NAME REDACTED		**OBJECT**	Submission ID: 217980
Organisation:	*N/A*	Key issues:	*Social impacts,Visual impacts, design and landscaping,Land use compatibility (surrounding land uses),Traffic,Other issues*
Location:	*New South Wales 2576*		
Attachment:	*Attached overleaf*		

Submission date: 11/25/2024 8:38:15 AM

Attached

SSD-9409987 - Water NSW submissions
Wingecarribee River - Catchment and River Health

Submission 1: The community should have an opportunity to comment on SSD-9409987 after Water NSW's annual catchment management report is submitted to IPART and made publicly available online.

Under clause 32(2) of its Operating Licence effective 1 July 2024, Water NSW is required by 30 November 2024 to submit its annual report on catchment management to IPART and publish the report.

For a considerable period of time, the Wingecarribee community has been seeking adequate information from Water NSW to enable them to engage meaningfully with issues relating to development on the Wingecarribee River floodplain. The community should have an opportunity to comment on SSD-9409987 after Water NSW's annual catchment management report is submitted to IPART and made publicly available online.

Submission 2: Wingecarribee Dam inundation maps should be made publicly available so that a risk-based approach can be taken to flooding in planning decisions.

Water NSW has refused to disclose DSEP inundation maps, which should be made publicly available so that a risk-based approach can be taken to flooding in planning decisions in respect of the Wingecarribee River floodplain.

a) PL2020 65 Wingecarribee Dam - DSEP Inundation Maps - PMF + Dam Break max depth – 2019 Document date: 18/05/2020

b) PL2020 66 Wingecarribee Dam - DSEP Inundation Maps - PMF + Dam Break - Gate fails to operate - max depth – 2019 Document date: 18/05/2020

c) PL2020 67 Wingecarribee Dam - DSEP Inundation Maps - SDF max depth – 2019 Document date: 18/05/2020

Submission 3: ***Water NSW is not acting in good faith in relation to the likelihood of land being flooded or the nature or extent of any such flooding under Local Government Act 1993*** **(NSW) s.733(1).**

With respect to the exercise of its functions in the Sydney catchment area, Water NSW is required to act in good faith in relation to the likelihood of land being flooded or the nature or extent of any such flooding, *Local Government Act 1993* s.733(1). Section 733 of the *Local Government Act 1993* applies to Water NSW with respect to the exercise of its functions in the Sydney catchment area in the same way as it applies to and in respect of a council, s.733(7)(e). Under s.733(4)(a), Water NSW is taken to have acted in good faith for the purposes of s.733 if the thing was done or omitted to be done substantially in accordance with the principles contained in the *Flood risk management manual.* There is no flood risk management study or plan for the Wingecarribee River and Water NSW is not entitled to rely on the presumption in s.733(4)(a).

On 23 August 2024, Water NSW disclosed information showing that Wingecarribee Dam spilled on 7 March 2022 (3650 ML), 8 March 2022 (4380 ML) & 9 March 2022 (2190 ML). On 24 October 2024, Water NSW disclosed information showing that Wingecarribee Dam spilled on 4 July 2022 (2820 ML), 5 July 2022 (6780 ML) & 6 July 2022 (640 ML).

In 2020, Council engaged WMA Water to prepare an updated site specific flood study as referred to in Water NSW's feedback on the *Local Housing Strategy.* The 2022 flood study 'update' was prepared without consultation with a floodplain risk committee as is typically the case, because the Wingecarribee River Floodplain Risk Management Committee was not functioning during the period of administration. Although the flood study 'update' was not adopted by the Administrator of Wingecarribee Shire Council until 20 July 2022, it failed to address the release of water from Wingecarribee Dam in significant rainfall events (including dam spill incidents in March and July 2022) and/or a dam failure. Amongst other flaws, the flood study 'update' excluded 40% of the areal extent of the Wingecarribee River catchment leading to significant underestimates of flood extents and severity.

Submission 4: ***Flood planning levels for the proposed Plasrefine development have not been set in accordance with relevant laws.***

The *State Significant Development Assessment Report* dated October 2024 pp 1-2 contains incorrect information in relation to water flows. The proposed development is downstream from Wingecarribee Dam and Wingecarribee Reservoir. Flood planning levels for proposed development on the Wingecarribee River floodplain have not been set in accordance with the *Flood risk management manual* and Planning Circular PS 24-*001Update on addressing flood risk in planning decisions*.

Submission 5: ***The Moss Vale Plastics Recycling Facility State Significant Development Assessment Report (SSD-9409987) dated October 2024 fails to adopt a risk-based approach to flooding in planning decisions.***

In a letter to Judy Hannan MP dated 21 June 2024, Paul Scully MP stated that flood planning for the Wingecarribee floodplain was using the 2022 flood study 'update' as well as 'a further study completed after the flood event in March 2022'.

The Assessment Report states on p.44 that, 'The development is located on flood prone land and has the potential to impact flood behaviour and offsite flood levels'. It goes on to state, 'The Department's assessment concludes the potential water impacts can be minimised and managed by the Applicant via the implementation of proposed water management measures and consent conditions which include the requirement to finalise design of the north-eastern dam spillway in consultation with BCS, prepare and implement an OWMP and a CSWMP to ensure water use and management remain as predicted and the Sydney Drinking Water Catchment is protected.'

The Department's conclusion fails to take into account the absence of flood risk management arrangements, the 2022 Wingecarribee Dam spill incidents and the flawed flood study 'update' dated 20 July 2022.

Submission 6: ***The Moss Vale Plastics Recycling Facility State Significant Development Assessment Report (SSD-9409987) dated October 2024 incorrectly summarises the advice provided by Water NSW.***

The Assessment Report states on p.43, 'Water NSW advised it was satisfied with the changes in the amended stormwater strategy'. No such advice has been uploaded to the NSW Planning Portal in the Agency Advice section.

Appendix C of the Report contains a summary of Agency Advice. Camilla Edmunds, Manager Environment and Catchment Protection, Water NSW wrote to Emma Barnet on 25 October 2023 in relation to the Amendment Report, noting that previous concerns regarding alternative wastewater management options remained. The letter does not say that Water NSW is satisfied with the changes in the amended stormwater strategy. This is also not stated in the letter from Camilla Edmunds to Emma Barnet dated 29 March 2023 in relation to Response to Submissions.

The correspondence from Camilla Edmunds, Manager Environment and Catchment Protection, Water NSW makes no mention of other issues with catchment management and protection in the Wingecarribee including the absence of a conservation management plan and flood risk management arrangements, the 2022 Wingecarribee Dam spill incidents and the flawed flood study 'update' dated 20 July 2022.

Submission 7: ***The Moss Vale Plastics Recycling Facility State Significant Development Assessment Report (SSD-9409987) dated October 2024 incorrectly concludes that the development would have a neutral effect on water quality.***

The Assessment Report states on p.69, '*State Environmental Planning Policy (Biodiversity and Conservation) 2021*. The Biodiversity and Conservation SEPP aims to protect biodiversity, regulate vegetation clearing and protect water catchments. The project area is within the Sydney Drinking Water Catchment. Chapter 6 of the Biodiversity and Conservation SEPP provides that a consent authority must not grant consent to the carrying out of development on land in the catchment unless it is satisfied the carrying out of the proposed development would have a neutral or beneficial effect (NorBE) on water quality The Department has undertaken an assessment of the development's impacts on water quality in consultation with WaterNSW in Section 6 of this report. The assessment concluded the development would have a neutral effect on water quality.'

It is not clear on what basis Water NSW has made its determination that the proposed Plasrefine facility would have a neutral effect on water quality. This conclusion is not supported by the correspondence from Water NSW's Manager of Environment and Catchment Protection on the NSW Planning Portal.

The Agency Advice section of the NSW Planning Portal does not contain advice from Water NSW that supports the conclusion that the development would have a neutral effect on water quality.

Submission 8: ***Water NSW is not managing and protecting the Sydney water catchment in a way that furthers its objectives under the Water NSW Act 2014*** **(NSW).**

The principal objectives of Water NSW are to ensure that declared catchment areas are managed and protected so as to promote water quality, the protection of public health and public safety, and the protection of the environment, *Water NSW Act 2014* s.6(1)(c). The proposed Plasrefine development would negatively impact the ability of Water NSW to achieve these objectives.

Other objectives include to exhibit a sense of social responsibility by having regard to the interests of the community in which it operates, s.6(2)(b), and to conduct its operations in compliance with the principles of ecologically sustainable development, s.6(2)(d), see also *State Owned Corporations Act 1989* (NSW) s.20E. Water NSW is not engaging with the community regularly and has not provided the community with adequate information to enable them to engage meaningfully with the relevant issues as required by its Operating Licence.

Submission 9: ***Water NSW is not managing and protecting the Sydney water catchment in a way that supports it to exercise its functions under the Water NSW Act 2014.***

The functions of Water NSW may only be exercised in accordance with its Operating Licence, *Water NSW Act 2014* s.7(3)(a).

The functions of Water NSW include to protect and enhance the quality and quantity of water in declared catchment areas see s.7(1)(g), to manage and protect declared catchment areas see s.7(1)(h), to undertake flood mitigation and management see s.7(1)(i), and to undertake research on catchments generally, and in particular on the health of declared catchment areas see s.7(1)(j). The information on the NSW Planning Portal in relation to SSD-9409987 indicates that Water NSW is not managing and protecting the Sydney water catchment in a way that supports it to exercise its functions under the *Water NSW Act 2014*.

Submission 10: ***Water NSW has not established and not is not keeping a Water NSW Heritage and Conservation Register and is not complying with its obligation sunder the Heritage Act 1977.***

Wingecarribee Swamp has been listed on the NSW State Heritage Inventory (SHR #00784) since 4 February 1999.

