibility of sub-clustering within the higher order clusters. This was repeated in succession until the level of admixture (representing genetic interchange between clusters) became too high to clearly identify distinct clusters. Thus, a hierarchical set of population clusters was established.

To confirm the results and to extend the analysis further the program BAPS VER. 5.3 (Corander and Marttinen 2006; Corander *et al.* 2006a; Corander *et al.* 2006b; Tang *et al.* 2009) was used. BAPS uses a more integrative Bayesian approach to achieve population clustering conceptually similar to that in STRUCTURE. The initial BAPS analytical run (population mixture analysis for clustering of groups of individuals) was set for a maximum K of 20. As the initial results from BAPS confirmed the results from the hierarchical STRUCTURE analysis, subsequent runs were based on 20 replicates of the optimum K from the initial BAPS run ± 3 in order to verify the outcome. In addition, by assigning the maximum value of K to match that first detected in STRUCTURE and re-running the original data set we were able to verify the first level division.

BAPS was then used to look for signs of admixture between regions and clusters (minimum pop. size = 5, iterations = 100, reference individuals = 200, reference iterations = 10). Admixture analysis within BAPS uses the output file from the mixture analysis as a starting point, in order to reduce the chance of spuriously high results. To aid in the interpretation of admixture, the raw admixture results were used to map the relative gene flow occurring between clusters ($\alpha = 0.05$, pruned to 0.01). To gain additional detail in the gene flow map, the admixture results were also regenerated forcing the original population groupings ($\alpha = 0.05$, pruned to 0.01). This allowed for the visualisation of the amount of relative gene flow that occurred between all the sample localities within the clusters that demonstrated admixture.

In addition to the Bayesian clustering analyses preformed, ARLEQUIN VER. 2.0 (Schneider *et al.* 2000) was used to calculate F_{st} as a widely used measure of differentiation between populations. FSTAT VER 2.93 (Goudet 1995) was used to determine the state of Hardy-Weinberg equilibrium (HWE) for the overall population, all loci, and individual clusters. Sequential Bonferroni corrections (Holm 1979; Rice 1989) were applied to the HWE results to prevent the likelihood of high type-I statistical error due to multiple tests. Finally, GENALEX VER. 6.3 (Peakall and Smouse 2006) was used to calculate distance matrices and run Mantel tests (10,000 permutations; pairwise genetic distance v. linear geographic distance) in order to look for the signal of an isolation-by-distance (IBD) effect across individuals. Isolation-by-distance for clusters was tested using a regression analysis of Slatkin's (1995) linearised FST transformation (FST/1 – FST) onto the natural log of geographic distance (km) between the closest individuals between clusters (Rousset 1997). A Mantel test (10, 000 permutations) was performed in POPTOOLS (Hood 2002) to determine the significance of this relationship.

Results

Population Clusters

Both STRUCTURE and BAPS identified a total of three top level clusters (i.e. K=3). These clusters divide the platypus distribution into three regions (Figure 6.3) corresponding to: northern mainland Australia (Region 1), southern mainland Australia (Region 2) and Tasmania (Region 3). At this level there were no admixed individuals detected.

The next level of population structure, also in agreement between STRUCTURE and BAPS, indicated the presence of six population clusters (Figure 6.3). Clusters one, two and three together form Region 1; clusters four and five form Region 2; and cluster six forms Region 3 (Figure 6.3). With the exception of clusters four and five, there is also no detectable admixture between clusters at this level of population structure. Attempts to find further levels of structuring that might be relevant were unsuccessful due to high admixture occurring within each cluster.

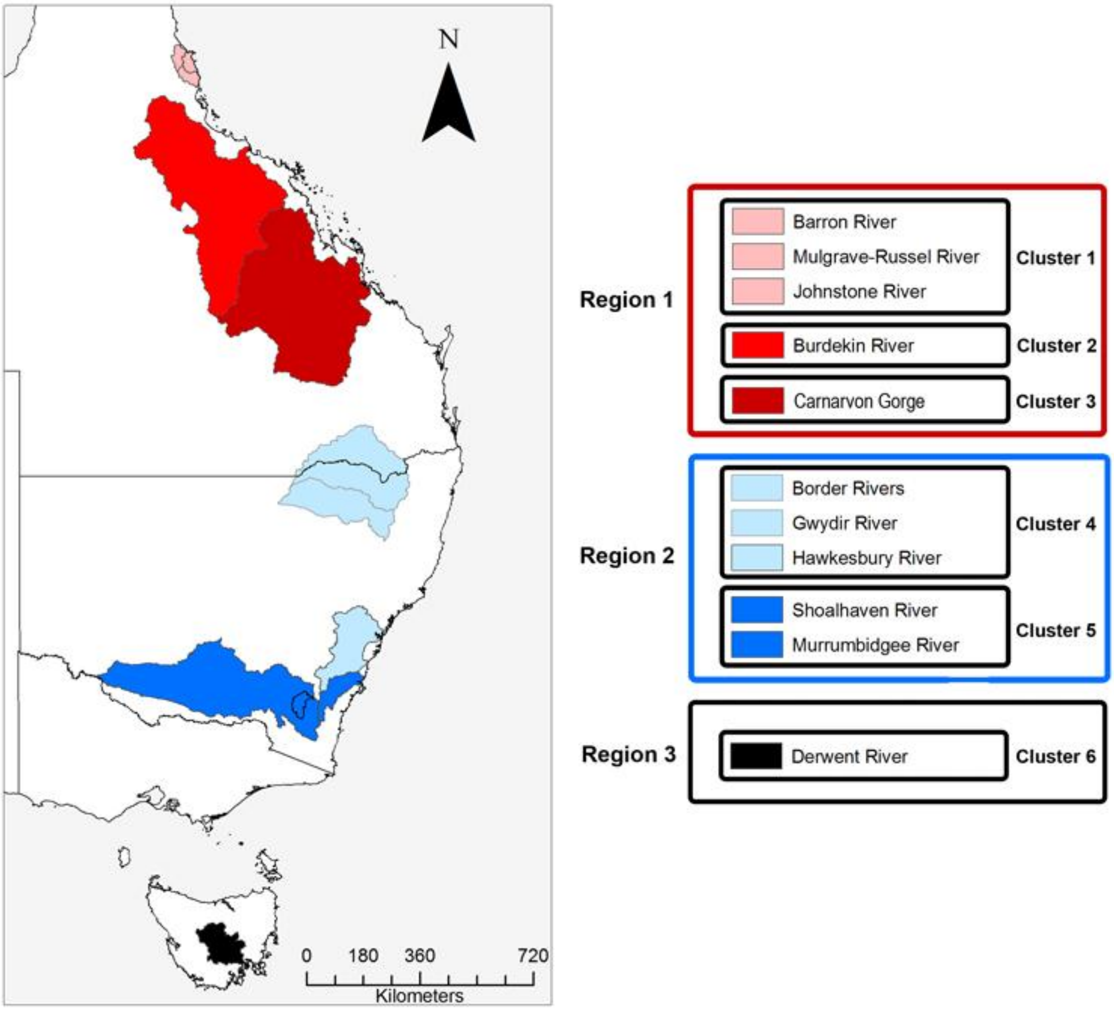


Figure 6.3 The relationships of detected Regions and population clusters.

Gene flow maps were produced to aid the visualisation of the inter-cluster relationships (Figure 6.4). The initial map indicated the amount of relative gene flow occurring at the cluster level. A second map, incorporating the original sample localities (Figure 6.2), produced a much more detailed view of the complex currents of gene flow that occur between clusters four and five (Figure 6.4).

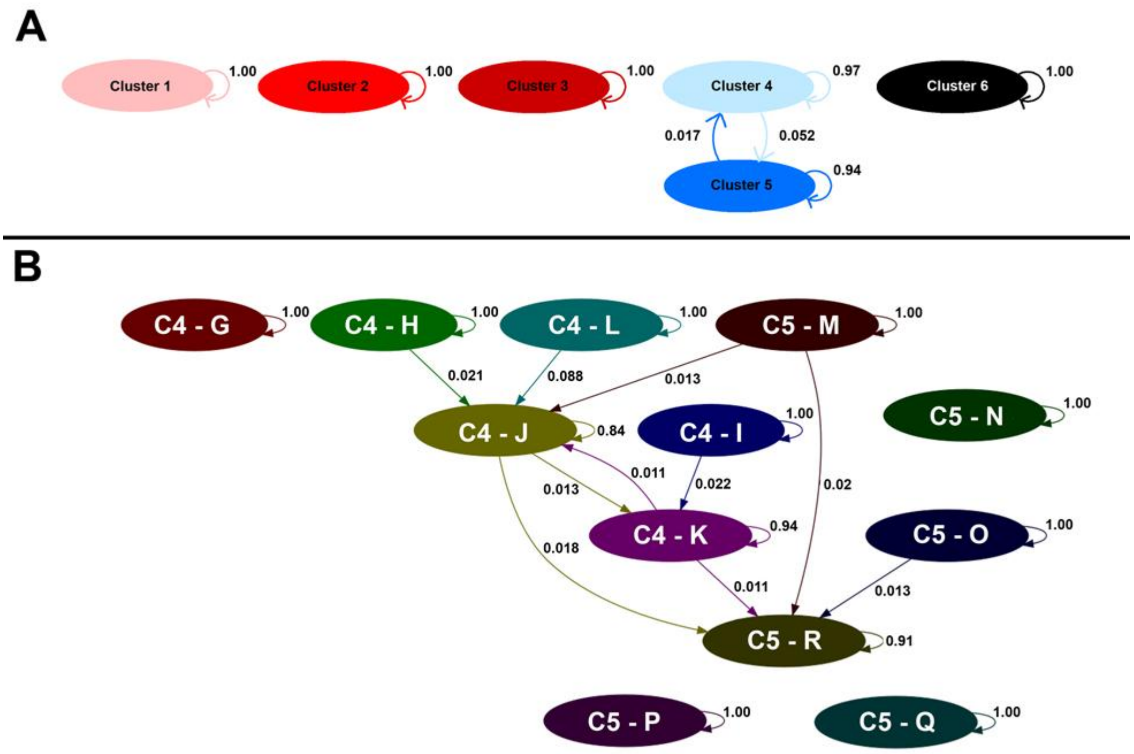


Figure 6.4 Map of relative gene flow detected in BAPS: A - gene flow at the cluster level, B - detail of clusters four and five demonstrating the complexities of gene flow between sample locales (due to sample size effects this level of detail is demonstrative only). The numbers near arrows indicate the amount of relative gene flow occurring and the direction of flow. For map B, C4 or C5 indicates whether that locality belongs to cluster 4 or cluster 5 respectively. The letter following the hyphen is a reference to the locality codes given in Figure 2 (Cluster 4: G - Tenterfield Creek; H - Laura Creek; I - Upper Nepean River A; J - Wingecarribee River; K - Upper Nepean River B; L - Wollondilly River. Cluster 5: M - Cotter River A; N - Cotter River B; O - Talpa; P - Flowerdale Lagoon; Q - Murrumbidgee River; R - Shoalhaven River.)

Population differentiation

Pairwise F_{st} ranged from 0.065 to 0.368 between regions and 0.037 and 0.479 between population clusters (Table 6.1). At the regional levels the largest F_{st} was between Tasmania and either of the two mainland regions. At the cluster level both Cluster 6 (Tasmania) and Cluster 3 (Carnarvon Gorge, Queensland) showed particularly high pairwise F_{st} values, ranging from 0.284 – 0.479 and 0.140 – 0.479 respectively.

Table 6.1 - Pairwise Fst Values between regions (A) and clusters (B). All results were **significant** with $p < 0.0001$.

A	Northern Australia	Southern Australia	Tasmania
Northern Australia	0.000		
Southern Australia	**0.065**	0.000	
Tasmania	**0.368**	**0.282**	0.000

B	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cluster 1	0.000					
Cluster 2	**0.103**	0.000				
Cluster 3	**0.177**	**0.224**	0.000			
Cluster 4	**0.098**	**0.112**	**0.140**	0.000		
Cluster 5	**0.087**	**0.103**	**0.172**	**0.037**	0.000	
Cluster 6	**0.398**	**0.412**	**0.479**	**0.284**	**0.304**	0.000

While both the platypus population as a whole and the loci used in the study were in Hardy-Weinberg equilibrium (HWE; $p > 0.05$ after sequential Bonferroni correction), two of the clusters were not. Clusters 4 and 5 both showed significant deviation from HWE.