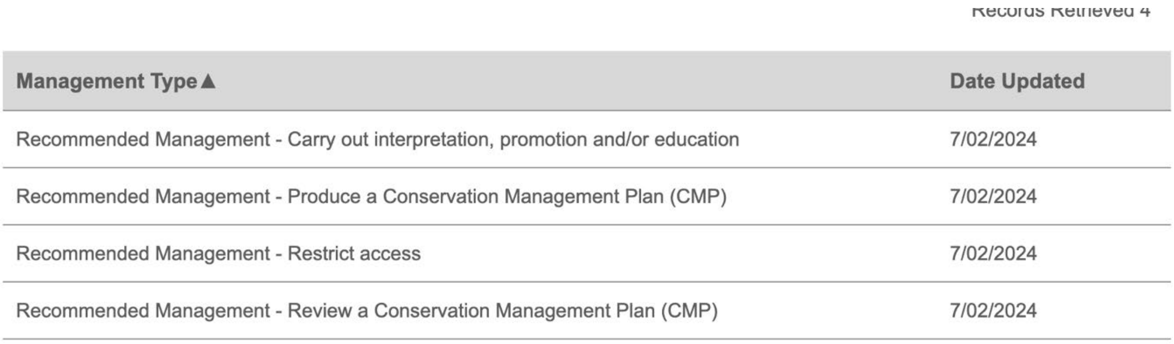
Under *Heritage Act 1977* (NSW) s.170, a government entity is required to establish and keep a “Heritage and Conservation Register”. The information currently on the NSW State Heritage Inventory for Wingecarribee Swamp was taken from a register prepared by the Sydney Catchment Authority (SCA) to meet the requirements of section 170 of the *Heritage Act 1977*, draft dated June 2010 (available in the Heritage Online Library). The inventory states, ‘The Sydney Catchment Authority (SCA) and Department of Conservation & Climate Change (DECC) are joint sponsors of the Wingecarribee Swamp and Special Area Plan of Management (WSSAPoM) 2007. The SCA, as land manager for Wingecarribee Swamp, has the primary responsibility for delivery of the actions under the plan and DECC's primary role is provision of expert and technical advice. The WSSAPoM was approved in 2007 by the then Minister for the Environment, Bob Debus MLA, and built on works implemented under the previous plan of management - WSSAPoM 2001-6 (Knowles, 2008, 2).’

Water NSW was established from 1 January 2015 (when the SCA was merged with State Water). Water NSW should comply with its obligations under the *Heritage Act 1977* in respect of Wingecarribee Swamp and the catchment.

Submission 11: ***Water NSW does not have a Conservation Management Plan in place for Wingecarribee Swamp.***

Under *Heritage Act 1977* s.170A(2), government instrumentalities are responsible for ensuring that the items entered on its register under section 170 and items and land to which a listing on the State Heritage Register applies that are under its care, control or management are maintained with due diligence in accordance with State Owned Heritage Management Principles.

The State Heritage Inventory was updated on 7 February 2024 in relation to Recommended Management, including Produce a Conservation Management Plan and Review a Conservation Management Plan.



Records Retrieved 4

Management Type▲	Date Updated
Recommended Management - Carry out interpretation, promotion and/or education	7/02/2024
Recommended Management - Produce a Conservation Management Plan (CMP)	7/02/2024
Recommended Management - Restrict access	7/02/2024
Recommended Management - Review a Conservation Management Plan (CMP)	7/02/2024

Water NSW does not have a Conservation Management Plan in place for Wingecarribee Swamp.

Submission 12: ***Water NSW should identify the Wingecarribee River as a geographical priority area likely to benefit from research considering the significance of the catchment and river health issues and the opportunities to improve water security, water quality and biodiversity in the catchment and downstream rivers e.g. Wollondilly River.***

Water NSW’s Operating Licence requires it to develop a strategy for an ongoing research program for catchment health and downstream river health by 30 November 2025, see clause 33.

The Wingecarribee community requests that Water NSW identify the Wingecarribee as a geographical priority area likely to benefit from research considering the significance of the catchment and the opportunities to improve water security, water quality and biodiversity in the catchment and downstream rivers such as the Wollondilly River.

A 2024 research paper prepared by the UNSW Water Research Laboratory and Water Research Centre with assistance from Water NSW, *Mining impacts peatland hydrology reducing discharge and water storage volumes*, recommended broader catchment management initiatives to prevent or limit further water extraction from upland swamps. This paper fails to mention the impacts of mining on Wingecarribee Swamp.

Submission 13: ***Water NSW should be considering the effects on platypus populations of water of the proposed plastics recycling and reprocessing facility.***

No information has been provided on the chemical composition of the wastewater stream. Water NSW should not assume that the human and ecological risks are acceptable, as the wastewater is likely to have toxic pollutants which will pass through the sewerage treatment plant and into Wingecarribee River.

Submission 14: ***Water NSW's ongoing research program for catchment health and downstream river health for the Wingecarribee River should acknowledge that there are platypus in the Wingecarribee River and identify opportunities to support research that increases biodiversity.***

Local sightings of platypus in the Wingecarribee River are well documented. A 2022 article in 'Communications Biology' that studied the Wingecarribee River is *Fragmentation by major dams and implications for the future viability of platypus populations* (2022 5:1127) written by Jose Mijangos, Gilad Bino et al. Abstract: The evolutionarily unique platypus (Ornithorhynchus anatinus) has experienced major declines and extinctions from a range of historical and recent interacting human-mediated threats. Although spending most of their time in the water, platypuses can move over land. Nevertheless, uncertainties remain whether dams are barriers to movement, thus limiting gene flow and dispersal, essential to evolution and ecology. Here we examined disruption of gene flow between platypus groups below and above five major dams, matched to four adjacent rivers without major dams. Genetic differentiation (FST) across dams was 4- to 20-fold higher than along similar stretches of adjacent undammed rivers; FST across dams was similar to differentiation between adjacent river systems. This indicates that major dams represent major barriers for platypus movements. Furthermore, FST between groups was correlated with the year in which the dam was built, increasing by 0.011 every generation, reflecting the effects of these barriers on platypus genetics. This study provides evidence of gene flow restriction, which jeopardises the long-term viability of platypus populations when groups are fragmented by major dams. Mitigation strategies, such as building of by-pass structures and translocation between upstream and downstream of the dam, should be considered in conservation and management planning.

Another study in the local area is *Instream flow requirements for the platypus (Ornithorhynchus anatinus): High flows. Studies of water transfers from the Shoalhaven River system to the Hawkesbury-Nepean River system* (1998) 'Australian mammalogy' Vol.20(2) p.304. Description: During periods of low flows into the storages of the Hawkesbury-Nepean River system, Sydney Water pumps water from Lake Yarrunga, a storage at the junction of the Shoalhaven and Kangaroo Rivers. to the Wingecarribee Dam in the southern tablelands of New South Wales. From there it can be released into the upper Nepean River storages and/or the Wingecarribee River, which drains into the Wollondilly River and hence into the stored water behind Warragamba Dam. Prior to the formulation of an operational release strategy for this system, controlled releases of water were made into both the systems. During these releases the effects of flows on bank stability, water birds, benthic organisms and platypus populations and habitat were assessed, and a regime of maximum releases formulated. Potential impact on platypus populations was assessed by determining the height and/or area of bank left available for use by platypuses at the various flows. Subjective assessments were also made of the usable sections of the rivers for foraging at various flows. Broken white water was assumed to be unsuitable for foraging. The various investigations indicated that flows of 400 ML/day during September to March (water bird and platypus breeding and nesting season) and 600 ML/day at other times would have minimal impact on the ecology of the two river systems. Monitoring, studies involving netting and observations of platypuses in the upper Nepean River system indicated that operational releases within the suggested regime between July 1994 and May 1995 may have led to slightly reduced body condition in some animals during the higher flows in the winter of 1994. However the smaller discharges during the latter period of releases did not appear to result in platypuses entering the winter of 1995 in poor condition. Assessment of the usefulness of some sections of the river for foraging indicated that minor adjustments to the higher end of the flow regime may be necessary for future releases and that further monitoring needs to be done. Capture and observational monitoring studies indicated that releases of up to 500 ML/day in the Wingecarribee River between February and June 1995 had no noticeable effect on platypus activity or populations. Further monitoring needs to be done prior to, during and after higher flows and/or for longer periods in the Wingecarribee River.

Submission 15: ***Water NSW should be monitoring the ecological effects of water releases and spills from Wingecarribee Dam.***

Water NSW is the owner of Wingecarribee Dam, a declared dam in the Sydney catchment area. Under the *Dams Safety Regulation 2019* (NSW) reg 13(1)(e), dam owners must implement a Dams Safety Management System that is compliant with AS ISO 55001:2014. An owner needs to determine the 'requirements and expectations' of their stakeholders which should be documented within the DSMS document and communicated. Water NSW has not determined Wingecarribee Dam stakeholders, their requirements and expectations, or stakeholder requirements for recording and reporting on dam spills and water releases (or communicated this information to them).

The *NSW State Flood Plan* dated 2 December 2021 outlines the arrangements for the management of downstream consequences of flooding due to dam failure. It states on p.42 that owners of declared dams must: 'Advise the downstream community of prospective and actual water releases.' Water NSW has not been advising the downstream community of prospective and actual water releases from Wingecarribee Dam.

On 23 August 2024, Water NSW disclosed information showing that Wingecarribee Dam spilled on 7 March 2022 (3650 ML), 8 March 2022 (4380 ML) & 9 March 2022 (2190 ML). On 24 October 2024, Water NSW disclosed information showing that Wingecarribee Dam spilled on 4 July 2022 (2820 ML), 5 July 2022 (6780 ML) & 6 July 2022 (640 ML). The incidents caused concern to members of the public but were not reported to Dams Safety NSW by the Chief Executive Officer of Water NSW as required by *Dams Safety Regulation 2019* reg 19(1)(f) and the data was not included in the Wingecarribee River flood study 'update' dated 20 July 2022.

Water NSW should be monitoring the ecological effects of water releases and spills from Wingecarribee Dam.

Submission 16: 74-76 Beaconsfield Road Moss Vale is NOT THE RIGHT SITE for a plastics recycling and reprocessing facility.

Instream Flow Requirements for The Platypus (Ornithorhynchus anatinus): High Flows. Studies of Water Transfers from The Shoalhaven River System to The Hawkesbury-Nepean River System

- **Authors / creators:** Grant, T. ; McDonald, G.
- **Is part of:** Australian mammalogy, 1998, Vol.20 (2), p.304
- **Description:** During periods of low flows into the storages of the Hawkesbury-Nepean River system, Sydney Water pumps water from Lake Yarrunga, a storage at the junction of the Shoalhaven and Kangaroo Rivers. to the Wingecarribee Dam in the southern tablelands of New South Wales. From there it can be released into the upper Nepean River storages and/or the Wingecarribee River, which drains into the Wollondilly River and hence into the stored water behind Warragamba Dam. Prior to the formulation of an operational release strategy for this system, controlled releases of water were made into both the systems. During these releases the effects of flows on bank stability, water birds, benthic organisms and platypus populations and habitat were assessed, and a regime of maximum releases formulated. Potential impact on platypus populations was assessed by determining the height and/or area of bank left available for use by platypuses at the various flows. Subjective assessments were also made of the usable sections of the rivers for foraging at various flows. Broken white water was assumed to be unsuitable for foraging. The various investigations indicated that flows of 400 ML/day during September to March (water bird and platypus breeding and nesting season) and 600 ML/day at other times would have minimal impact on the ecology of the two river systems. Monitoring, studies involving netting and observations of platypuses in the upper Nepean River system indicated that operational releases within the suggested regime between July 1994 and May 1995 may have led to slightly reduced body condition in some animals during the higher flows in the winter of 1994. However the smaller discharges during the latter period of releases did not appear to result in platypuses entering the winter of 1995 in poor condition. Assessment of the usefulness of some sections of the river for foraging indicated that minor adjustments to the higher end of the flow regime may be necessary for future releases and that further monitoring needs to be done. Capture and observational monitoring studies indicated that releases of up to 500 ML/day in the Wingecarribee River between February and June 1995 had no noticeable effect on platypus activity or populations. Further monitoring needs to be done prior to, during and after higher flows and/or for longer periods in the Wingecarribee River.
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ARTICLE

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Fragmentation by major dams and implications for the future viability of platypus populations

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The evolutionarily unique platypus (*Ornithorhynchus anatinus*) has experienced major declines and extinctions from a range of historical and recent interacting human-mediated threats. Although spending most of their time in the water, platypuses can move over land. Nevertheless, uncertainties remain whether dams are barriers to movement, thus limiting gene flow and dispersal, essential to evolution and ecology. Here we examined disruption of gene flow between platypus groups below and above five major dams, matched to four adjacent rivers without major dams. Genetic differentiation (F_{ST}) across dams was 4- to 20-fold higher than along similar stretches of adjacent undammed rivers; F_{ST} across dams was similar to differentiation between adjacent river systems. This indicates that major dams represent major barriers for platypus movements. Furthermore, F_{ST} between groups was correlated with the year in which the dam was built, increasing by 0.011 every generation, reflecting the effects of these barriers on platypus genetics. This study provides evidence of gene flow restriction, which jeopardises the long-term viability of platypus populations when groups are fragmented by major dams. Mitigation strategies, such as building of by-pass structures and translocation between upstream and downstream of the dam, should be considered in conservation and management planning.

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The semi-aquatic platypus (*Ornithorhynchus anatinus*), along with echidnas, belong to the order Monotremata, the most species-poor ($n = 5$) and most basal branch of mammals, which diverged from marsupials and eutherians 187 Mya[1]. Platypuses have a unique combination of features, including oviparity, venomous spurs in males, electroreception used to locate freshwater macroinvertebrates, biofluorescent pelage, and multiple sex chromosomes (five pairs instead of one[2–4]). The uniqueness and rarity of platypus features (*sensu* Pavoine et al.[5]) and its evolutionary distinctiveness[6] make it arguably one of the most irreplaceable mammals existing today.

The platypus is currently listed as 'Near Threatened' by the International Union for Conservation of Nature (IUCN[7]), 'Endangered' in South Australia (*National Parks and Wildlife Act 1972*) and 'Vulnerable' in Victoria[8].

There is increasing evidence of larger numbers of platypuses in historical times[9] and ongoing declines and extinctions of local populations[2,10,11]. Declines have been driven by multiple and synergistic threats, including river regulation, loss and modification of habitats, climate change, pollution, by-catch mortality and predation by invasive species[2,9–11]. Continued declines due to current and future climate change are predicted as a result of increased frequency and severity of droughts[2,12,13], as well as elevated temperature conditions which could lead to the loss of more than 30% of suitable habitat by 2070[12,14].

Threats to freshwater ecosystems are commonly synergistic and are intensified by the construction of major dams that can have immediate and long-term impacts[15]. Nearly half of the world's river discharge is impacted by flow regulation and fragmentation[16]. Dams pose a major threat to global freshwater biodiversity[17]. Large dams form major barriers for aquatic organisms, limiting critical ecological processes, such as fish migration[18]. Water impoundments behind major dams form wind-exposed, deep, and standing (lentic) ecosystems which can offer little resources for flow-dependant species[19]. In Australia, dams are one of the more serious threats for platypus conservation, given their potential broad impact on habitat[2,12,20]. Major dams are widespread across much of the platypus' distribution, where as many as 77% (383 out of 495) of the Australian major dams (wall height >10 m; ancold.org.au) coincide within the regions where platypuses occur (Fig. 1a; see also Bino et al.[11]). Immediate adverse effects of major dams extend over large areas both upstream and downstream. Below major dams, altered natural flow regimes, including changing of the timing of flows and important reduction in flow volumes have been found to significantly impact platypus abundances and demographics[21]. Conditions below and above major dams represent poor foraging and burrowing habitat for platypuses, given lower productivity of macroinvertebrate prey species[10,22–25].

Long-term effects of major dams may include reduction in the ability of platypuses to move between potential habitat areas. This fragmentation has twofold impact; first, it restricts the ability to recolonise available habitat or migrate to areas with more suitable conditions[26]. Secondly, fragmentation also simultaneously reduces both local population size and gene flow, each of which is expected to lead to increased inbreeding and reduction of the genetic variation necessary for adaptation to changes including threats[27]. One adverse consequence of small population size is lower survival and lower reproduction output due either to inbreeding depression or to catastrophic stochastic events. Another adverse consequence is reduced variation between individuals, necessary for adaptation to changes such as the threats listed above[28]. These genetic changes may be prevented by immigration because gene flow replenishes the gene pool of populations, but of course, this will only happen if the small population is not a fragmented isolate[29,30].

For platypuses, major dams are predicted to be a barrier for dispersal[31,32], with potential long-term ramifications for gene flow, genetic variation, and adaptation to threats. However, both the restriction of dispersal and the genetic consequences remain largely unquantified. When major dams are assumed to pose barriers for movements, population viability analyses demonstrate considerable impacts by major dams, particularly in synergy with lower habitat quality and droughts, which are projected to increase[11]. In addition, since the introduction of red foxes (*Vulpes vulpes*) to Australia, overland movements of platypuses carry an increased risk of predation[24], effectively increasing the impact of dams as barriers to platypuses. However, the extent to which major dams restrict platypus dispersal remains unclear because landscape connectivity varies due to both the species' life history and landscape features[26]. Platypuses are known to be able to climb around dams up to 10 m high (Dr Tom Grant & Dr Anne Musser, personal communication, June 23, 2021), although their ability to find their way around higher structures is currently unknown. Their ability to swim across the large deep-water impoundments above the dam is also unclear.

Therefore, our research uses genetic methods to focus on the connectivity of platypus groups above and below major dams. Genetic-based methods used to infer patterns of dispersal and gene flow[33] commonly examine the positive relationship between the amount of genetic differentiation between populations or individuals and the geographic distance separating them[34]. The presence of a dispersal barrier could be inferred by testing whether populations or individuals, separated by potential barriers, are more genetically differentiated than populations or individuals in landscapes lacking such barriers but separated by a similar distance. Genetic differentiation can increase due to dispersal barriers within one to 15 generations during computer simulations[35], but is unlikely to arise if population size is large (>50 individuals[36]).

To determine whether major dams have reduced dispersal and gene flow between platypus groups, we analysed genetic data from platypuses sampled in nine rivers; five rivers were regulated by major dams, and four were unregulated (Fig. 1). If major dams adversely affected gene flow between platypus groups, we predicted the following: (a) individuals and groups separated by a major dam in a river should be more differentiated than in an unregulated river, and; (b) genetic differentiation across major dams should correlate with the time since the dam was built.

Results

Genetic variation within groups. Mean single nucleotide variation (SNP) genetic variation across all rivers (expected heterozygosity) was $He = 0.140$. He was significantly different between all groups within one river system (except for Severn above the dam/Severn below the dam; p-value >0.05; Table 1). He was also significantly different between regions (except for Snowy Rivers/Upper Murray Rivers; p-value >0.05; Table 1). Border Rivers, located in the north, had the lowest He (range: 0.130–0.135), followed by the Snowy Rivers (0.139–0.144) and the Upper Murray Rivers (0.140–0.152), river regions in the south (Fig. 1). Estimates of allelic richness follow the same trend as heterozygosity estimates. Inbreeding estimates (F_{IS}) were close to zero except for the microsatellite dataset (Table 1).