A Mantel test, looking for a correlation between genetic distance and geographic distance at both the individual ($r^2 = 0.0015$, $p = 0.120$) and cluster level ($r^2 = 0.170$, $p = 0.989$), indicated that there was no isolation-by-distance (IBD) pattern in effect..

Discussion

Regions as evolutionarily significant units

Using microsatellite DNA markers to analyse a distribution-wide sampling of the platypus we have found that the distribution of the platypus can be divided into three major regions based on genetic population clustering (Figure 6.3). These major regions coincide with the geo-climatically isolated regions depicted in CHAPTER 2. The northern region is separated from the southern region by a series of areas (Figure 6.1) where platypuses have not been recorded and climatic modelling indicates that conditions are not suitable for the platypus (CHAPTER 2). The southern region is in turn isolated from the Tasmanian region by the Bass

Strait. These features have established a long-standing barrier to gene flow between regions that was estimated to have been in effect for well over 400,000 years (divergence estimate based on mitochondrial sequence data: Swan *et al. in review*). This predates the most recent separation of Tasmania from the mainland (aprox. 8 kya: George *et al.* 1990) and suggests that the divergence is due to more than simple terrestrial connectivity. The pairwise F_{st} values detected between Tasmania and either mainland region were much larger than that between each mainland region (Table 6.1). This suggests either a less complete or more recent separation of the mainland regions from each other than from Tasmania.

On the basis that these regions are genetically discrete, geographically isolated from each other (CHAPTER 2) and are congruent with the reciprocally monophyletic clades recently identified using mitochondrial DNA sequence (*Swan et al. in review*), we suggest their recognition as three evolutionarily significant units (ESUs) within *O. anatinus*: Northern, Southern, and Tasmanian. Combined with the results of Swan *et al.* (in review) these divisions meet the accepted definition (Ryder 1986) and proposed criteria (Moritz 1994; Paetkau 1999; Palsbøll *et al.* 2006) for ESUs.

Clusters and management units

The six population clusters (Figure 6.3) that were detected below the regional ESU level still appear to be based on geological isolation instead of geographical distance. This is indicated by the lack of any signal supporting an isolation-by-distance pattern. At this level, the river basins or localities within each cluster either share a recent common ancestor or share genes contemporarily as any differentiation between them is not enough to result in independent population clusters. Based on significant levels of genetic differentiation (Moritz 1994; Palsbøll *et al.* 2006) we grouped the six population clusters into five management units.

Cluster 1 encompasses three river basins on the Atherton Tableland region in tropical north Queensland. The headwaters and tributaries of these basins are in close proximity to each other and exhibit there are no strong natural barriers to inhibit genetic exchange between the basins.

The two sample localities that combine to form Cluster 2 occurred at the extreme latitudinal ends of the huge Burdekin River basin (~130,000 km^2, Dight 2009). The Burdekin River basin itself lacks any historical or contemporary reports of platypus presence apart from these populations on its margins. Both of these localities are in regions of cool upland rainforest, while the low-lying area between them is much warmer, more arid and, according to bioclimatic modelling, not suitable for the platypus (CHAPTER TWO). The highly erratic flow characteristics across the Burdekin River basin range from zero surface flow to discharges peaking at 40,000 m^3s^{-1} (Fielding and Alexander 2007). On rare occasions, climate conditions may create periods when conditions would allow dispersing platypuses to cross the otherwise inhospitable catchment.

Cluster 3 represents Carnarvon Gorge, an isolated rainforest-filled gorge juxtaposed in a semi-arid landscape more typical of inland central Queensland. Carnarvon Creek, which runs the length of the gorge and provides suitable habitat for the platypus, disappears underground soon after exiting the gorge. The platypus population in Carnarvon Creek is thus separated from the next suitable waterway by over 100 km of dry creek bed – in which platypus cannot live and across which they are unlikely to move. Connection to the rest of the Fitzroy River basin could only occur during infrequent high flow events. This cluster has the highest pairwise F_{st} values observed on the mainland (0.140 – 0.224), further supporting its relative isolation. The platypus is not the only species for which Carnarvon Gorge contains an isolated population (e.g. butterflies - Norgate *et al.* 2009, dragonflies – Watson and Theischinger 1984).

Combined, Clusters 4 and 5 represent the largest continuous area within the platypus distribution. The separation of these two clusters is not as complete as that of the other clusters. The pairwise F_{st} value between cluster four and cluster five was 0.037, the lowest, by far, of any cluster pair showing a lower level of differentiation between the two clusters. As these were also the only two clusters to show any admixture of individuals or detectable gene flow (Figure 6.4), this indicates that there is only partial isolation between them. This is further supported by the genetic evidence of cross catchment migrations inferred by Kolomyjec *et al.* (2009). As migration violates one of the key assumptions of Hardy-Weinberg Equilibrium and first generation migrants have been previously detected within the rivers of these clusters (Kolomyjec *et al.* 2009), this may also explain why these two clusters significantly deviated from HWE.

Finally, Cluster 6 is the most obvious. Cluster 6, representing Tasmania, has been isolated from the mainland since the formation of the Bass Strait after the last ice age, at least 7,000 year BP (Nevill 2010). However, the time of divergence between mainland and Tasmanian platypuses has recently been estimated by Swan *et al.* (*in review*) as 200 – 400 thousand years ago, indicating a period of isolation substantially pre-dating the most recent land bridge between Tasmania and mainland Australia. As would be expected for such a long period of isolation, the pairwise F_{st} values between Tasmania and the mainland clusters are very high (0.284 – 0.479).

By combining the outcomes of this research with knowledge of each of the detected clusters, we are able to recommend the recognition of a series of meaningful management units (MUs) for future monitoring and conservation of the platypus (Table 6.2).

Table 6.2 - Proposed management units for the platypus.

Name [Cluster]	Description	Relevant observations and suggestions
North Queensland [1]	Catchments and river basins north of the Burdekin, closely follows the Wet Tropics World Heritage Area in north Queensland	Large amounts of agricultural land use in this region interspersed with world heritage areas produces a high potential for habitat fragmentation.
Burdekin Catchment [2]	The Burdekin river catchment, particularly the highland areas of the Paluma Range and the area around Eungella, Queensland	While distance and political boundaries may make it practical to manage the two areas separately, any conservationally informed decisions need to consider them as one unit.
Carnarvon Gorge [3]	Highly isolated population within Carnarvon Gorge, central Queensland	The gorge falls entirely within a national park, managed by on-site rangers, and is probably relatively secure.
South-eastern Australia [4 & 5]	Virtually all the distribution not included in the above MUs, follows eastern side of the continent from southern Queensland southward	Largest continuous area of distribution observed in the platypus, spanning numerous habitat types. Water availability and habitat fragmentation are likely to be the key issues within this region. While many headwater streams are protected by national parks and reserves in this area, many waterways towards the coast and to the west of the Great Dividing Range are not.
Tasmania [6]	Tasmania	Further detailed studies of this region will likely subdivide the state (and King Island).

While future studies involving platypus population genetics that include river basins not covered in this study may identify additional, isolated MUs, the strong agreement between modelled species distribution and the evolutionarily significant units proposed in this paper suggests that many new locales will simply add to the continuity of the South-Eastern Australia MU. Either way any newly sampled rivers will fall most likely fall within the ESUs proposed in this paper. In particular, King Island (located between Tasmania and mainland Australia) will almost certainly be a separate management unit. One other island population does exist on Kangaroo Island (off the coast of South Australia). However, these platypuses were introduced in several intentional translocation events from the mainland and Tasmania (Grant 2007), do not represent a naturally-occurring population and so, should be managed accordingly.

This study is the first to identify and formally suggest a series of evolutionarily significant units based on patterns of genetic and geographic parameters for the platypus. While it is almost certain that there are additional management units within the identified ESUs. These were not detected by the current study due to the logistics of sampling platypuses over such a large geographic area, extending across almost 26 degrees of latitude. However, we have been able to provide a framework in which additional management units can easily be added if or when needed. By acknowledging these potential omissions we have also identified where future work in this field should be targeted.

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Stephen H Kolomyjec is a researcher interested in mammalian biology and population genetics. Tom Grant is involved in research, environmental impact and monitoring studies of the platypus. Chris Johnson is Professor of Wildlife Ecology and Conservation Biology at the University of Tasmania and James Cook University, and studies the ecology and conservation status of Australian mammals. David Blair is a Professor at James Cook University whose interests range from parasites to flying foxes.

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Section 4 – Conclusions

This section will examine the outcomes and general conclusions drawn from the study

Plate 4 Platypus returning to water
(Photo© S Kolomyjec)

Chapter 7 – Conclusion

Overview

The general aims of this thesis were to explore the biogeography of the platypus and the relationships between populations, especially those in northern Queensland. In meeting those aims, this study was the first to:

1. Produce a species distribution model for the platypus based on occurrence *and* climate data
2. Model platypus distribution onto other time frames (last glacial maximum and future climate change models)
3. Produce a published set of reliable microsatellite DNA markers for population genetic studies on the platypus
4. Infer the overland movement of dispersing platypuses between river systems
5. Use genetic markers to indentify the effects of a large dam on the population dynamics of *any* semi-aquatic mammal
6. Establish a series of evolutionarily significant units and management units based on genetic information and geographic distribution

In addition, this study represents one of the largest platypus sampling efforts to occur in the historically understudied northern aspect of the platypus distribution. And while Akiyama (1998) was the first person to attempt a distribution-wide study of the population genetics of the platypus, this is the first study to use modern molecular ecology tools and produced published results.

History

Since understanding a species' distribution and the limiting factors of that distribution is a vital step in understanding a species' ecology (Guisan and Zimmermann 2000), this study used bioclimatic modelling to produce a species distribution map (SDM) for the platypus. Not only does this depict the environmental space (aka climatically suitable habitat) available to the platypus, but it also allows for the identification of key environmental factors determining that environmental space (Chapter 2). The factors that are most important in determining this suitability in the platypus are ultimately temperature and water availability. If the temperature is too warm or there is not enough water to support the habitat than the platypus cannot survive. This is compounded by the fact that, for platypuses in the Wet Tropics of north Queensland, too much water can be a bad thing as it can wash away burrows and dependant young (Grant 2007).

This modelling also suggests that areas within the greater distribution that are known to lack observational evidence of the platypus are genuine distribution gaps resulting from climatically unsuitable habitat. By projecting these models onto climate data from the last glacial maximum (LGM) it becomes obvious that these gaps have existed for a substantial time period and are responsible for the large amount of genetic differentiation between the northern and southern regions of the platypus distribution (Chapter 6). Future ground truthing targeted at these areas of predicted climate insuitability may help confirm these findings.

By projecting the model forward, using various climate change scenarios, the effects of climate change on the platypus can be predicted. While individual scenario-models cover a wide spectrum of outcomes ranging from a slight increase (+3.5%) to a significant decline (-68%) in distribution, the aggregated mean suggests a more moderate decrease of approximately 15.2% by the year 2070.

Understanding the where and why of the platypus SDM will be a very useful tool for conservation planning and management. That usefulness if further strengthened by the ability to understand how that SDM has changed in the past and how it is expected to change in the future.

Relationships

The development and use of a reliable set of microsatellite markers (Chapter 3) has allowed the investigation into the interpopulation relationships across the platypus distribution (Chapters 4, 5 and 6). At the broad scale, the distribution can be divided into three evolutionarily significant units (ESUs) based on genetic and geographic isolation: Northern, Southern and Tasmanian (Chapter 6). These divisions meet the accepted definition (Ryder 1986) and proposed criteria (Moritz 1994; Paetkau 1999; Palsbøll *et al.* 2006) for ESU status. The first two ESUs are on the mainland and represent the same pattern of northern Queensland's isolation from the rest of Australia that was observed during distribution modelling (Chapter 2). The third ESU represents Tasmania, which is separated from the mainland by the Bass Strait. The genetic separation of Tasmania from the mainland has been documented previously (Akiyama 1998; Furlan et al. 2009). These major divisions are also supported by an, as of yet, unpublished mitochondrial study by Swan *et al.* (*in review*).