Connectivity between platypus groups—effects of major dams. For unregulated and regulated river comparisons, the river with the dam showed higher genetic differentiation: Mitta-Mitta above versus below dam had $F_{ST} = 0.024$, whereas Ovens above versus below had $F_{ST} = 0.002$; Nepean below versus above dam had $F_{ST} = 0.073$, whereas Wingecarribee above versus below had

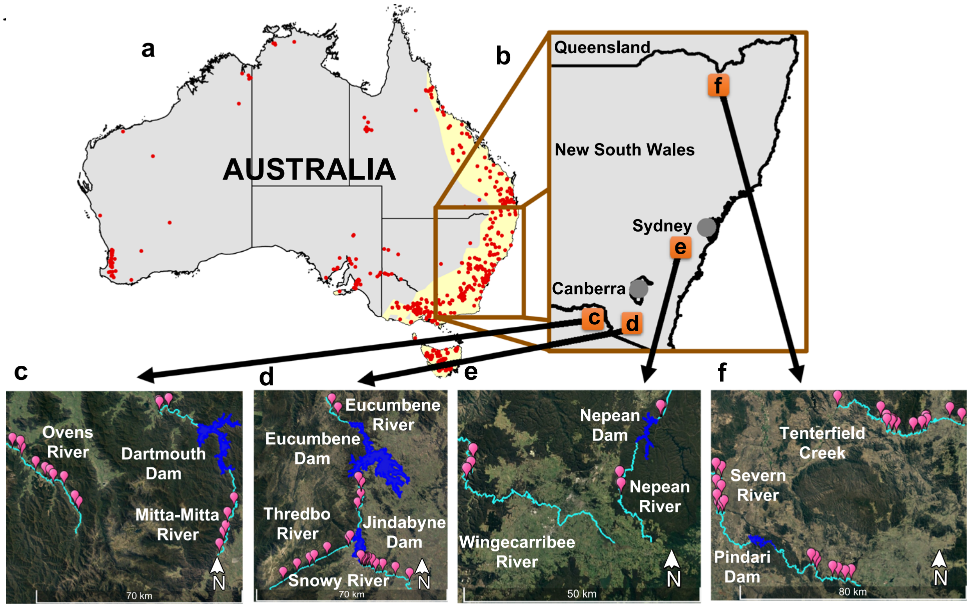

Fig. 1 Sampling sites. a Distribution of major dams (>10 m high; red points) within and outside the IUCN platypus distribution (yellow shade), and the focus regions for this study (brown inset). **b** Location of rivers in south-east Australia where platypuses were sampled (orange squares) in rivers that were regulated (with major dams) and unregulated (no major dams). **c** Upper Murray Rivers: Ovens (unregulated) and Mitta-Mitta Rivers (regulated, upstream sections are in the south, confluence with Ovens is out of the frame, in northwest). **d** Snowy Rivers (do not follow the paired experimental design, due to geographic constraints; see methods): Eucumbene (regulated), Thredbo (unregulated), and Snowy River (regulated, Snowy flows downstream to the southeast). **e** Central NSW Rivers: Wingecarribee River (unregulated) and Nepean River (regulated, downstream sections are in the north, there is no confluence with Wingecarribee). **f** Border Rivers: Tenterfield Creek (unregulated) and Severn River (regulated, upstream sections are to the east, confluence with Tenterfield is out of the frame, in northwest). Pink balloons represent the 81 sampling sites; rivers are coloured in light blue, and reservoirs behind major dams are in dark blue. Platypus distribution shapefile was downloaded from The IUCN Red List of Threatened Species[74]. Rivers and dams shapefiles were downloaded from Geoscience Australia[75,76]. Map of Australia shapefile was downloaded from Australian Bureau of Statistics[77]. Dams height and GPS coordinates were downloaded from Australian National Committee on Large Dams Incorporated[78].

$F_{ST} = 0.016$; and Severn below versus above dam had $F_{ST} = 0.061$, whereas Tenterfield above versus below had $F_{ST} = 0.007$ (Table 2). In each case, the dammed versus undammed F_{ST} values differed by more than two standard errors of the mean; the average F_{ST} for the three dammed rivers (0.053) was about six times higher than the paired undammed rivers (0.008). The relatively high within-locality variation for microsatellites has the potential to lower F_{ST} for microsatellites relative to SNPs[37], however, such a trend was not evident—in fact, the opposite trend was seen. Finally, in the more complex Snowy Rivers system (Fig. 1), this simple paired F_{ST} analysis was not easy to interpret, so we relied upon the other analyses presented below. Using Mutual information and Jost's D to assess genetic differentiation with and without major dams gave results that were comparable to those from F_{ST} (Supplementary Tables 3–4 and Supplementary Figure 2).

Over all four river systems, we observed a positive and significant relationship ($R^2 = 0.615$; p-value $= 0.013$) between F_{ST} and the number of platypus generations since the building of the dam (Fig. 2). We note again that potential bias towards lower F_{ST} values in microsatellites than in SNPs, mentioned above, was not evident—the oldest dam was in the river system analysed by microsatellites, and this system showed the highest F_{ST} (Fig. 2).

Spatial differentiation summarised by principal components analysis (PCA) of the Upper Murray Rivers (Mitta-Mitta and Ovens Rivers) did not show complete separation of samples for different locations, but there was noticeable clustering of platypuses into three groups: Ovens river (unregulated); below the dam in the Mitta-Mitta River, and above the dam in the Mitta-Mitta River (Fig. 3a). Snowy Rivers (Snowy, Thredbo and Eucumbene Rivers) did not follow the paired experimental design due to geographic constraints. PCA analyses showed that platypuses from the Snowy River formed a separated cluster to that of the Thredbo and Eucumbene Rivers (Fig. 3b), whereas platypuses from the two latter rivers overlapped somewhat on the PCA plot. Notably, platypuses from the Eucumbene River above the dam were closer to platypuses from Thredbo River than platypuses from the Eucumbene River below the dam. PCA analyses of the central New South Wales Rivers (Nepean and Wingecarribee Rivers) did not show a clear clustering pattern (Fig. 3c) possibly due to the low number of markers used in this analysis (12 microsatellites) compared to the other rivers systems (2641 SNPs). For the Border Rivers (Tenterfield Creek and Severn River), the principal component analysis (PCA) of these rivers indicated three well-separated clusters (Fig. 3d), with platypuses collected below and above the dam in the Severn River, and Tenterfield Creek forming different groups. 3D PCA plots showing the first three principal components are available in Supplementary Data 1–4.

Discussion

Dispersal and gene flow are essential for the viability of natural populations, critical for ecological and evolutionary processes such as recolonisation, dispersal to suitable habitats, increased genetic diversity to avoid inbreeding depression and allow adaptation[26,29,30]. There is increasing concern about the impacts of dams on aquatic biota and ecological processes[15,17] given this is a critical global issue for rivers, with at least 2.8 million reservoirs larger than 0.1 ha[38]. Our analyses suggest that major dams pose barriers to platypus dispersal and gene flow given that genetic differentiation increased proportionally with time after

Table 1 Summary statistics across the four river regions.

Region	River/Creek	Survey section (km)	Sample size	Proxy of abundance	Allelic richness	*Ho*	SE	*He*	SE	F_{IS}	SE
Upper Murray Rivers	Ovens	36	19	27	1.375	0.144	0.003	0.145	0.003	0.005	0.004
	Mitta-Mitta above dam	23	13	19	1.370	0.140	0.003	0.143	0.003	0.013	0.005
	Mitta-Mitta below dam	18	4	4	1.395	0.152	0.003	0.153	0.003	−0.019	0.009
Snowy Rivers	Snowy	26	56	46	1.365	0.139	0.002	0.141	0.002	0.010	0.002
	Thredbo	33	19	37	1.365	0.141	0.003	0.141	0.003	−0.003	0.004
	Eucumbene above dam	18	4	36	1.370	0.144	0.003	0.143	0.003	−0.026	0.009
	Eucumbene below dam	20	20	50	1.346	0.137	0.003	0.136	0.003	−0.001	0.004
Central NSW Rivers	Wingecarribee*	7	42	**	4.113	0.703	0.060	0.731	0.044	0.053	0.047
	Nepean above dam*	0.5	11	**	3.942	0.549	0.063	0.646	0.064	0.142	0.051
	Nepean below dam*	4	7	**	4.706	0.589	0.095	0.608	0.059	0.096	0.107
Border Rivers	Tenterfield	96	39	207	1.353	0.135	0.003	0.138	0.003	0.015	0.003
	Severn above dam	50	23	115	1.335	0.133	0.003	0.133	0.003	−0.003	0.004
	Severn below dam	60	17	83	1.333	0.130	0.003	0.131	0.003	0.005	0.005

The number of samples and a proxy of abundance calculated as (unique number of captures/number of sampling nights) × (length of the river surveyed) based on Hawke et al.[21]; *Ho*—observed heterozygosity; *He*—expected Hardy-Weinberg heterozygosity; F_{IS}—inbreeding coefficient.

Note that small sample sizes in Mitta-Mitta below the dam and Eucumbene above the dam (both 4 individuals) are likely to result in unreliable estimates of diversity.

SE standard error, *NSW* New South Wales.

*Microsatellite data.

**Comparable estimates are not available due to different survey techniques see Kolomyjec et al.[31,53,55].

Table 2 Genetic differentiation (F_{ST}) between rivers in different connectivity scenarios.

Region	River 1	River 2	F_{ST}	SE	Connectivity scenario
Border Rivers	Tenterfield	Severn above dam	0.063	0.002	Separated by a river system
	Tenterfield	Severn below dam	0.075	0.002	Separated by a river system
	Severn below dam	Severn above dam	0.061	0.002	Separated by dam for 47 years (*Circa* 1969)*
	Tenterfield above	Tenterfield below	0.007	0.001	No dam
Upper Murray Rivers	Ovens	Mitta-Mitta above dam	0.052	0.002	Contiguous river systems
	Ovens	Mitta-Mitta below dam	0.035	0.003	Contiguous river systems
	Mitta-Mitta above dam	Mitta-Mitta below dam	0.024	0.003	Separated by dam for 39 years (*Circa* 1979)
	Ovens above	Ovens below	0.002	0.002	No dam
Snowy Rivers	Snowy	Thredbo	0.024	0.001	Separated by dam for 50 years (*Circa* 1967)
	Snowy	Eucumbene above dam	0.042	0.002	Separated by dam for 59 years (*Circa* 1958)
	Snowy	Eucumbene below dam	0.045	0.001	Separated by dam for 50 years (*Circa* 1967)
	Thredbo	Eucumbene above dam	0.040	0.003	Separated by dam for 59 years (*Circa* 1958)
	Thredbo	Eucumbene below dam	0.031	0.002	Separated by lake for 50 years (*Circa* 1967)
	Eucumbene above dam	Eucumbene below dam	0.053	0.003	Separated by dam for 59 years (*Circa* 1958)
Central NSW Rivers	Wingecarribee**	Nepean above dam	0.060	0.023	Contiguous river systems
	Wingecarribee**	Nepean below dam	0.062	0.013	Contiguous river systems
	Nepean above dam**	Nepean below dam	0.073	0.018	Separated by dam for 74 years (*Circa* 1935)
	Wingecarribee above**	Wingecarribee below	0.016	0.007	No dam

SE standard error.
*Pindari Dam. The height of the dam wall was doubled from 45 m to 85 m in 1995.
**Microsatellite data.