Within each of these ESUs there are distinct population clusters which serve well for the establishment of management units (MUs) to facilitate conservation and further study. This study identifies five distinct management units, although there are likely to be additional, unidentified MUs within the identified ESUs.

At the finer scale this study has identified the catchment or river system to be the locally distinct population unit. Individual migrants moving between systems maintain genetic connectivity and establish an isolation-by-distance pattern between local populations (Chapters 4 and 5). This movement between rivers suggests a degree of overland movement hitherto unsuspected to occur in the platypus[1] (Chapter 4). This study has also demonstrated that anthropogenic modifications to stream flow (e.g. the construction of a large dam) can alter the rates of gene flow that occur within and between river systems (Chapter 5).

Future studies and applicability to other species

As with many scientific studies this one has created at least as many questions as it has answered. One of the potential areas of further study highlighted by the results of this study is that of palaeo-modelling. While we were able to project the distribution of the platypus back to the last glacial maximum and demonstrate the long term nature of the distribution gap between the Northern and Southern ESUs, this raises the question: When were these regions last

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[1] The exception to this is, perhaps, the Tasmanian platypus which is reputed to move over land quite frequently (Pers. Comm. Peter. Temple-Smith).

connected? As additional palaeo-climate data becomes available it may eventually become possible to answer this question.

Additionally, the fact that this study was able to detect migrants between river systems raises several additional questions as to: who is moving (male or female; dispersing juvenile or transient adults?), when they are moving (at what season?) and why they are moving? It may be possible to answer these questions with an intensive study along two adjacent river systems utilising genetic techniques and telemetry.

Another major question is one of taxonomy. If a regionalised analysis of morphometric character data were to indicate that observed differences in anatomy were statistically correlated with the distributional and genetic data instead of simply a pattern of phenotypic plasticity such as Bergmann's Rule, then a serious re-evaluation of platypus taxonomy would be required. This may warrant the elevation of the ESU groupings to subspecies level or, perhaps, even species level. In the past multiple subspecies of platypus were recognised (CHAPTER ONE). These included a northern subspecies (*O. anatinus phoxinus*) as well as a Tasmania one (*O. anatinus crispus*) (Iredale and Troughton 1934). So, if the differences between regions are deemed great enough than this may be a simple re-instatement of the previous nomenclature.

The relationship of climate, geography and genetics as observed in this study may be applicable to other species of semi-aquatic mammals (such as river otters [subfamily Lutrinae] and yapoks [*Chironectes minimus*]). At the very least, it provides an example for comparison to future studies on animals with similar niches; however, combined with similar studies on other semi-aquatic mammals for comparison it may become possible to establish a set of critical parameters that would be applicable across the group and would allow effective conservation and management plans for these highly specialised animals to the established.

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Appendix

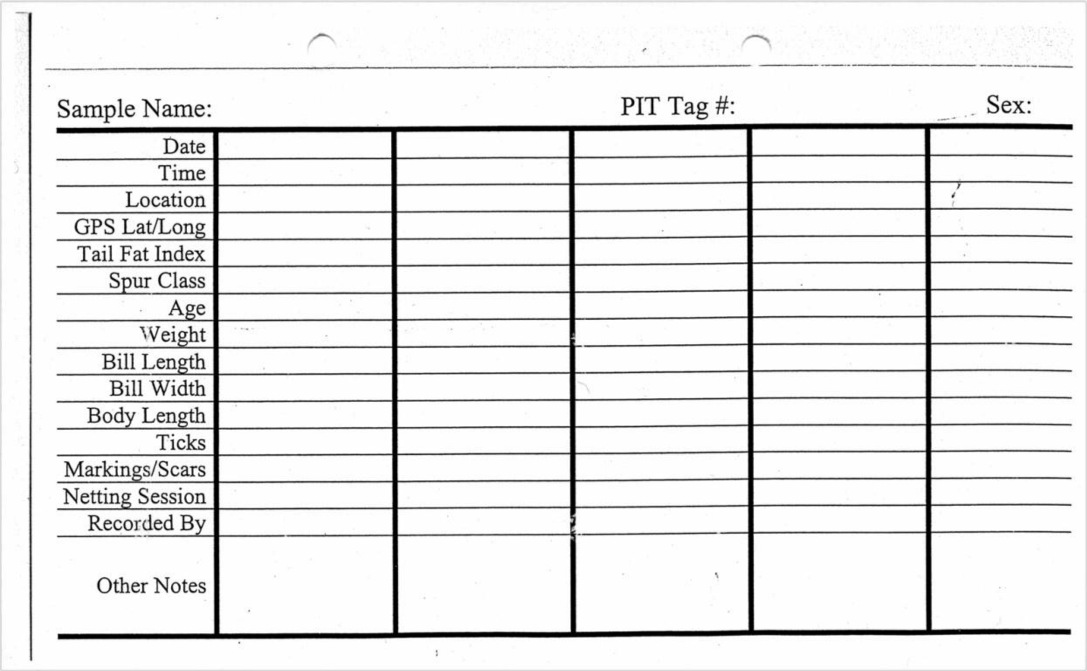
Relevant material that is not included within the main body of the thesis, including: permits, data sheets and raw data.

Plate 5. Collecting morphometric data from a platypus.
(Photo S Kolomyjec)

Appendix 1 – Permits

ADMINISTRATIVE DOCUMENTATION HAS BEEN REMOVED

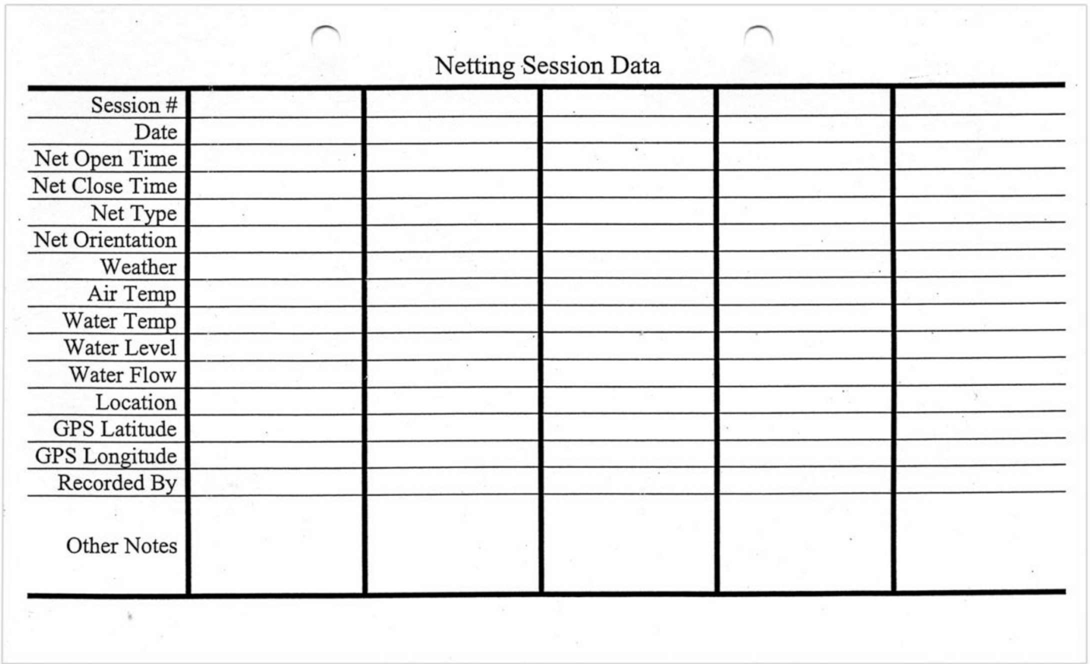
Appendix 2 – Data Sheets



Sample Name: PIT Tag #: Sex:

Date					
Time					
Location					
GPS Lat/Long					
Tail Fat Index					
Spur Class					
Age					
Weight					
Bill Length					
Bill Width					
Body Length					
Ticks					
Markings/Scars					
Netting Session					
Recorded By					
Other Notes					

A.



Netting Session Data

Session #					
Date					
Net Open Time					
Net Close Time					
Net Type					
Net Orientation					
Weather					
Air Temp					
Water Temp					
Water Level					
Water Flow					
Location					
GPS Latitude					
GPS Longitude					
Recorded By					
Other Notes					

B.

Data sheets used to collect field data. Sheet A contains information on the individual animal, while Sheet B records information on each netting session.

Appendix 3 – Raw Data

The following pages contain all raw data collected during this study.

Pages 132-144 contain the raw genotypic data collected for this study. Each subset of genotypic the data is preceded by location code tables, except for the Tasmania data which is all from the Derwent River basin.

Pages 145-146 contain the data collected on all platypuses caught by the author.

Page 147 contains summary trapping statistics and pp.148-160 contains the details of the cumulative trapping effort.

This data is the intellectual property of the author (S. Kolomyjec) and collecting researchers (Tom Grant – New South Wales samples, Joanne Connolly – Murrumbidgee samples and Rachelle Olsson-Herrin – Tasmanian samples) and any derivative analysis requires written permission.

Queensland Location Codes

Catchment	Code	Location	Latitude	Longitude
Barron river	Q-BRR-APS	Atherton Pumping Station	-17.26118	145.53753
	Q-BRR-POL	Pollumbo's	-17.29203	145.60449
Russel River	Q-RR-CAZ	Cazzonelli's Dam	-17.40474	145.72754
North Johnstone	Q-NJ-CHP	Chapman	-17.38329	145.55541
	Q-NJ-HIL	Hillcrest (Dam)	-17.39181	145.60498
	Q-NJ-MK3	McKenna P3	-17.45319	145.58948
	Q-NJ-MKR	McKenna - Riffle	-17.45379	145.58854
	Q-NJ-MK4	McKenna P4	-17.45394	145.59000
Hidden Valley (Running River)	Q-HV-3G	"Old Place"/Large Pool	-18.95521	146.07456
	Q-HV-M	Martin's place	-18.95632	146.04690
	Q-HV-B	Bridge	-18.98039	146.02572
Broken River	Q-BRK-CRD	Crediton Loop 3	-21.18030	148.54273
	Q-BRK-E4	Sheehy's Dam	-21.20374	148.52836
	Q-BRK-E6	Dam Adjacent to Sheehy	-21.20711	148.52961
	Q-BRK-E3	Grogan's Dam	-21.22868	148.55714
Carnarvon	Q-CG-X6	Carnarvon Gorge crossing 6	-25.05139	148.21686
	Q-CG-FN3	Carnarvon Gorge FN3	-25.05711	148.24204
	Q-CG-FN1	Carnarvon Gorge FN1	-25.05821	148.24017
	Q-CG-TK	Carnarvon Gorge - Takaraka Resort	-25.07332	148.26892

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New South Wales Locations Codes

Catchment	Code	River/Site	Latitude	Longitude
Border Rivers	N-TFC-TMC	Tenterfield Creek - 10 mile creek	-29.025	151.8667
	N-TFC-NEH	Tenterfield Creek - New England HWY	-28.985	151.9499
Gwydir	N-LAC-BUB	Laura Creek - Bundarra Bridge	-30.137296	151.024972
	N-LAC-BAB	Laura Creek - Baldersleigh Bridge	-30.262729	151.419383
	N-LAC-LAS	Laura Creek -Laura Station	-30.2334	151.1503
	N-LAC-HUC	Laura Creek - Hudson Crossing	-30.2335	151.4756
	N-LAC-RDS	Laura Creek - Roadside	-30.239	151.4741
Hawkesbury-Nepean	N-NPR-ADR	Upper Nepean - Avon Dam Road (below dam) Upstream	-34.330219	150.625536
	N-NPR-ADR	Upper Nepean - Avon Dam Road (below dam) Downstream	-34.330156	150.628464
	N-NPR-BLC	Upper Nepean - Belmore Crossing	-34.510724	150.548012
	N-NPR-CHC	Upper Nepean - Chalkers Crossing	-34.455473	150.530832
	N-NPR-MGC	Upper Nepean - McGuires Crossing	-34.476935	150.534132
	N-NPR-TRB	Upper Nepean - Tourist Road Bridge	-34.526412	150.582073
	N-NPR-WCJ	Upper Nepean - Wallaby Creek juntion	-34.523615	150.574954
	N-WRR-S1	Wingecarribee 1 -	-34.4118	150.195105
	N-WRR-S2	Wingecarribee 2 -	-34.437121	150.189959
	N-WRR-S3	Wingecarribee 3 -	-34.448776	150.18002
	N-WLR-PRJ	Wollondilly - Paddys R Junction	-34.580444	150.082829
Shoalhaven	N-SHR-S1	Cattle Co.	-35.62699	149.612899
	N-SHR-S2	Main-Lower (d/s of main riffles)	-35.641144	149.61074
	N-SHR-S3	Jerrabattgulla Creek	-35.656026	149.598831
	N-SHR-S4	Junction Creek	-35.639397	149.607285
	N-SHR-S5	Moga Station	-35.639397	149.607285
Cotter	A-COT-VC	Vanitys Crossing	-35.346092	148.889704

*Cotter is ACT

sample processed in full or part by JYT Chong

ID Code Extras

FA - Female Adult
FJ-FemaleJuvenile
MA-MaleAdult
MJ-MaleJuvenile

TABLES OF RAW DATA HAVE BEEN REMOVED

Location Codes for Samples From Joanne Connolly

Catchment	Code	River	Site	Latitude	Longitude
Murrumbidgee	A-COT-CG	Cotter River	Campground	-35.326	148.948
	NSW-FLW-BLY	Flowerdale Lagoon	Bailey	-35.1	147.3
	NSW-FLW-KD	Flowerdale Lagoon	Kebab Dan	-35.112	147.340
	NSW-GDR-DH	Goodradigbee	Dip hole	-35.151	148.695
	NSW-KD-EE	Kunama Dam	East end	-35.544	148.118
	NSW-MUR-BW	Murrumbidgee	Berembed weir	-34.879	146.837
	NSW-MUR-CDA	Murrumbidgee	Childowla	-34.902	148.517
	NSW-MUR-SDL	Murrumbidgee	Scottsdale	-35.883	149.130
	NSW-MUR-TD	Murrumbidgee	Tantangara dam DS	-35.796	148.665
	NSW-QBN-TLP	Queanbeyan	Talpa	-35.403	149.256
	NSW-TUM-JB	Tumut River	Jones Bridge	-35.378	148.244
	NSW-TUM-JMUR	Tumut River	Junction with Murrumb	-35.025	148.182
	NSW-YAS-HRD	Yass River	Hardwicke Stud	-34.860	148.946

TABLES OF RAW DATA HAVE BEEN REMOVED

Buccal Swab Samples From Derwent River via Rachelle Olsson-Herrin

TABLES OF RAW DATA HAVE BEEN REMOVED

TABLES OF RAW DATA HAVE BEEN REMOVED

Trapping Statistics

Total Nights	90
Total Captures	74
Total Platypuses	68
Total Nets*	371
Total Net Hours	2933.75
Capture per net hour	0.0252
Platypus per net hour	0.0232
Capture per night	0.8222
platypus per night	0.7556
Capture per Trap night	0.1995
platypus per trap night	0.1833

*adjusted for miscounting on net # assignment (5 additional nets)

Trap Night	Net #	Date	Open Time	Close Time	Hours open	Type	Orientation	Weather	Air temp	Water Temp	Water Level	Water Flow	Location	Latitude	Longitude
1	1	15-Sep-07	4:30pm	10:45pm	6.25	Fyke	Upstream	clear	22-16	16	Normal	slow	Birthday Creek	-18.97804	146.16438
2	2	05-Oct-07	4:30pm	10PM	5.5	Gill	Parallel	clear			Normal	slow	Birthday Creek	-18.97804	146.16438
	3	05-Oct-07	4:30pm	10PM	5.5	Fyke	Upstream	clear			Normal	slow	Birthday Creek	-18.97804	146.16438
3	4	24-Oct-07	6.45pm	11.15pm	4.5	Gill	Across	l. cloud	26	26	2+ meters	slow	HV-M	-18.95642	146.04658
	5	24-Oct-07	5pm	10pm	5	Gill	Across	l. cloud	26	26	2+ meters	slow	HV-M	-18.95642	146.04658
4	6	26-Dec-07	2pm		36	Gill	Parallel	rain	26		rising	normal	HV-M	-18.95603	146.04711
	7	26-Dec-07	2.30pm		36	Gill	Parallel	rain	26		rising	normal	HV-M	-18.95632	146.0469
	8	26-Jan-08	1.30pm	Midnight	10.5	Gill	Parallel	clear	26	26	high	still	HV-M	-18.95632	146.0469
	9	26-Jan-08	1.30pm	Midnight	10.5	Gill	Parallel	clear	26	26	high	still	HV-M	-18.95632	146.0469
	10	26-Jan-08	3pm	Midnight	9	Fyke	Upstream	clear	26	26	shallow/sanc	slow	HV-M	-18.95632	146.0469
5	11	27-Jan-08	3pm	11pm	8	modifie	Across	clear	30+	26	high	still	HV-M	-18.95632	146.0469
	12	27-Jan-08	3pm	11pm	8	large' gi	Parallel	clear	30+	26	high	still	HV-M	-18.95632	146.0469
	13	27-Jan-08	3pm	11pm	8	Gill	Parallel	clear	30+	26	high	still	HV-M	-18.95632	146.0469
	14	27-Jan-08	2.30pm	6am	15.5	Fyke	Downstream	clear	30+	26	high	still	HV-M	-18.95632	146.0469
	15	27-Jan-08	2.30pm	6am	15.5	Fyke	Downstream	clear	30+	26	high	still	HV-M	-18.95632	146.0469
6	16	17-May-08	6pm	11.30pm	5.5	Gill	Parallel	clear	16	19.5	Normal	slow-modera	McKenna's (P1)	-17.45503	145.58818
	17	17-May-08	7pm	11.30pm	4.5	Fyke	Upstream	clear	16	19.5	Normal	slow-modera	McKenna's Riffle		
7	18	18-May-08	4pm	11.30pm	7.5	Gill	Enclosure	clear	12	18.75	low	still	Pollumbo	-17.29203	145.60449
	19	18-May-08	4pm	11.30pm	7.5	Gill	Enclosure	clear	12	18.75	low	still	Pollumbo	-17.29203	145.60449
	20	18-May-08	4pm	11.30pm	7.5	Gill	Enclosure	clear	12	18.75	low	still	Pollumbo	-17.29203	145.60449
8	21	19-May-08	2.30pm	11.30pm	9	Gill	Enclosure	clear	18	18.5	Normal	slow	McKenna (P2)	-17.45482	145.58756
	22	19-May-08	2.30pm	11.30pm	9	Gill	Enclosure	clear	18	18.5	Normal	slow	McKenna (P2)	-17.45482	145.58756
	23	19-May-08	2.30pm	11.30pm	9	Gill	Enclosure	clear	18	18.5	Normal	slow	McKenna (P2)	-17.45482	145.58756
	24	19-May-08	3pm	11.30pm	8.5	Fyke	Upstream	clear	18	18.5	shallow	fast	McKenna's Riffle	-17.45379	145.58854
9	25	24-May-08	4pm	Midnight	8	Fyke	Upstream	sprinkle	23	18.5	shallow	fast	McKenna's Riffle	-17.45379	145.58854
	26	24-May-08	3pm	Midnight	9	Gill	Enclosure	sprinkle	23	18.5	Normal	slow	McKenna (P3)	-17.45319	145.58948
	27	24-May-08	3pm	Midnight	9	Gill	Enclosure	sprinkle	23	18.5	Normal	slow	McKenna (P3)	-17.45319	145.58948
	28	24-May-08	3pm	Midnight	9	Gill	Enclosure	sprinkle	23	18.5	Normal	slow	McKenna (P3)	-17.45319	145.58948

	29	24-May-08	3pm	Midnight	9	Gill	Enclosure	sprinkle	23	18.5	Normal	slow	McKenna (P3)	-17.45319	145.58948
	30	24-May-08	3pm	Midnight	9	Gill	Enclosure	sprinkle	23	18.5	Normal	slow	McKenna (P3)	-17.45319	145.58948
	31	24-May-08	3.30pm	Midnight	8.5	Fyke	Upstream	sprinkle	23	18	Normal	fast	McKenna	-17.45349	145.58973
10	32	25-May-08	4.30pm	Midnight	7.5	Fyke	Upstream	rain	21		Normal	fast	McKenna	-17.45379	145.58854
	33	25-May-08	4.30pm	Midnight	7.5	Fyke	Upstream	rain	21		Normal	fast	McKenna	-17.45379	145.58854
	34	25-May-08	4pm	Midnight	8	Gill	Enclosure	rain	21		Normal	slow	McKenna (P3)	-17.45319	145.58948
	35	25-May-08	4pm	Midnight	8	Gill	Enclosure	rain	21		Normal	slow	McKenna (P3)	-17.45319	145.58948
	36	25-May-08	4pm	Midnight	8	Gill	Enclosure	rain	21		Normal	slow	McKenna (P3)	-17.45319	145.58948
	37	25-May-08	4pm	Midnight	8	Gill	Enclosure	rain	21		Normal	slow	McKenna (P3)	-17.45319	145.58948
	38	25-May-08	4pm	Midnight	8	Gill	Enclosure	rain	21		Normal	slow	McKenna (P3)	-17.45319	145.58948
11	39	31-May-08	4pm	Midnight	8	Gill	Enclosure	rain	18.5	16.5	Normal	slow	McKenna (P3)	-17.45319	145.58948
	40	31-May-08	4pm	Midnight	8	Gill	Enclosure	rain	18.5	16.5	Normal	slow	McKenna (P3)	-17.45319	145.58948
	41	31-May-08	4pm	Midnight	8	Gill	Enclosure	rain	18.5	16.5	Normal	slow	McKenna (P3)	-17.45319	145.58948
	42	31-May-08	4pm	Midnight	8	Gill	Enclosure	rain	18.5	16.5	Normal	slow	McKenna (P3)	-17.45319	145.58948
	43	31-May-08	4pm	Midnight	8	Gill	Enclosure	rain	18.5	16.5	Normal	slow	McKenna (P3)	-17.45319	145.58948
	44	none													
	45	31-May-08	4.30pm	10.30pm	6	Fyke	Upstream	clear	18.5	16.5	shallow	fast	McKenna	-17.45379	145.58854
	46	31-May-08	4.30pm	10.30pm	6	Fyke	Upstream	clear	18.5	16.5	shallow	fast	McKenna	-17.45379	145.58854
12	47	01-Jun-08	4pm	11pm	7	Gill	Enclosure	clear			Normal	slow	McKenna (P3)	-17.45319	145.58948
	48	01-Jun-08	4pm	11pm	7	Gill	Enclosure	clear			Normal	slow	McKenna (P3)	-17.45319	145.58948
	49	01-Jun-08	4pm	11pm	7	Gill	Enclosure	clear			Normal	slow	McKenna (P3)	-17.45319	145.58948
	50	01-Jun-08	4pm	11pm	7	Gill	Enclosure	clear			Normal	slow	McKenna (P3)	-17.45319	145.58948
	51	01-Jun-08	4pm	11pm	7	Gill	Enclosure	clear			Normal	slow	McKenna (P3)	-17.45319	145.58948
	52	01-Jun-08	4.30pm	11pm	6.5	Fyke	Up/Downstre	clear			Normal	fast	McKenna	-17.45379	145.58854
	53	01-Jun-08	4.30pm	11pm	6.5	Fyke	Up/Downstre	clear			Normal	fast	McKenna	-17.45379	145.58854
13	54	03-Jun-08	5.30pm	11pm	5.5	Gill	Enclosure	clear	20		Normal	slow	McKenna (P3)	-17.45319	145.58948
	55	03-Jun-08	5.30pm	11pm	5.5	Gill	Enclosure	clear	20		Normal	slow	McKenna (P3)	-17.45319	145.58948
	56	03-Jun-08	5.30pm	11pm	5.5	Gill	Enclosure	clear	20		Normal	slow	McKenna (P3)	-17.45319	145.58948
	57	03-Jun-08	5.30pm	11pm	5.5	Gill	Enclosure	clear	20		Normal	slow	McKenna (P3)	-17.45319	145.58948
	58	03-Jun-08	5.30pm	11pm	5.5	Gill	Enclosure	clear	20		Normal	slow	McKenna (P3)	-17.45319	145.58948