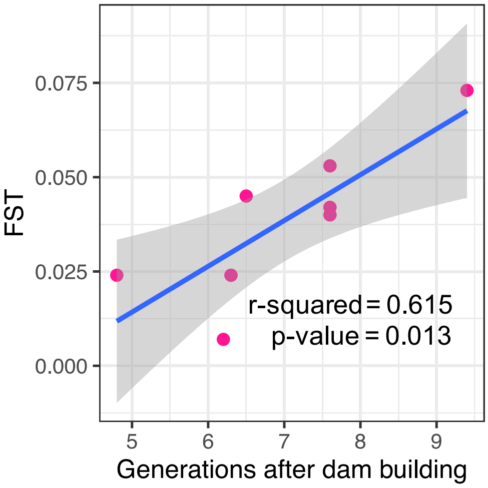

Fig. 2 Genetic differentiation against dam age. Relationship between genetic differentiation (F_{ST}) between platypus groups separated by major dams ($n = 8$ major dams) and the number of platypus generations (7.9 years[68]) since the building of the dam. Genetic differentiation increased at a rate of 0.011 per generation.

the building of a dam and was higher in dammed than undammed rivers.

In relation to whether major dams affect the connectivity between platypus groups, F_{ST} values were higher when there was a dam, and some F_{ST} values between groups separated by a dam were as high as F_{ST} values between groups in different rivers (Table 2). In addition, we found a significant association between F_{ST} and the number of platypus generations since dam construction (Fig. 2), suggesting that F_{ST} increases at a rate of 0.011 by generation. Even though the Nepean dam, built in 1935, was analysed with a different type of molecular marker (microsatellites, not SNPs), recent research indicates that estimates of F_{ST} using SNPs and microsatellites are comparable[39,40]. If anything, we would expect the microsatellites used in this system to have lower F_{ST} due to the effect of their high within-group variation[37,41], but in fact the opposite trend was seen. We noticed that F_{ST} values in the Snowy Rivers were higher between groups separated by the Jindabyne Dam (Eucumbene below dam/Snowy; $F_{ST} = 0.045$) than between groups divided by the Jindabyne reservoir but not a dam (Eucumbene below dam/Thredbo; $F_{ST} = 0.031$). This observation suggests that some limited gene flow might have occurred across the Jindabyne reservoir.

Overall, our results are consistent with the notion that major dams and their associated waterbodies may be considerable barriers for platypuses. Despite platypuses being able to move substantial distances (e.g., male juveniles can move >40 km[42–44]), the effect of major dams on genetic differentiation was considerable. Such impacts can be directly related to the dam walls representing a barrier dissuading platypuses from attempting to bypass the wall through overland movements as well as indirectly by increasing predation risk by introduced predators such as foxes, cats, and dogs[24].

Major dams represent dispersal barriers for most freshwater species[45,46], requiring mitigation strategies to offset negative demographic impacts. For instance, human-mediated relocation of individuals between populations has been implemented successfully to limit the effects of population isolation and small population size[47]. A common rule of thumb in conservation suggests that one dispersing individual per generation would minimise the effects of population isolation[48]. Another strategy to improve connectivity between populations, despite some limitations and caveats, is the construction of dam bypass structures that increase dispersal of freshwater species, including fishways[49–51], although there are adverse consequences of connectivity, such as disease risks[52]. Such by-pass structures have not yet been considered for the platypus.

We have found that platypus connectivity between groups is adversely affected by major dams, and it is known that reduced connectivity can lead to the adverse long-term conservation outcomes described above[26–30]. Therefore there will be a need for the management of platypuses to consider ways such as those just described to minimise detrimental effects of river regulation on the platypus (and other species). Some of the long-term effects of major dams might be reduced by rare natural dispersal events between rivers[53], but our results indicate that this has not been enough to offset the divisive effect of the major dams, so more active management is required. Firstly, new dams within the

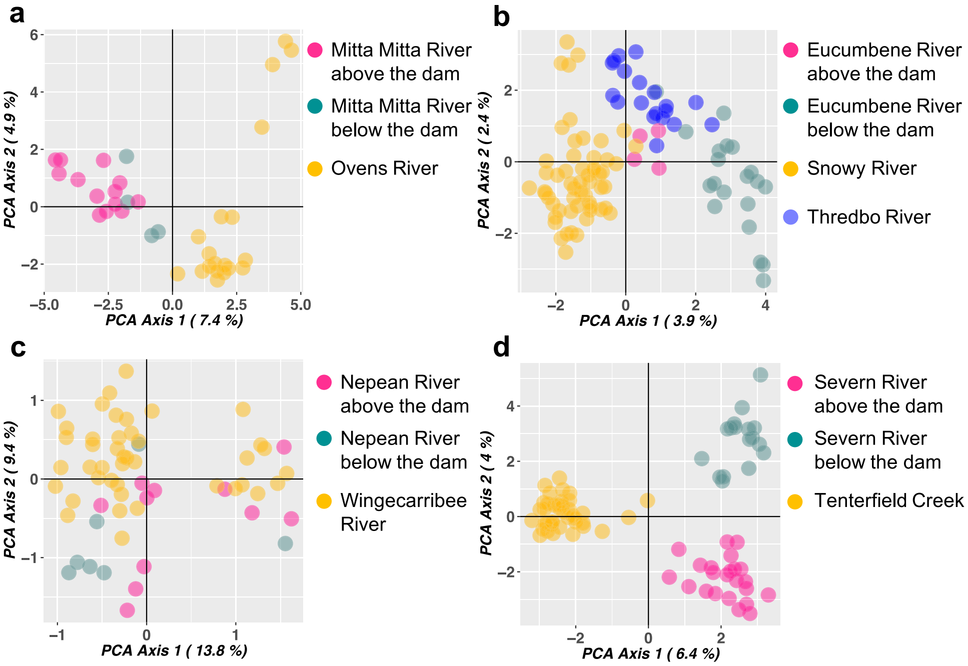

Fig. 3 Principal coordinates analyses. **a** Upper Murray Rivers: unregulated (no dam) Ovens and regulated (dam) Mitta-Mitta Rivers. **b** Snowy Rivers: regulated (dam) Snowy, unregulated (no dam) Thredbo and regulated (dam) Eucumbene Rivers. These rivers do not follow the paired experimental design due to geographic constraints. **c** Central NSW Rivers: regulated (dam) Nepean and unregulated (no dam) Wingecarribee Rivers. **d** Border Rivers: unregulated (no dam) Tenterfield Creek and regulated (dam) Severn River. Numbers between parentheses in the axis labels show the percentage of variation captured by each axis. Each point represents a platypus individual.

Table 3 The four study systems and the major dams.

Region	River/Creek	Dam name	Year of completion	Dam height (m)	Dam volume (GL)
Upper Murray Rivers[c]	Ovens	–	–	–	–
	Mitta-Mitta	Dartmouth	1979	180	3856
Snowy Rivers[d]	Snowy	Jindabyne	1967	72	688
	Thredbo	–	–	–	–
	Eucumbene	Eucumbene	1958	116	4798
Central NSW Rivers[e]	Wingecarribee	–	–	–	–
	Nepean	Nepean	1935	85	68
Border Rivers[f]	Tenterfield	–	–	–	–
	Severn	Pindari*	1969	85	312

See Fig. 1 for details of geography. The letters c, d, e and f refer to panels in Fig. 1.
GL gigalitres.
*Pindari Dam. The height of the dam wall was doubled from 45 m to 85 m in 1995.

platypus distribution need to be avoided, for example, by pumping from the river into an off-stream storage without the necessity for a dam on the river itself, as is done for metropolitan water supplies in both the Manning and Hastings Rivers, in New South Wales. Secondly, for existing major dams, it might be possible to devise platypus-specific versions of methods that have been used to ameliorate dam effects in other species, such as human-mediated relocation of individuals or by-pass structures that increase dispersal.

In this study, we compared regulated rivers, with major dams, to adjacent unregulated rivers with no major dams and identified that major dams were barriers to movement of platypuses within a river system, reflected in genetic variation. Major dams restricted dispersal and gene flow between groups and therefore increased the possibility of inbreeding depression, loss of adaptive genetic variation, failure to recolonise areas where local extinctions have occurred and failure to disperse to areas with more suitable conditions. Synergistic with reduced habitat quality, these are all expected to lower the long-term viability of the platypus[11]. Our analyses reinforce the growing evidence on the negative impacts of major dams on platypus populations. These studies are relevant to inform the decision-making process of conservation managers and could be used in viability analysis and decision analysis[54] to develop strategies that ensure the long-term persistence of the unique platypus. This study adds to the growing evidence about the impacts of dams on aquatic biota and their viability.

Methods

Study areas and fieldwork. Samples from platypuses were collected from nine different rivers (five regulated by major dams and four unregulated) across four regions in south-east Australia (see Fig. 1 and Table 3), also described in Hawke et al.[21] and Kolomyjec et al.[55,56]. River flows upstream of major dams were minimally regulated, contrasting with heavily regulated downstream flows. Throughout their range, the platypus comprises four major geographically defined genetic clusters: North Queensland, central Queensland, New South Wales and Tasmania[57]. The samples used in this study belong to the New South Wales cluster.

Table 4 SNP filtering.

Filter	Variation between groups	Variation within groups
Reproducibility (RepAvg)	>100%	>100%
Retain only one SNP per read	Used	Used
Departure from Hardy-Weinberg proportions	<0.05	<0.05
Mapped to chromosome	Used	Used
BLAST alignment E-value	<1e−20	<1e−20
Missing data by site	>90%	>100%
Minor allele count (MAC)	>3	Not used
Linkage disequilibrium (r^2)	<0.2	Not used
Remove sites located within coding regions	Used	Not used
Remove sites located within sex chromosomes	Not used	Used
Total SNPs after filtering	2641	4551

Filters and their thresholds used for SNPs to remove genomic sites for the analyses based on variation between groups and variation within groups.

Platypuses were captured across 81 sites (Fig. 1). In this study, we used two different molecular markers: single nucleotide polymorphism (SNPs) for all samples except Central NSW, and microsatellites for Central NSW[55,56]. Sampling for microsatellites in Central NSW is described in Kolomyjec et al.[55,56]. For SNPs at all other sites, we aimed to cover a minimum of 40 km of each unregulated river and 20 km of river above and below major dams on regulated rivers. The procedure of trapping and sampling platypuses, including details of anaesthesia, used in this study have been described elsewhere[21,58]. Briefly, platypuses were captured using fyke nets or unweighted mesh (gill) nets and implanted with a Passive Integrated Transponder (PIT) tag (Trovan) to identify recaptured individuals. Platypuses were then weighed, measured, sexed, aged, and blood collected (~2 ml) and stored in Qiagen RNAprotect® animal blood tubes (Qiagen, Hilden, Germany). For the SNP sampling, our proxy of abundance for each river was the following metric: unique number of captures/number of sampling nights x length of the river surveyed (see Hawke et al.[21]).

Laboratory work. For SNPs (single nucleotide polymorphisms), genomic DNA was extracted from whole blood using a Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). DNA quality and concentration were visualised using agarose gel electrophoresis and quantified fluorimetrically with a Qubit 2.0 (Thermo Fisher Scientific). Samples were genotyped using DArTseq^{TM} (DArT Pty Ltd, Canberra, ACT, Australia). DArT's procedure uses a combination of genome complexity reduction methods using restriction enzymes, implicit fragment size selection and next-generation sequencing to produce thousands of SNPs randomly distributed throughout the genome[59]. Read sequences were processed using proprietary DArT analytical pipelines[59] and mapped to the representative platypus genome (mOrnAna1.p.v1, GenBank assembly accession: GCA_004115215.2; total sequence length of 1.8 Gbps, 305 scaffolds with an N50 of 83 Mbp). Refer to Georges et al.[60] for details of DArT sequencing, genotyping and filtering processes. DArT's genotyping has various advantages such as limiting the potential for ascertainment bias[61], providing metadata for each locus with various quality and BLAST alignment measures, including the proportion of replicates for which the marker score is consistent (RepAvg) and the average of the polymorphism information content of the reference and SNP allele (AvgPIC).

For microsatellites, genomic DNA was extracted from toe-web biopsies (2 × 2 mm specimens stored in 70% ethanol) using a proteinase K/salt precipitation method[62]. Twelve published microsatellite sites were amplified and scored according to standard techniques[55,56].

SNP filtering. The criterion for SNP filtering used to analyse variation between groups (e.g., F_{ST}) can bias estimates of variation within groups (e.g., heterozygosity[63]). Therefore, we used different filtering settings for each type of analysis (Table 4). Detailed description of the filtering processes can be found in the Supplementary Information document.

For SNPs, a total of 295 platypuses were captured and blood sampled across four river regions in southeast Australia (Supplementary Table 2). DNA extraction and DArT^{TM} sequencing were successful in 218 blood samples from individuals. Two samples, each collected in a different river (V30 in Ovens and V32 in Mitta-Mitta), showed contrasting genetic patterns relative to samples collected in the same river (Supplementary Figure 1). Relatedness analyses performed in the R package *related*[64] revealed these two samples had closer relatives in the opposite river (Supplementary Table 1). In addition, the locations of these two samples were separated by 46 km, steep mountainous terrain, and a river system. Under these conditions, we considered that dispersal events were unlikely and concluded that samples were mislabelled and therefore assigned them to the presumed correct river and site. Relatedness analyses also identified two pairs of samples in which each pair was collected from the same individual (i.e., recaptures; samples T3-T5 and T28-T42; Supplementary Table 1). Consequently, we removed one sample from each pair. In the unlikely event that these were pairs of identical twins, it would still be appropriate to remove one of each pair.

For SNPs, sequencing provider DArT^{TM} (Canberra) successfully genotyped 17,631 single nucleotide polymorphism (SNP) sites. After stringent filtering, our dataset for analysing genetic variation between groups comprised 2641 SNPs genotyped in 214 platypus samples (108 females, 106 males). After filtering, our SNP dataset for analysing genetic variation within groups comprised 4551 SNPs genotyped in 214 platypus samples (108 females, 106 males).

Data analyses

Genetic variation within groups. To measure genetic variation within rivers, we calculated observed heterozygosity (*Ho*), expected heterozygosity (*He*) and allelic richness using the R package *Hierfstat*[65]. After identifying that the data did not conform to a normal distribution, using a Shapiro–Wilk test of normality (R function *shapiro.test*), we tested whether *He* was significantly different between groups using a non-parametric Mann–Whitney U test (R function *wilcox.test* with option paired = FALSE). In addition, we calculated the inbreeding coefficient (F_{IS}) of each river group using *Hierfstat*.

Investigating whether major dams affect connectivity between platypus groups. We used multiple approaches to investigate whether major dams affect gene flow between platypus groups. Firstly, to test whether groups separated by major dams are more genetically different than otherwise, we divided the sampling sites of each pair of rivers into comparable upstream and downstream groups. For regulated rivers (Nepean, Severn and Mitta-Mitta), the dam, ignoring the reservoir, was used as reference point for the division. For unregulated rivers (Wingecarribee, Tenterfield and Ovens), the division point was chosen at a comparable position to the dam in the paired regulated river. We then calculated the genetic differentiation using F_{ST} following Nei's method[66] between the two groups within each river. We tested the significance of the difference of F_{ST} values between dammed and unregulated rivers using a Mann–Whitney U test (R function *wilcox.test* with option paired = FALSE). In addition, we used Mutual Information[41] and Jost's D[67] two measures that assess between-group differentiation independently of within-group variation.

Secondly, to test whether the number of platypus generations since the building of the dams can predict the genetic differentiation of SNPs and microsatellites between groups (F_{ST}), we used univariate linear regression models (R function *lm*). We considered one platypus generation to be 7.9 years based on Pacifici et al.[68], who used information on age at first reproduction and reproductive life span to estimate generation length in platypus.

Thirdly, to visualise the spatial distribution of genetic variation of the sampled individuals, we performed principal component analysis (PCA) using the R package *dartR*[69] using our two datasets of SNP's and microsatellites. PCA is a statistical method that summarises the variance in the data and projects the top principal components onto a series of orthogonal axes[70]. We chose to use PCA because it has an exact mathematical relationship to the biological coalescent, or genealogy[70], and provides two-dimensional and three-dimensional displays, which are not available in other methods such as STRUCTURE[71].

Statistics and reproducibility. Sample sizes and statistical parameters used in each analysis are indicated in the relevant 'Methods' and 'Results' sections, as well as in tables when applicable. All statistical analyses were performed in R (v4.0.5)[72].

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The datasets used for this research work are stored in GitHub: https://github.com/mijangos81/Platypus and have been archived within the Zenodo repository: https://doi.org/10.5281/zenodo.7039778[73].

Code availability

The R scripts used for this research work are stored in GitHub: https://github.com/mijangos81/Platypus and have been archived within the Zenodo repository: https://doi.org/10.5281/zenodo.7039778[73].

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Author contributions

W.B.S., G.B., R.T.K., J.G. and others conceived the project and acquired the research funds; H.S. and W.B.S. supervised; J.L.M., J.D., K.N.D. and J.G. performed DNA extraction; G.B., T.H. and T.G. carried out fieldwork and collected samples; S.H.K. performed the microsatellite analyses; J.L.M. analysed the data; J.L.M. wrote the manuscript with support from W.B.S. T.G. and S.H.K. first pondered on the possible effect major dams may be having on the genetics of the platypus, carried out the initial research and encouraged others to further investigate this aspect of platypus conservation. All authors discussed the results and contributed to the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Genetic structure and phylogeography of platypuses revealed by mitochondrial DNA

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Abstract

The platypus *Ornithorhynchus anatinus* is an endemic monotreme species with a wide latitudinal distribution in eastern Australia, including Tasmania. Understanding of the phylogeography within this species is very limited at present and represents a gap in the documentation of Australia's unique biodiversity. We analysed mitochondrial DNA sequences (partial control region and complete cytochrome *b*, including portions of flanking tRNAs) of 74 individuals from across the distribution of the species. Phylogenetic analysis of the concatenated sequences corroborated the primary split within the platypus, showing two major clades: one from mainland Australia and the other from Tasmania/King Island. Estimates of divergence times suggest that these clades last shared a common mitochondrial ancestor ~0.7–0.94 Ma. Using an extended dataset of partial control region sequences from 284 individuals, we found evidence of genetic structure between river basins, primarily within mainland Australia, as well as an additional divergent lineage in North-eastern Australia. Overall, few haplotypes were shared between river basins. Analyses of molecular variance of the control region sequences indicated low rates of gene flow and significant divergence, particularly at the river basin and geographical area scales.

Introduction

The platypus *Ornithorhynchus anatinus* is endemic to Australia and exhibits a fascinating suite of characteristics, being a specialized semi-aquatic, fossorial, carnivorous and egg-laying mammal (Grant, 2007). It occurs naturally in freshwater streams, rivers and lakes of eastern Australia, including Tasmania and King Island, and there is a small introduced population on Kangaroo Island in South Australia (Fleay, 1980, Grant 2007) (Fig. 1). Although the platypus is considered by the IUCN to be a 'species of least concern' (Lunney *et al*., 2008), it is potentially vulnerable owing to its dependence on water bodies, from which it must obtain its food. There is some evidence of decline in abundance and restriction or fragmentation of distribution in a number of catchments, particularly in urban and agricultural landscapes (Serena *et al*., 1998; Otley, 2001; Grant, 2007).