	59	03-Jun-08	6.30pm	11pm	4.5	Fyke	Up/Downstre	clear	20		Normal	fast	McKenna	-17.45379	145.58854
	60	03-Jun-08	6.30pm	11pm	4.5	Fyke	Up/Downstre	clear	20		Normal	fast	McKenna	-17.45379	145.58854
14	61	05-Jun-08	7am	6pm	11	Gill	Across	rain to sun	20		Normal	slow	McKenna (P3)	-17.45319	145.58948
	62	05-Jun-08	7.30am	6pm	10.5	Gill	Enclosure	rain to sun	20		Normal	slow	McKenna (P3)	-17.45319	145.58948
	63	05-Jun-08	7.30am	6pm	10.5	Gill	Enclosure	rain to sun	20		Normal	slow	McKenna (P3)	-17.45319	145.58948
	64	05-Jun-08	7.30am	6pm	10.5	Gill	Enclosure	rain to sun	20		Normal	slow	McKenna (P3)	-17.45319	145.58948
	65	05-Jun-08	7.30am	6pm	10.5	Gill	Enclosure	rain to sun	20		Normal	slow	McKenna (P3)	-17.45319	145.58948
15	66	06-Jun-08	5am	6pm	13	Gill	Enclosure	clear	warm-hot		Normal	slow	McKenna (P3)	-17.45319	145.58948
	67	06-Jun-08	5am	6pm	13	Gill	Enclosure	clear	warm-hot		Normal	slow	McKenna (P3)	-17.45319	145.58948
	68	06-Jun-08	5am	6pm	13	Gill	Enclosure	clear	warm-hot		Normal	slow	McKenna (P3)	-17.45319	145.58948
	69	06-Jun-08	5am	6pm	13	Gill	Enclosure	clear	warm-hot		Normal	slow	McKenna (P3)	-17.45319	145.58948
	70	06-Jun-08	5am	6pm	13	Gill	Enclosure	clear	warm-hot		Normal	fast	McKenna	-17.45379	145.58854
	71	06-Jun-08	5.30am	6pm	12.5	Fyke	Up/Downstre	clear	warm-hot		Normal	fast	McKenna	-17.45379	145.58854
	72	06-Jun-08	5.30am	6pm	12.5	Fyke	Up/Downstre	clear	warm-hot		Normal	fast	McKenna	-17.45379	145.58854
16	73	25-Jul-08	4pm	10pm	6	Fyke	Up/Downstre	clear	warm	17	slightly up	sedimentate	McKenna	-17.45379	145.58854
	74	25-Jul-08	4pm	10pm	6	Fyke	Up/Downstre	clear	warm	17	slightly up	sedimentate	McKenna	-17.45379	145.58854
	75	25-Jul-08	3pm	10pm	7	Gill	Enclosure	clear	warm	17	slightly up	sedimentate	McKenna (P3)	-17.45319	145.58948
	76	25-Jul-08	3pm	10pm	7	Gill	Enclosure	clear	warm	17	slightly up	sedimentate	McKenna (P3)	-17.45319	145.58948
	77	25-Jul-08	3pm	10pm	7	Gill	Enclosure	clear	warm	17	slightly up	sedimentate	McKenna (P3)	-17.45319	145.58948
	78	25-Jul-08	3pm	10pm	7	Gill	Enclosure	clear	warm	17	slightly up	sedimentate	McKenna (P3)	-17.45319	145.58948
	79	25-Jul-08	3pm	10pm	7	Gill	Enclosure	clear	warm	17	slightly up	sedimentate	McKenna (P3)	-17.45319	145.58948
17	80	26-Jul-08	3pm	10pm	7	Gill	Enclosure	clear	warm		Normal	moderate	McKenna (P4)	-17.45394	145.59000
	81	26-Jul-08	3pm	10pm	7	Gill	Enclosure	clear	warm		Normal	moderate	McKenna (P4)	-17.45394	145.59000
	82	26-Jul-08	3pm	10pm	7	Gill	Enclosure	clear	warm		Normal	moderate	McKenna (P4)	-17.45394	145.59000
	83	26-Jul-08	3pm	10pm	7	Gill	Enclosure	clear	warm		Normal	moderate	McKenna (P4)	-17.45394	145.59000
	84	26-Jul-08	3pm	10pm	7	Gill	Enclosure	clear	warm		Normal	moderate	McKenna (P4)	-17.45394	145.59000
	85	26-Jul-08	4pm	11pm	7	Fyke	Up/Downstre	clear	warm		Normal	fast	McKenna	-17.45482	145.58756
	86	26-Jul-08	4pm	11pm	7	Fyke	Up/Downstre	clear	warm		Normal	fast	McKenna	-17.45482	145.58756
	87	26-Jul-08	4pm	11pm	7	Fyke	Up/Downstre	clear	warm		Normal	fast	McKenna	-17.45482	145.58756
18	88	28-Jul-08	5am	6pm	13	Gill	Enclosure	clear	warm		Normal	swift	McKenna (P4)	-17.45394	145.59000

	89	28-Jul-08	5am	6pm	13	Gill	Enclosure	clear	warm		Normal	swift	McKenna (P4)	-17.45394	145.59000
	90	28-Jul-08	5am	6pm	13	Gill	Enclosure	clear	warm		Normal	swift	McKenna (P4)	-17.45394	145.59000
	91	28-Jul-08	5am	6pm	13	Gill	Enclosure	clear	warm		Normal	swift	McKenna (P4)	-17.45394	145.59000
	92	28-Jul-08	5am	6pm	13	Gill	Enclosure	clear	warm		Normal	swift	McKenna (P4)	-17.45394	145.59000
	93	28-Jul-08	6am	6pm	12	Fyke	Up/Downstre	clear	warm		Normal	swift	McKenna	-17.45379	145.58854
	94	28-Jul-08	6am	6pm	12	Fyke	Up/Downstre	clear	warm		Normal	swift	McKenna	-17.45379	145.58854
	95	28-Jul-08	12.30pm	10pm	9.5	Gill	Across	clear	warm	17	Normal	smooth	McKenna (P2)	-17.45482	145.58756
19	96	29-Jul-08	3pm	10pm	7	Gill	Parallel	clear	v. cold after dar		Normal	v.slow	Birchill	-17.29127	145.59493
	97	29-Jul-08	3pm	10pm	7	Gill	Parallel	clear	v. cold after dar		Normal	v.slow	Birchill	-17.29127	145.59493
	98	29-Jul-08	3.30pm	10pm	6.5	Fyke	Up/Downstre	clear	v. cold after dar		Normal	v.slow	Birchill	-17.29127	145.59493
	99	29-Jul-08	3.30pm	10pm	6.5	Fyke	Up/Downstre	clear	v. cold after dar		Normal	v.slow	Birchill	-17.29127	145.59493
20	100	30-Jul-08	2pm	8.30pm	6.5	Gill	Across Dam	windy	cold	17	Normal	nil	Cazzonelli	-17.40474	145.72754
	101	30-Jul-08	2pm	8.30pm	6.5	Gill	Across Dam	windy	cold	17	Normal	nil	Cazzonelli	-17.40474	145.72754
	102	30-Jul-08	2pm	8.30pm	6.5	Gill	Across Dam	windy	cold	17	Normal	nil	Cazzonelli	-17.40474	145.72754
	103	30-Jul-08	2pm	8.30pm	6.5	Gill	Across Dam	windy	cold	17	Normal	nil	Cazzonelli	-17.40474	145.72754
	104	30-Jul-08	2pm	8.30pm	6.5	Gill	Across Dam	windy	cold	17	Normal	nil	Cazzonelli	-17.40474	145.72754
21	105	31-Jul-08	2pm	9pm	7	Gill	Across Dam	windy	cold	17	Normal	nil	Cazzonelli	-17.40474	145.72754
	106	31-Jul-08	2pm	9pm	7	Gill	Across Dam	windy	cold	17	Normal	nil	Cazzonelli	-17.40474	145.72754
	107	31-Jul-08	2pm	9pm	7	Gill	Across Dam	windy	cold	17	Normal	nil	Cazzonelli	-17.40474	145.72754
	108	31-Jul-08	2pm	9pm	7	Gill	Across Dam	windy	cold	17	Normal	nil	Cazzonelli	-17.40474	145.72754
	109	31-Jul-08	2pm	9pm	7	Gill	Across Dam	windy	cold	17	Normal	nil	Cazzonelli	-17.40474	145.72754
	110	31-Jul-08	2pm	9pm	7	Gill	Across Dam	windy	cold	17	Normal	nil	Cazzonelli	-17.40474	145.72754
22	111	01-Aug-08	4pm	10.30pm	6.5	Fyke	Up/Downstre	clear	warm		shallow	moderate	Chapman	-17.38329	145.55541
	112	01-Aug-08	4pm	10.30pm	6.5	Fyke	Up/Downstre	clear	warm		shallow	moderate	Chapman	-17.38329	145.55541
	113	01-Aug-08	4pm	10.30pm	6.5	Fyke	Up/Downstre	clear	warm		shallow	moderate	Chapman	-17.38329	145.55541
	114	01-Aug-08	4pm	10.30pm	6.5	Fyke	Up/Downstre	clear	warm		shallow	moderate	Chapman	-17.38329	145.55541
23	115	02-Aug-08	4pm	10.30pm	6.5	Fyke	Up/Downstre	clear	warm		shallow	moderate	Chapman	-17.38329	145.55541
	116	02-Aug-08	4pm	10.30pm	6.5	Fyke	Up/Downstre	clear	warm		shallow	moderate	Chapman	-17.38329	145.55541
	117	02-Aug-08	4pm	10.30pm	6.5	Fyke	Up/Downstre	clear	warm		shallow	moderate	Chapman	-17.38329	145.55541
	118	02-Aug-08	4pm	10.30pm	6.5	Fyke	Up/Downstre	clear	warm		shallow	moderate	Chapman	-17.38329	145.55541