The platypus is regarded as a single species across its geographical distribution despite some morphological and behavioural variation (Grant, 2007). In particular, body size increases substantially from north to south and in streams flowing inland from the Great Dividing Range on the Australian mainland (Grant & Temple-Smith, 1983; Akiyama, 1998). However, individuals from King Island are smaller than their Tasmanian neighbours (Akiyama, 1998). Recapture and radio-tracking data show considerable mobility by adults and much more dispersal by juvenile platypuses compared with adults (Grant, 2007), providing a potential avenue for gene flow between geographical areas and river systems. Molecular genetics is an important tool for elucidating these

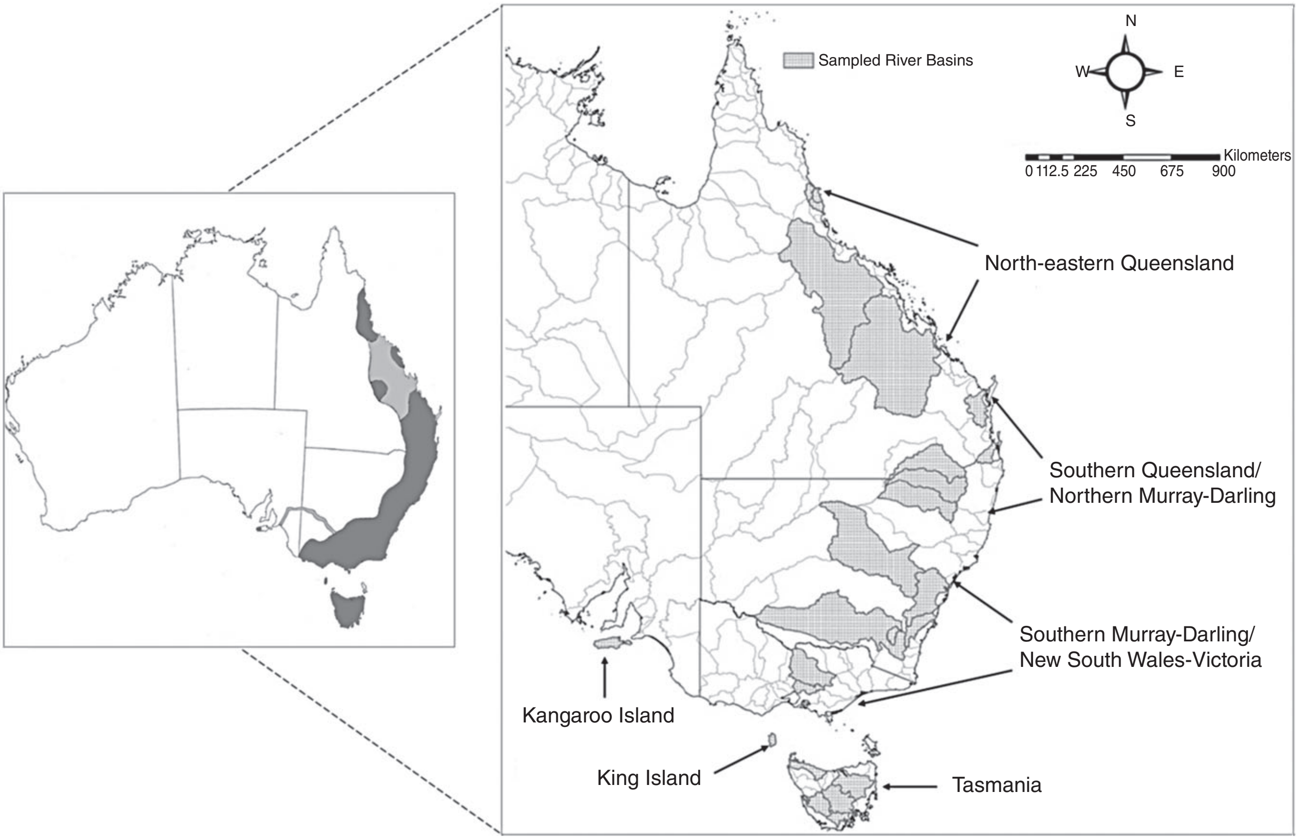


Figure 1 Left: map of Australia showing platypus distribution. Light shading indicates areas of sparse distribution, absence of records or presence of transient animals only (sourced from Grant, 2007). Right: map of eastern Australia showing drainage basins in grey from which samples were obtained.

aspects of a species' biology where conventional field techniques cannot.

There have been few genetic studies on the platypus to date. An early study using mitochondrial restriction-fragment length polymorphisms and a limited number of individuals (Gemmell *et al.*, 1992), showed some geographic partitioning between neighbouring populations in a southern New South Wales river basin (Gemmell *et al.*, 1992). Analyses of microsatellites (Akiyama, 1998; Furlan *et al.*, 2010) and nuclear LINE-2/Mon-1 elements (Warren *et al.*, 2008) suggested a degree of genetic divergence between Tasmanian and Australian mainland platypuses. However, it remains unclear when this divergence occurred. In addition, the patterns of genetic structure/substructure and relationships across mainland Australia are at present poorly understood for a species showing an immense latitudinal range (between about 16°S and 43°S), which might have promoted diversity and divergence. The current study investigates the phylogenetic relationships and levels of genetic differentiation between river systems/basins across the species' distribution. We analysed the entire mtDNA cytochrome *b* gene and flanking tRNAs, as well as a portion of the control region from specimens from mainland Australia, Tasmania, and King and Kangaroo Islands.

Methods

Sample collection

Toe-web biopsies and hair samples (n = 286) were opportunistically collected from platypuses in various locations during other studies (Akiyama, 1998; Grant, 2004, 2006; Kolomyjec *et al.*, 2009). The samples used here represent 38 stream systems (rivers, creeks, dams and lakes) of 22 river basins across the distribution of the platypus (Tables S1 and S2).

DNA extraction, amplification and sequencing

DNA was extracted from platypus tissue samples using the QIAamp©Mini Kit (QIAGEN, Doncaster, Australia). Primers were designed from the platypus mitochondrial genome sequence X83427 (Janke *et al.*, 1996). Two mtDNA fragments of 1.8 kb (control region) and 1.3 kb (cytochrome *b* and portions of the flanking tRNA-Glu and tRNA-Thr) from 74 platypuses, representing most of the sampling locations, were amplified using the following primer pairs, respectively: L15418-(5′-CCATCAACTCCCAAAGCTGA-3′) and H50-(5′-GCTCGGGTTCAGTTACGAAT-3′); and L14041-(5′-GGAGAAGGTTTAGAAGCGAAAGCCA-3′) and-H15398-(5′-TCTGAGGCCTTAGGAGGAAGTTTCA-3′). These fragments were used for phylogenetic inference and estimation of divergence times. Of the 1.8 kb control region sequence, only the first 580 bp could be used in analyses. This was because of the presence of two heteroplasmic tandem repeat motifs towards the 3′ end (Janke *et al.*, 1996). To allow a more detailed analysis of phylogeographic patterns, mtDNA control region sequences were obtained from an additional 212 individuals from the river basins described in Tables S1 and S2. Thus 286 sequences were used for this component of the study. A published sequence (X83427), from the Goulburn River in Victoria (Janke *et al.*, 1996), was added to the dataset.

Most of the PCRs reported here (Appendix S1) were undertaken at the University of Sydney. Sequencing of the first 580 bp of the mtDNA control region in 286 platypuses and the entire cytochrome *b* (1140 bp) and the two flanking tRNA genes (88 bp) in 74 of these specimens was performed as described in Appendix S2. The mtDNA haplotype sequences generated in the current study have been deposited in GenBank (accession numbers HQ379855 through HQ379936). Data from one Tasmanian and two mainland platypuses were excluded from the analyses (footnote to Table S1). Consequently, only 74 concatenated and 284 control region sequences were used in the analyses described below.

Phylogenetic inference

Sequences were aligned using CLUSTAL X (Larkin *et al.*, 2007). One site containing an indel was removed prior to phylogenetic analyses. Two echidna sequences (*Tachyglossus aculeatus* NC_003321; *Zaglossus bruijni* NC_006364) were included for the purpose of rooting trees and for age calibration (described below). Bayesian phylogenetic analysis was performed using a concatenated alignment (1808 bp) of the mitochondrial sequences from 74 platypuses, representing most of the sampling locations in the current study (Tables S1 and S2). To test for potential conflict in phylogenetic signals between cytochrome *b* (1140 bp) and the control region (580 bp), a partition-homogeneity test was conducted using the software PAUP* version 4.0b10 (Swofford, 2010). This test was performed using a heuristic parsimony search with 1000 replicates.

The phylogeny and divergence times of the concatenated mtDNA and separate cytochrome *b* and control region sequences from 74 platypuses were co-estimated using the Bayesian phylogenetic software *BEAST 1.5.4 (Drummond & Rambaut, 2007). The HKY+G model of nucleotide substitution was chosen by comparing values of the Bayesian information criterion, calculated using MODELGENERATOR (Keane *et al.*, 2006); models incorporating a proportion of invariable sites were disregarded. Rate heterogeneity among sites was modelled using a discrete gamma distribution with six rate categories. Owing to the generally intra-specific nature of the dataset, we employed a strict molecular clock. The position of the root was fixed by enforcing mutual monophyly on the in-group (platypuses) and out-group (echidnas). The constant-size coalescent prior model was selected and specified for the in-group (platypuses) as described in Appendix S3. A uniform prior was placed on the out-group branches (echidnas) and the branch joining the out-group and in-group, following previous implementations of this approach (Ho *et al.*, 2008). Estimates of the posterior distribution were obtained via Markov chain Monte Carlo sampling as described in Appendix S3.