24	119	13-Sep-08	2pm	11pm	9	Gill	Enclosure	clear			Normal	slow	McKenna (P4)	-17.45394	145.59000
	120	13-Sep-08	2pm	11pm	9	Gill	Enclosure	clear			Normal	slow	McKenna (P4)	-17.45394	145.59000
	121	13-Sep-08	2pm	11pm	9	Gill	Enclosure	clear			Normal	slow	McKenna (P4)	-17.45394	145.59000
	122	13-Sep-08	2pm	11pm	9	Gill	Enclosure	clear			Normal	slow	McKenna (P4)	-17.45394	145.59000
	123	13-Sep-08	2pm	11pm	9	Gill	Enclosure	clear			Normal	slow	McKenna (P3)	-17.45319	145.58948
	124	13-Sep-08	2pm	11pm	9	Gill	Enclosure	clear			Normal	slow	McKenna (P3)	-17.45319	145.58948
	125	13-Sep-08	3pm	2pm	69	Fyke	Up/Downstream				deep	moderate	McKenna (P2)	-17.45482	145.58756
	126	13-Sep-08	3pm	2pm	23	Fyke	Upstream				deep	moderate	McKenna (P2)	-17.45482	145.58756
25	127	14-Sep-08	5am	2.30pm	9.5	Gill	Enclosure	clear			Normal	slow	McKenna (P3)	-17.45319	145.58948
	128	14-Sep-08	5am	2.30pm	9.5	Gill	Enclosure	clear			Normal	slow	McKenna (P3)	-17.45319	145.58948
	129	14-Sep-08	5am	2.30pm	9.5	Gill	Enclosure	clear			Normal	slow	McKenna (P3)	-17.45319	145.58948
	130	14-Sep-08	5am	2.30pm	9.5	Gill	Enclosure	clear			Normal	slow	McKenna (P3)	-17.45319	145.58948
26	131	16-Oct-08	2pm	10pm	8	Gill	Across	clear, breezy	28 - 17.	23	low-normal	slow	Hidden Valley (HVE	-18.98039	146.02572
	132	16-Oct-08	2pm	10pm	8	Gill	Parallel	clear, breezy	28 - 17.	23	low-normal	slow	Hidden Valley (HVE	-18.98039	146.02572
	133	16-Oct-08	3pm	10pm	7	Fyke	Up/Downstre	clear, breezy	28 - 17.	23	low-normal	slow	Hidden Valley (HVE	-18.98039	146.02572
	134	16-Oct-08	3pm	10pm	7	Fyke	Up/Downstre	clear, breezy	28 - 17.	23	low-normal	slow	Hidden Valley (HVE	-18.98039	146.02572
	135	16-Oct-08	3pm	10pm	7	Fyke	Upstream	clear, breezy	28 - 17.	23	low-normal	slow	Hidden Valley (HVE	-18.98039	146.02572
27	136	17-Oct-08	2pm	10pm	8	Gill	Across	clear, breezy	28 - 17.	23	low-normal	slow	Hidden Valley (HVE	-18.98039	146.02572
	137	17-Oct-08	2pm	10pm	8	Fyke	Upstream	clear, breezy	28 - 17.	23	low-normal	slow	Hidden Valley (HVE	-18.98039	146.02572
	138	17-Oct-08	2pm	10pm	8	Gill	Parallel	clear, breezy	28 - 17.	23	low-normal	slow	Hidden Valley (HVE	-18.98039	146.02572
	139	17-Oct-08	2pm	10pm	8	Fyke	Up/Downstre	clear, breezy	28 - 17.	23	low-normal	slow	Hidden Valley (HVE	-18.98039	146.02572
	140	17-Oct-08	2pm	10pm	8	Fyke	Up/Downstre	clear, breezy	28 - 17.	23	low-normal	slow	Hidden Valley (HVE	-18.98039	146.02572
	141	17-Oct-08	2pm	10pm	8	Fyke	Up/Downstre	clear, breezy	28 - 17.	23	low-normal	slow	Hidden Valley (HVE	-18.98039	146.02572
28	142	18-Oct-08	3.30pm	10pm	6.5	Gill	Parallel	clear, sprinkle			DEEP	slow	Hidden Valley (HV3	-18.95521	146.07456
	143	18-Oct-08	3.30pm	10pm	6.5	Gill	Parallel	clear, sprinkle			DEEP	slow	Hidden Valley (HV3	-18.95521	146.07456
	144	18-Oct-08	3.30pm	10pm	6.5	Gill	Across	clear, sprinkle			DEEP	slow	Hidden Valley (HV3	-18.95521	146.07456
	145	18-Oct-08	3.30pm	10pm	6.5	Gill	Parallel	clear, sprinkle			DEEP	slow	Hidden Valley (HV3	-18.95521	146.07456
	146	18-Oct-08	3.30pm	10pm	6.5	Gill	Parallel	clear, sprinkle			DEEP	slow	Hidden Valley (HV3	-18.95521	146.07456
29	147	20-Oct-08	3.30pm	10pm	6.5	Gill	Parallel	clear, sprinkle			rain swollen	slow	Hidden Valley (HV3	-18.95521	146.07456
	148	20-Oct-08	3.30pm	10pm	6.5	Gill	Parallel	clear, sprinkle			rain swollen	slow	Hidden Valley (HV3	-18.95521	146.07456

	149	20-Oct-08	3.30pm	10pm	6.5	Gill	Parallel	clear, sprinkle		rain swollen	slow	Hidden Valley (HV3	-18.95521	146.07456
	150	20-Oct-08	3.30pm	10pm	6.5	Gill	Parallel	clear, sprinkle		rain swollen	slow	Hidden Valley (HV3	-18.95521	146.07456
	151	20-Oct-08	3.30pm	10pm	6.5	Gill	Parallel	clear, sprinkle		rain swollen	slow	Hidden Valley (HV3	-18.95521	146.07456
30	152	22-Oct-08	2pm	10pm	8	Gill	Parallel	clear, sprinkle		Normal	slow	Hidden Valley (HV3	-18.95521	146.07456
	153	22-Oct-08	2pm	10pm	8	Gill	Parallel	clear, sprinkle		Normal	slow	Hidden Valley (HV3	-18.95521	146.07456
	154	22-Oct-08	2pm	10pm	8	Gill	Parallel	clear, sprinkle		Normal	slow	Hidden Valley (HV3	-18.95521	146.07456
	155	22-Oct-08	2pm	10pm	8	Gill	Parallel	clear, sprinkle		Normal	slow	Hidden Valley (HV3	-18.95521	146.07456
	156	22-Oct-08	2pm	10pm	8	Gill	Parallel	clear, sprinkle		Normal	slow	Hidden Valley (HV3	-18.95521	146.07456
31	157	23-Oct-08	3.30pm	10pm	6.5	Fyke	Up/Downstre	slight cloud		shallow	slow	Hidden Valley (HVJ	-18.98633	146.01230
	158	23-Oct-08	3.30pm	10pm	6.5	Fyke	Up/Downstre	slight cloud		shallow	slow	Hidden Valley (HVJ	-18.98633	146.01230
	159	23-Oct-08	3.30pm	10pm	6.5	Fyke	Up/Downstre	slight cloud		shallow	slow	Hidden Valley (HVJ	-18.98633	146.01230
	160	23-Oct-08	3.30pm	10pm	6.5	Fyke	Up/Downstre	slight cloud		shallow	slow	Hidden Valley (HVJ	-18.98633	146.01230
32	161	24-Oct-08	3pm	10pm	7	Gill	Parallel	clear, sprinkle		Normal	slow	Hidden Valley (HV3	-18.95521	146.07456
	162	24-Oct-08	3pm	10pm	7	Gill	Parallel	clear, sprinkle		Normal	slow	Hidden Valley (HV3	-18.95521	146.07456
	163	24-Oct-08	3pm	10pm	7	Gill	Parallel	clear, sprinkle		Normal	slow	Hidden Valley (HV3	-18.95521	146.07456
	164	24-Oct-08	3pm	10pm	7	Gill	Parallel	clear, sprinkle		Normal	slow	Hidden Valley (HV3	-18.95521	146.07456
33	165	11-Nov-08	4pm	5am	13	Fyke	Up/Downstre	clear	warm	shallow	slow	Carnarvon Gorge (x2	-25.05852	148.22888
	166	11-Nov-08	4pm	5am	13	Fyke	Up/Downstre	clear	warm	shallow	slow	Carnarvon Gorge (x2	-25.05852	148.22888
	167	11-Nov-08	4pm	5am	13	Fyke	Up/Downstre	clear	warm	shallow	slow	Carnarvon Gorge (x3	-25.05539	148.22637
	168	11-Nov-08	4pm	5am	13	Fyke	Up/Downstre	clear	warm	shallow	slow	Carnarvon Gorge (x3	-25.05539	148.22637
	169	11-Nov-08	4pm	5am	13	Fyke	Up/Downstre	clear	warm	shallow	slow	Carnarvon Gorge (x4	-25.05488	148.22364
	170	11-Nov-08	4pm	5am	13	Fyke	Up/Downstre	clear	warm	shallow	slow	Carnarvon Gorge (x4	-25.05488	148.22364
	171	11-Nov-08	4pm	5am	13	Fyke	Up/Downstre	clear	warm	shallow	slow	Carnarvon Gorge (x5	-25.05338	148.2209
	172	11-Nov-08	4pm	5am	13	Fyke	Up/Downstre	clear	warm	shallow	slow	Carnarvon Gorge (x5	-25.05338	148.2209
	173	11-Nov-08	5pm	Midnight	7	Gill	Parallel	clear	warm	chest deep	slow	Carnarvon Gorge (x6	-25.05139	148.21686
	174	11-Nov-08	5pm	Midnight	7	Gill	Parallel	clear	warm	chest deep	slow	Carnarvon Gorge (x6	-25.05139	148.21686
	175	11-Nov-08	5pm	Midnight	7	Gill	Parallel	clear	warm	chest deep	slow	Carnarvon Gorge (x6	-25.05139	148.21686
34	176	12-Nov-08	5am	Midnight	19	Gill	Parallel	clear	warm	deep	slow	Carnarvon Gorge (x8	-25.05075	148.21167
	177	12-Nov-08	5am	Midnight	19	Gill	Parallel	clear	warm	deep	slow	Carnarvon Gorge (x8	-25.05075	148.21167
35	178	13-Nov-08	4pm	5am	13	Fyke	Up/Downstre	clear	warm	shallow	slow	Carnarvon Gorge (FI	-25.05821	148.24017

	179	13-Nov-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm			shallow	slow	Carnarvon Gorge (F	-25.05821	148.24017
	180	13-Nov-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm			shallow	slow	Carnarvon Gorge (F	-25.05821	148.24017
	181	13-Nov-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm			shallow	slow	Carnarvon Gorge (F	-25.05821	148.24017
	182	13-Nov-08	4pm	Midnight	8	Gill	Parallel	clear	warm			shallow	slow	Carnarvon Gorge (N	-25.05821	148.24017
	183	13-Nov-08	4pm	Midnight	8	Gill	Parallel	clear	warm			shallow	slow	Carnarvon Gorge (N	-25.05821	148.24017
	184	13-Nov-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm			shallow	slow	Carnarvon Gorge (F	-25.05711	148.24204
	185	13-Nov-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm			shallow	slow	Carnarvon Gorge (F	-25.05711	148.24204
36	186	14-Nov-08	4pm	Midnight	8	Gill	Parallel	clear	warm day cold			shallow	slow	Carnarvon Gorge (x1)		
	187	14-Nov-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm day cold			shallow	slow	Carnarvon Gorge (x1)		
	188	14-Nov-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm day cold			shallow	slow	Carnarvon Gorge (x1)		
	189	14-Nov-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm day cold			shallow	slow	Carnarvon Gorge (x1)		
	190	14-Nov-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm day cold			shallow	slow	Carnarvon Gorge (x1)		
	191	14-Nov-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm day cold			shallow	slow	Carnarvon Gorge (x1)		
	192	14-Nov-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm day cold			shallow	slow	Carnarvon Gorge (x1)		
	193	14-Nov-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm day cold			shallow	slow	Carnarvon Gorge (x1)		
37	194	15-Nov-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm day cold			shallow	slow	Carnarvon Gorge (T	-25.07332	148.26892
	195	15-Nov-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm day cold			shallow	slow	Carnarvon Gorge (T	-25.07332	148.26892
	196	15-Nov-08	4pm	Midnight	8	Gill	Parallel	clear	warm day cold			shallow	slow	Carnarvon Gorge (T	-25.07332	148.26892
	197	15-Nov-08	4pm	Midnight	8	Gill	Parallel	clear	warm day cold			shallow	slow	Carnarvon Gorge (T	-25.07332	148.26892
	198	15-Nov-08	4pm	Midnight	8	Gill	Parallel	clear	warm day cold			shallow	slow	Carnarvon Gorge (T	-25.07332	148.26892
38	199	06-Dec-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm			Normal	slow	McKenna (P2)	-17.45482	145.58756
	200	06-Dec-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm			Normal	slow	McKenna (P2)	-17.45482	145.58756
	201	06-Dec-08	4pm	5am	13	Fyke	Up/Downstr	clear	warm			Normal	slow	McKenna (P2)	-17.45482	145.58756
	202	06-Dec-08	4pm	midnight	8	Gill	Enclosure	clear	warm			Normal	slow	McKenna (P3)	-17.45319	145.58948
	203	06-Dec-08	4pm	midnight	8	Gill	Enclosure	clear	warm			Normal	slow	McKenna (P3)	-17.45319	145.58948
	204	06-Dec-08	4pm	midnight	8	Gill	Enclosure	clear	warm			Normal	slow	McKenna (P3)	-17.45319	145.58948
	205	06-Dec-08	4pm	midnight	8	Gill	Enclosure	clear	warm			Normal	slow	McKenna (P3)	-17.45319	145.58948
	206	06-Dec-08	4pm	midnight	8	Gill	Enclosure	clear	warm			Normal	slow	McKenna (P3)	-17.45319	145.58948
39	207	07-Dec-08	6am	2pm	8	Gill	Parallel	clear	warm			Normal	slow	McKenna (P4)	-17.45394	145.59000
40	208	20-Apr-09	4.30pm	10pm	5.5	Gill	Across	clear		23	20.5	high, 1m ab	swift	Hidden Valley (HVE	-18.98039	146.02572

	209	20-Apr-09	4.30pm	10pm	5.5	Gill	Parallel	clear	23	20.5	high, 1m abc	swift	Hidden Valley (HVE	-18.98039	146.02572
	210	20-Apr-09	4.30pm	10pm	5.5	Gill	Parallel	clear	23	20.5	high, 1m abc	swift	Hidden Valley (HVE	-18.98039	146.02572
41	211	21-Apr-09	4.30pm	10pm	5.5	Gill	Parallel	clear	23	20.5	high	swift	Hidden Valley (HV3	-18.95521	146.07456
	212	21-Apr-09	4.30pm	10pm	5.5	Gill	Parallel	clear	23	20.5	high	swift	Hidden Valley (HV3	-18.95521	146.07456
	213	21-Apr-09	4.30pm	10pm	5.5	Gill	Parallel	clear	23	20.5	high	swift	Hidden Valley (HV3	-18.95521	146.07456
	214	21-Apr-09	4.30pm	10pm	5.5	Gill	Parallel	clear	23	20.5	high	swift	Hidden Valley (HV3	-18.95521	146.07456
42	215	22-Apr-09	4.30pm	10pm	5.5	Gill	Parallel	clear	23	20.5	high	swift	Hidden Valley (HVE	-18.98039	146.02572
	216	22-Apr-09	4.30pm	10pm	5.5	Gill	Parallel	clear	23	20.5	high	swift	Hidden Valley (HVE	-18.98039	146.02572
	217	22-Apr-09	4.30pm	10pm	5.5	Gill	Parallel	clear	23	20.5	high	swift	Hidden Valley (HVE	-18.98039	146.02572
43	218	23-Apr-09	5pm	10pm	5	Gill	Parallel	clear	23	20.5	high	swift	Hidden Valley (HV3	-18.95521	146.07456
	219	23-Apr-09	5pm	10pm	5	Gill	Parallel	clear	23	20.5	high	swift	Hidden Valley (HV3	-18.95521	146.07456
	220	23-Apr-09	5pm	10pm	5	Gill	Parallel	clear	23	20.5	high	swift	Hidden Valley (HV3	-18.95521	146.07456
	221	23-Apr-09	5pm	10pm	5	Gill	Parallel	clear	23	20.5	high	swift	Hidden Valley (HV3	-18.95521	146.07456
44	222	25-Apr-09	4.15am	7.30am	3.25	Gill	Parallel	clear	19				Hidden Valley (HVE	-18.98039	146.02572
	223	25-Apr-09	4.15am	7.30am	3.25	Gill	Parallel	clear	19				Hidden Valley (HVE	-18.98039	146.02572
45	224	26-Apr-09	4.30am	8am	3.5	Gill	Parallel	clear	19				Hidden Valley (HVE	-18.98039	146.02572
	225	26-Apr-09	4.30am	8am	3.5	Gill	Parallel	clear	19				Hidden Valley (HVE	-18.98039	146.02572
	226	26-Apr-09	4.30am	8am	3.5	Gill	Parallel	clear	19				Hidden Valley (HVE	-18.98039	146.02572
46	227	30-Apr-09	4.45pm	7am	14.25	Gill	Parallel	clear	19				Hidden Valley (HVE	-18.98039	146.02572
	228	30-Apr-09	4.45pm	7am	14.25	Gill	Parallel	clear	19				Hidden Valley (HVE	-18.98039	146.02572
	229	30-Apr-09	4.45pm	7am	14.25	Gill	Parallel	clear	19				Hidden Valley (HVE	-18.98039	146.02572
47	230	01-May-09	4pm	7pm	3	Gill	Parallel	clear	19				Hidden Valley (HVE	-18.98039	146.02572
	231	01-May-09	4pm	7pm	3	Gill	Parallel	clear	19				Hidden Valley (HVE	-18.98039	146.02572
	232	01-May-09	5pm	6pm	1	Gill	Parallel	clear	19				Hidden Valley (HVM	-18.95642	146.04658
	233	01-May-09	5pm	6pm	1	Gill	Parallel	clear	19				Hidden Valley (HVM	-18.95642	146.04658
48	234	25-May-09	4.30pm	10pm	5.5	Gill	Parallel	drizzle		17	Normal	slow	Hidden Valley (HV3	-18.95521	146.07456
	235	25-May-09	4.30pm	10pm	5.5	Gill	Parallel	drizzle		17	Normal	slow	Hidden Valley (HV3	-18.95521	146.07456
	236	25-May-09	4.30pm	10pm	5.5	Gill	Parallel	drizzle		17	Normal	slow	Hidden Valley (HV3	-18.95521	146.07456
49	237	26-May-09	4pm	10pm	6	Gill	Parallel	overcast			high		Hidden Valley (HVE	-18.98039	146.02572
	238	26-May-09	4pm	10pm	6	Gill	Parallel	overcast			high		Hidden Valley (HVE	-18.98039	146.02572

50	239	27-May-09	4.30pm	10pm	5.5	Gill	Parallel	partial clou	warm					Hidden Valley (HV3	-18.95521	146.07456
	240	27-May-09	4.30pm	10pm	5.5	Gill	Parallel	partial clou	warm					Hidden Valley (HV3	-18.95521	146.07456
	241	27-May-09	4.30pm	10pm	5.5	Gill	Parallel	partial clou	warm					Hidden Valley (HV3	-18.95521	146.07456
51	242	28-May-09	5pm	10pm	5	Gill	Parallel	clear						Hidden Valley (HVE	-18.98039	146.02572
	243	28-May-09	5pm	10pm	5	Gill	Parallel	clear						Hidden Valley (HVE	-18.98039	146.02572
52	244	29-May-09	4pm	10pm	6	Gill	Parallel	clear						Hidden Valley (HV3	-18.95521	146.07456
	245	29-May-09	4pm	10pm	6	Gill	Parallel	clear						Hidden Valley (HV3	-18.95521	146.07456
	246	29-May-09	4pm	10pm	6	Gill	Parallel	clear						Hidden Valley (HV3	-18.95521	146.07456
53	247	30-May-09	4pm	10pm	6	Gill	Parallel	clear						Hidden Valley (HVE	-18.98039	146.02572
	248	30-May-09	4pm	10pm	6	Gill	Parallel	clear						Hidden Valley (HVE	-18.98039	146.02572
54	249	16-Jun-09	6pm	10pm	4	Gill	Parallel	clear	cool			Normal	slow	CRED1	-21.18079	148.54402
	250	16-Jun-09	6pm	10pm	4	Gill	Parallel	clear	cool			Normal	slow	CRED1	-21.18079	148.54402
	251	16-Jun-09	6pm	10pm	4	Fyke	Up/Downstre	clear	cool			Normal	slow	CRED1	-21.18079	148.54402
	252	16-Jun-09	6pm	10pm	4	Fyke	Up/Downstre	clear	cool			Normal	slow	CRED1	-21.18079	148.54402
55	253	17-Jun-09	2.30pm	9pm	6.5	Gill	Parallel	clear	cool			Normal	slow	CRED1	-21.18079	148.54402
	254	17-Jun-09	2.30pm	9pm	6.5	Gill	Parallel	clear	cool			Normal	slow	CRED1	-21.18079	148.54402
56	255	18-Jun-09	2pm	9pm	7	Gill	Parallel	clear	cool			Normal	slow	CRED2	-21.18075	148.54371
	256	18-Jun-09	2pm	9pm	7	Gill	Parallel	clear	cool			Normal	slow	CRED2	-21.18075	148.54371
57	257	19-Jun-09	2pm	10pm	8	Gill	V	clear	cool			Normal	moderate	CRED3	-21.1803	148.54273
	258	19-Jun-09	2pm	10pm	8	Gill	V	clear	cool			Normal	moderate	CRED3	-21.1803	148.54273
58	259	20-Jun-09	2pm	10pm	8	Gill	V	clear, sprin	cool			Normal	moderate	CRED3	-21.1803	148.54273
	260	20-Jun-09	2pm	10pm	8	Gill	V	clear, sprin	cool			Normal	moderate	CRED3	-21.1803	148.54273
59	261	14-Jul-09	3.30pm	10pm	6.5	Gill	Enclosure	partial clou	17	20		Normal	slow	Atherton Pumping S	-17.26118	145.53753
	262	14-Jul-09	3.30pm	10pm	6.5	Gill	Enclosure	partial clou	17	20		Normal	slow	Atherton Pumping S	-17.26118	145.53753
	263	14-Jul-09	3.30pm	10pm	6.5	Gill	Enclosure	partial clou	17	20		Normal	slow	Atherton Pumping S	-17.26118	145.53753
	264	14-Jul-09	3.30pm	10pm	6.5	Gill	Enclosure	partial clou	17	20		Normal	slow	Atherton Pumping S	-17.26118	145.53753
60	265	15-Jul-09	5pm	10.30pm	5.5	Gill	Enclosure	clear	17	20		Normal	slow	Atherton Pumping S	-17.26118	145.53753
	266	15-Jul-09	5pm	10.30pm	5.5	Gill	Enclosure	clear	17	20		Normal	slow	Atherton Pumping S	-17.26118	145.53753
	267	15-Jul-09	5pm	10.30pm	5.5	Gill	Enclosure	clear	17	20		Normal	slow	Atherton Pumping S	-17.26118	145.53753
61	268	16-Jul-09	3.30pm	10pm	6.5	Gill	Enclosure	clear	17	20		Normal	slow	Atherton Pumping S	-17.26118	145.53753

	269	16-Jul-09	3.30pm	10pm	6.5	Gill	Enclosure	clear	17	20	Normal	slow	Atherton Pumping St	-17.26118	145.53753
	270	16-Jul-09	3.30pm	10pm	6.5	Gill	Enclosure	clear	17	20	Normal	slow	Atherton Pumping St	-17.26118	145.53753
	271	16-Jul-09	3.30pm	10pm	6.5	Gill	Enclosure	clear	17	20	Normal	slow	Atherton Pumping St	-17.26118	145.53753
62	272	17-Jun-09	4.30pm	10pm	5.5	Gill	zig-zag	clear	COLD		below norma	nil	Hillcrest	-17.39181	145.60498
	273	17-Jun-09	4.30pm	10pm	5.5	Gill	zig-zag	clear	COLD		below norma	nil	Hillcrest	-17.39181	145.60498
	274	17-Jun-09	4.30pm	10pm	5.5	Gill	zig-zag	clear	COLD		below norma	nil	Hillcrest	-17.39181	145.60498
	275	17-Jun-09	4.30pm	10pm	5.5	Gill	zig-zag	clear	COLD		below norma	nil	Hillcrest	-17.39181	145.60498
63	276	18-Jul-09	2.30pm	10pm	7.5	Gill	zig-zag	clear	COLD		below norma	nil	Hillcrest	-17.39181	145.60498
	277	18-Jul-09	2.30pm	10pm	7.5	Gill	zig-zag	clear	COLD		below norma	nil	Hillcrest	-17.39181	145.60498
	278	18-Jul-09	2.30pm	10pm	7.5	Gill	zig-zag	clear	COLD		below norma	nil	Hillcrest	-17.39181	145.60498
	279	18-Jul-09	2.30pm	10pm	7.5	Gill	zig-zag	clear	COLD		below norma	nil	Hillcrest	-17.39181	145.60498
64	280	20-Aug-09	3.30pm	11pm	7.5	Gill	Enclosure	clear			Normal	slow	Atherton Pumping St	-17.26118	145.53753
	281	20-Aug-09	3.30pm	11pm	7.5	Gill	Enclosure	clear			Normal	slow	Atherton Pumping St	-17.26118	145.53753
	282	20-Aug-09	3.30pm	11pm	7.5	Gill	Enclosure	clear			Normal	slow	Atherton Pumping St	-17.26118	145.53753
65	283	21-Aug-09	2.30pm	10.30pm	8	Gill	Enclosure	clear			Normal	slow	Atherton Pumping St	-17.26118	145.53753
	284	21-Aug-09	2.30pm	10.30pm	8	Gill	Enclosure	clear			Normal	slow	Atherton Pumping St	-17.26118	145.53753
	285	21-Aug-09	2.30pm	10.30pm	8	Gill	Enclosure	clear			Normal	slow	Atherton Pumping St	-17.26118	145.53753
66	286	22-Aug-09	3.30pm	10.30pm	7	Gill	Enclosure	clear	warm		Normal	slow	McKenna (P3)	-17.45319	145.58948
	287	22-Aug-09	3.30pm	10.30pm	7	Gill	Enclosure	clear	warm		Normal	slow	McKenna (P3)	-17.45319	145.58948
	288	22-Aug-09	3.30pm	10.30pm	7	Gill	Enclosure	clear	warm		Normal	slow	McKenna (P3)	-17.45319	145.58948
	289	22-Aug-09	3.30pm	10.30pm	7	Gill	Enclosure	clear	warm		Normal	slow	McKenna (P3)	-17.45319	145.58948
	290	22-Aug-09	4.30pm	10.30pm	6	Fyke	Upstream	clear	warm		Normal	slow	McKenna	-17.45319	145.58948
67	291	23-Aug-09	3pm	10pm	7	Gill	Parallel	clear			Normal	slow	McKenna (P4)	-17.45394	145.59000
	292	23-Aug-09	3pm	10pm	7	Gill	Parallel	clear			Normal	slow	McKenna (P4)	-17.45394	145.59000
	293	23-Aug-09	3pm	10pm	7	Gill	Parallel	clear			Normal	slow	McKenna (P4)	-17.45394	145.59000
68	294	16-Sep-09	4pm	10pm	6	Gill	Enclosure	clear			Normal	slow	Atherton Pumping St	-17.26118	145.53753
	295	16-Sep-09	4pm	10pm	6	Gill	Enclosure	clear			Normal	slow	Atherton Pumping St	-17.26118	145.53753
	296	16-Sep-09	4pm	10pm	6	Gill	Enclosure	clear			Normal	slow	Atherton Pumping St	-17.26118	145.53753
69	297	17-Sep-09	4pm	10pm	6	Gill	Enclosure	clear			Normal	slow	Atherton Pumping St	-17.26118	145.53753
	298	17-Sep-09	4pm	10pm	6	Gill	Enclosure	clear			Normal	slow	Atherton Pumping St	-17.26118	145.53753

	299	17-Sep-09	4pm	10pm	6	Gill	Enclosure	clear			Normal	slow	Atherton Pumping S	-17.26118	145.53753
70	300	18-Sep-09	3pm	10pm	7	Gill	Enclosure	clear			Normal	slow	Atherton Pumping S	-17.26118	145.53753
	301	18-Sep-09	3pm	10pm	7	Gill	Enclosure	clear			Normal	slow	Atherton Pumping S	-17.26118	145.53753
	302	18-Sep-09	3pm	10pm	7	Gill	Enclosure	clear			Normal	slow	Atherton Pumping S	-17.26118	145.53753
71	303	19-Sep-09	4pm	10pm	6	Gill	Enclosure	clear			Normal	slow	Atherton Pumping S	-17.26118	145.53753
	304	19-Sep-09	5.30pm	10pm	4.5	Gill	Enclosure	clear			Normal	slow	Atherton Pumping S	-17.26118	145.53753
	305	19-Sep-09	5.30pm	10pm	4.5	Gill	Enclosure	clear			Normal	slow	Atherton Pumping S	-17.26118	145.53753
72	306	28-Sep-09	3.30pm	10pm	6.5	Gill	Enclosure	clear			Normal	slow	Atherton Pumping S	-17.26118	145.53753
	307	28-Sep-09	3.30pm	10pm	6.5	Gill	Enclosure	clear			Normal	slow	Atherton Pumping S	-17.26118	145.53753
	308	28-Sep-09	3.30pm	10pm	6.5	Gill	Enclosure	clear			Normal	slow	Atherton Pumping S	-17.26118	145.53753
73	309	29-Sep-09	3.45pm	10pm	6.25	Gill	Across Dam	windy	warm	warm	Very Low	nil	Cazzonelli	-17.40474	145.72754
	310	29-Sep-09	3.45pm	10pm	6.25	Gill	Across Dam	windy	warm	warm	Very Low	nil	Cazzonelli	-17.40474	145.72754
	311	29-Sep-09	4.30pm	10pm	5.5	Gill	Across Dam	windy	warm	warm	Very Low	nil	Cazzonelli	-17.40474	145.72754
74	312	30-Sep-09	3pm	10pm	7	Gill	Across Dam	windy	warm	warm	Very Low	nil	Cazzonelli	-17.40474	145.72754
	313	30-Sep-09	3pm	10pm	7	Gill	Across Dam	windy	warm	warm	Very Low	nil	Cazzonelli	-17.40474	145.72754
75	314	01-Oct-09	3.15pm	10pm	6.75	Gill	zig-zag				slightly low	nil	Hillcrest	-17.39181	145.60498
	315	01-Oct-09	3.15pm	10pm	6.75	Gill	zig-zag				slightly low	nil	Hillcrest	-17.39181	145.60498
	316	01-Oct-09	3.15pm	10pm	6.75	Gill	zig-zag				slightly low	nil	Hillcrest	-17.39181	145.60498
	317	01-Oct-09	3.15pm	10pm	6.75	Gill	zig-zag				slightly low	nil	Hillcrest	-17.39181	145.60498
76	318	02-Oct-09	3pm	10pm	7	Gill	zig-zag				slightly low	nil	Hillcrest	-17.39181	145.60498
	319	02-Oct-09	3pm	10pm	7	Gill	zig-zag				slightly low	nil	Hillcrest	-17.39181	145.60498
	320	02-Oct-09	3pm	10pm	14	Gill	zig-zag				slightly low	nil	Hillcrest	-17.39181	145.60498
77	321	03-Oct-09	3.30pm	10pm	6.5	Gill	Enclosure	clear			Normal	slow	Atherton Pumping S	-17.26118	145.53753
	322	03-Oct-09	3.30pm	10pm	6.5	Gill	Enclosure	clear			Normal	slow	Atherton Pumping S	-17.26118	145.53753
	323	03-Oct-09	3.30pm	10pm	6.5	Gill	Enclosure	clear			Normal	slow	Atherton Pumping S	-17.26118	145.53753
78	324	15-Oct-09	4.30pm	10pm	5.5	Gill	zig-zag	clear	warm	cool	Normal	nil	E2	-21.20533	148.522
	325	15-Oct-09	4.30pm	10pm	5.5	Gill	zig-zag	clear	warm	cool	Normal	nil	E2	-21.20533	148.522
	326	15-Oct-09	4.30pm	10pm	5.5	Fyke	Upstream	clear	warm	cool	Shallow	nil	E2	-21.20533	148.522
79	327	16-Oct-09	3.30pm	10pm	6.5	Gill	Enclosure	clear			Deep	nil	E3	-21.22868	148.55714
	328	16-Oct-09	3.30pm	10pm	6.5	Gill	Enclosure	clear			Deep	nil	E3	-21.22868	148.55714

	329	16-Oct-09	3.30pm	10pm	6.5	Gill	Enclosure	clear		Deep	nil	E3	-21.22868	148.55714
	330	16-Oct-09	3.30pm	10pm	6.5	Gill	Enclosure	clear		Deep	nil	E3	-21.22868	148.55714
80	331	17-Oct-09	3.30pm	10pm	6.5	Gill	zig-zag	clear		Normal	nil	E4	-21.20374	148.52836
	332	17-Oct-09	3.30pm	10pm	6.5	Gill	zig-zag	clear		Normal	nil	E4	-21.20374	148.52836
	333	17-Oct-09	3.30pm	10pm	6.5	Gill	zig-zag	clear		Normal	nil	E4	-21.20374	148.52836
81	334	18-Oct-09	4.30pm	10pm	5.5	Gill	Enclosure	clear		Deep	nil	E3	-21.22868	148.55714
	335	18-Oct-09	4.30pm	10pm	5.5	Gill	Enclosure	clear		Deep	nil	E3	-21.22868	148.55714
	336	18-Oct-09	5.30pm	10pm	4.5	Gill	Enclosure	clear		Deep	nil	E3	-21.22868	148.55714
	337	18-Oct-09	5.30pm	10pm	4.5	Gill	Enclosure	clear		Deep	nil	E3	-21.22868	148.55714
82	338	02-Nov-09	5pm	10pm	5	Gill	zig-zag	clear		Normal	nil	E4	-21.20374	148.52836
	339	02-Nov-09	5pm	10pm	5	Gill	zig-zag	clear		Normal	nil	E4	-21.20374	148.52836
	340	02-Nov-09	5pm	10pm	5	Gill	zig-zag	clear		Normal	nil	E4	-21.20374	148.52836
83	341	03-Nov-09	3pm	10pm	7	Gill	zig-zag	clear		Normal	nil	E4	-21.20374	148.52836
	342	03-Nov-09	3pm	10pm	7	Gill	zig-zag	clear		Normal	nil	E4	-21.20374	148.52836
	343	03-Nov-09	3pm	10pm	7	Gill	zig-zag	clear		Normal	nil	E4	-21.20374	148.52836
84	344	04-Nov-09	2pm	10pm	8	Fyke	Upstream	clear		Normal	slow	E5	-21.20181	148.52721
	345	04-Nov-09	3.30pm	10pm	26	Gill	zig-zag	clear		Normal	nil	E6	-21.20711	148.52961
85	346	05-Nov-09	4pm	10pm	6	Gill	Enclosure	clear		Normal	nil	E3	-21.22868	148.55714
	347	05-Nov-09	4pm	10pm	6	Gill	Enclosure	clear		Normal	nil	E3	-21.22868	148.55714
	348	05-Nov-09	4pm	10pm	6	Gill	Enclosure	clear		Normal	nil	E3	-21.22868	148.55714
	349	05-Nov-09	4pm	10pm	6	Gill	Enclosure	clear		Normal	nil	E3	-21.22868	148.55714
86	350	06-Nov-09	2pm	10pm	8	Gill	Enclosure	clear		Normal	nil	E3	-21.22868	148.55714
	351	06-Nov-09	2pm	10pm	8	Gill	Enclosure	clear		Normal	nil	E3	-21.22868	148.55714
	352	06-Nov-09	2pm	10pm	8	Gill	Enclosure	clear		Normal	nil	E3	-21.22868	148.55714
	353	06-Nov-09	2pm	10pm	8	Gill	Enclosure	clear		Normal	nil	E3	-21.22868	148.55714
87	354	07-Nov-09	3.30pm	10pm	6.5	Gill	Enclosure	wet, misty, windy		Normal	nil	E3	-21.22868	148.55714
	355	07-Nov-09	3.30pm	10pm	6.5	Gill	Enclosure	wet, misty, windy		Normal	nil	E3	-21.22868	148.55714
	356	07-Nov-09	3.30pm	10pm	6.5	Gill	Enclosure	wet, misty, windy		Normal	nil	E3	-21.22868	148.55714
	357	07-Nov-09	3.30pm	10pm	6.5	Gill	Enclosure	wet, misty, windy		Normal	nil	E3	-21.22868	148.55714
88	358	19-Nov-09	4.15pm	10pm	5.75	Gill	Enclosure	clear	warm/hot	up	slow	Atherton Pumping S	-17.26118	145.53753

	359	19-Nov-09	4.15pm	10pm	5.75	Gill	Enclosure	clear	warm/hot	up	slow	Atherton Pumping St	-17.26118	145.53753
	360	19-Nov-09	4.15pm	10pm	5.75	Gill	Enclosure	clear	warm/hot	up	slow	Atherton Pumping St	-17.26118	145.53753
89	361	20-Nov-09	4pm	10pm	6	Gill	Parallel	clear	warm/hot	low	slow	McKenna (P4)	-17.45394	145.59000
	362	20-Nov-09	4pm	10pm	6	Gill	Parallel	clear	warm/hot	low	slow	McKenna (P4)	-17.45394	145.59000
	363	20-Nov-09	4pm	10pm	6	Gill	Parallel	clear	warm/hot	low	slow	McKenna (P4)	-17.45394	145.59000
90	364	21-Nov-09	4pm	10pm	6	Gill	Parallel	clear	warm/hot	low	slow	McKenna (P3)	-17.45319	145.58948
	365	21-Nov-09	4pm	10pm	6	Gill	Parallel	clear	warm/hot	low	slow	McKenna (P3)	-17.45319	145.58948
	366	21-Nov-09	4pm	10pm	6	Gill	Parallel	sprinkles	cool	low	slow	McKenna (P3)	-17.45319	145.58948