Divergence time and mutation rate estimates

To obtain estimates of rates and divergence times, it is necessary to include some form of independent calibration information. Owing to the paucity of fossil evidence for the echidna and platypus lineages, we chose to employ a secondary calibration based on a recent molecular estimate (Phillips, Bennet & Lee, 2009). This estimate was obtained using seven fossil-based calibrations on a multi-gene dataset containing 14 vertebrate taxa, and suggested that the platypus and echidna diverged between 19 and 38 Ma, which is consistent with other published estimates (Appendix S4). We modelled their molecular estimate using a normal distribution with a mean of 32 Ma and a standard deviation of 7.14 Ma (Ho & Phillips, 2009) to estimate the upper limit of divergence for the most recent common ancestor (MRCA) of modern platypuses. This was specified as the prior distribution of the age of the root node, representing the divergence between echidnas and platypuses. To alleviate potential biases in date estimates (Appendix S4) caused by incomplete purifying selection (Ho *et al.*, 2005), we repeated our phylogenetic analysis using only the third codon sites of cytochrome *b*. The TrN+G substitution model was selected by comparing values of the Bayesian information criterion. All other settings were the same as those used in the analysis described above.

To investigate the likelihood of the mtDNA divergence of Australian mainland and Tasmanian platypuses being the result of the last geographical isolation between these two regions, a further analysis to estimate the mtDNA mutation rate was performed using this biogeographic hypothesis to inform the age calibration (described in Appendix S5).

Phylogeographic analysis

In order to assess the intraspecific relationships between individuals and the level of genetic structure between river basins, a dataset consisting of mtDNA control region (580 bp) sequences from 284 platypuses, including the 74 sequences described above and the published sequence (X83427), was analysed. The control region was chosen over cytochrome *b* for this purpose because of its higher mutation rate, providing greater resolution for intraspecific analyses. Median-joining network (MJN) analyses were performed on this dataset using

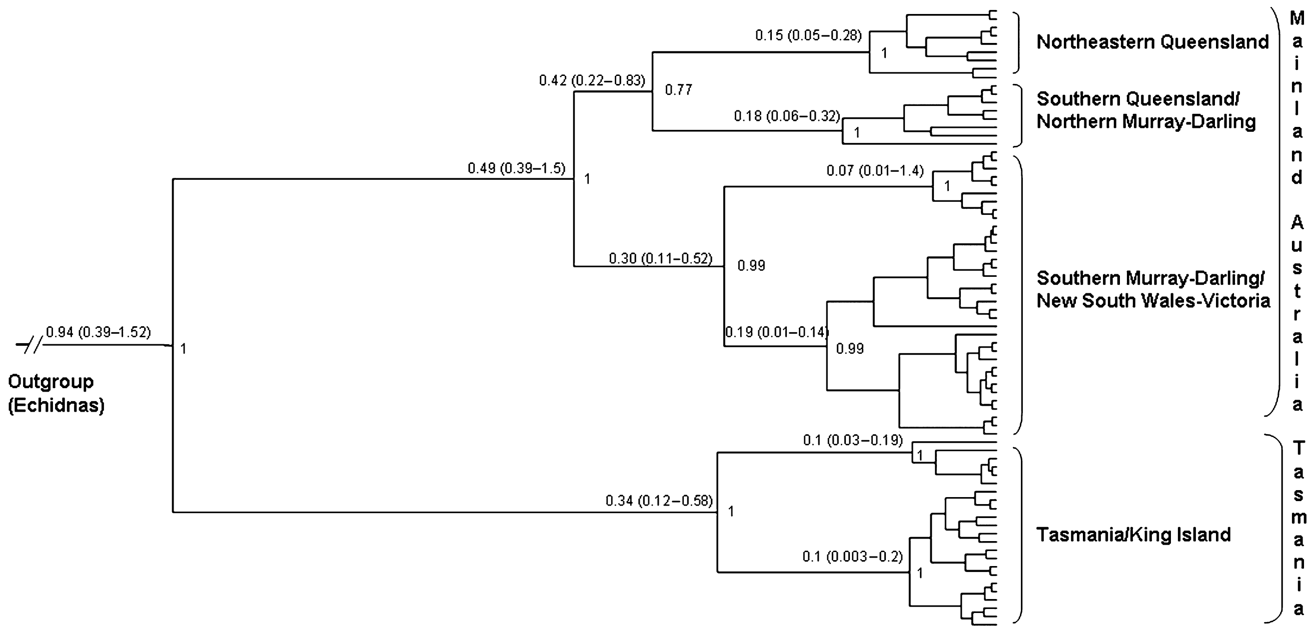


Figure 2 Bayesian phylogenetic tree of concatenated mtDNA sequences from 74 platypuses from across eastern Australia, including Tasmania and King Island, and an introduced population on Kangaroo Island. The right-most brackets show the broad geographical origin of platypus sequences while others indicate the major split between Australian mainland and Tasmanian platypuses. Clades were defined on the basis of their monophyletic groupings and posterior probabilities. Naming of clades was based on their broad geographical origin as described in Fig. 1. Divergence time estimates in Ma with 95% credibility intervals in Ma (in brackets) are on branches. Posterior probabilities are provided immediately after nodes. Divergence time estimates using cytochrome *b* are shown in Fig. S1. The maximum limit for the divergence between the short-beaked (*Tachglossus aculeatus*) and long-beaked (*Zaglossus bruijni*) echidnas was 3.3–3.8 Ma.

the NETWORK 4.1.1.2 program (Bandelt, Forster & Rohl, 1999) with the standard settings.

Analysis of molecular variance

Control region sequences from 254 platypuses were used for analysis of molecular variance (AMOVA) using ARLEQUIN 3.11 (Excoffier, Laval & Schneider, 2005) using 10 000 permutations and a significance level (P) of 0.05. The rest of the sequences ($n = 33$) were excluded as their sampling location information was incomplete. To assess the level of differentiation, the 37 sample locations (populations) were grouped into 22 river basins (groups). Higher hierarchical groups were also used, one constraining river basins into drainage divisions and the other into more broad-scale geographic areas based on the clades and haplogroups inferred from the Bayesian and MJN analyses. The purpose of these analyses was to assess whether these high-level groupings are associated with significant levels of differentiation.

As some proportion of the samples analysed in the current study were also used in published population analyses using nuclear LINE-2/Mon-1 retrotransposons (Warren *et al.*, 2008) and microsatellites (Kolomyjec *et al.*, 2009), we attempted to identify the common patterns among all of these datasets where possible.

Results

Phylogenetic analysis of concatenated sequences

Numbers of haplotypes in concatenated and individual cytochrome *b* and control region are explained in Fig. S1. Bayesian phylogenetic analyses of concatenated mtDNA sequences from 74 platypuses show a number of clades, most of which group in concordance with their overall geographic origin (Fig. 2). Although cytochrome *b* and the control region apparently show differences in the major split of the platypus tree (Fig. S2), the partition-homogeneity test shows that the two partitions do not significantly differ ($P = 0.117$) in the information they provide. Consequently, these mtDNA partitions were concatenated for phylogenetic analyses. What is clear, however, is that the control region partition has more homoplasy. The consistency indices for parsimony-informative sites in the cytochrome *b* gene and control region are 0.89 and 0.56, respectively.

Bayesian analyses of 74 concatenated mtDNA sequences show that platypuses formed two major clades, one representing mainland Australia and the other Tasmania, both with posterior probabilities of 1.00 (Fig. 2). These two major clades are differentiated by 21 diagnostic sites (Fig. S1), 17 of which are located within cytochrome *b*. Australian mainland platypuses split into two subclades, one consisting of specimens from the North-eastern Queensland, Southern Queensland/Northern Murray-Darling areas and the other consisting of the Southern Murray-Darling/New South Wales-Victoria region (Fig. 2). Each of these subclades was divided into a further two with high support values. Two well-supported subclades were also observed within Tasmania, showing apparently no geographical correlation except that mtDNA sequences from platypuses from King Island clustered within one of these subclades. However, it is possible that the lack of information about the specific origin of many of the Tasmanian samples impedes the recognition of any geographical pattern.

Upper age limits for the most recent common ancestor of modern platypuses

Using the concatenated mtDNA dataset and the third codon sites of cytochrome *b*, estimates of the upper limit for the MRCA of modern platypus major clades (mainland Australia/Kangaroo Island vs. Tasmania) are 0.94 and 0.70 Ma, respectively (Fig. 2, Fig. S2), with 95% credibility intervals (CIs) of 0.39–1.52 Ma and 0.10–1.46 Ma, respectively. This maximum age limit falls between the two biochronological epochs (Middle Pleistocene and Early Pliocene) for the earliest distribution of modern platypuses (Musser, 1998; Long *et al.*, 2002).

Based on the estimates from the concatenated sequences, Australian mainland platypus clades appear to have diverged from their most recent common ancestor about 0.49 Ma (95% CI: 0.39–1.50 Ma), while divergence within Tasmania could have occurred about 0.34 Ma (95% CI: 0.12–0.58 Ma). However, cytochrome *b* sequences alone suggest that these events occurred later (0.21 and 0.22 Ma, respectively), with a similar 95% CI of 0.02–0.44/0.48 Ma (Fig. S2). North-eastern Queensland and Southern Queensland-Northern Murray Darling subclades (Fig. 2) diverged from their most recent common ancestor ~0.42 Ma, with a 95% CI of 0.22–0.83. Estimates also suggest that further divergence within the former subclade occurred ~0.15 Ma (95% CI: 0.05–0.28).

Genetic patterns and shared haplotypes

The 47 haplotypes of the control region dataset (580 bp) observed among the 284 individuals grouped according to their geographical origin, most of them in concordance with their river basin (Fig. 3, Tables S1 and S2). There were four plausible major haplogroups, which is consistent with the clades in the estimated phylogeny shown in Fig. 2. Twenty-eight control region haplotypes were observed among the Australian mainland/Kangaroo Island platypuses (Fig. 3, Table S1), while 19 were observed among specimens from Tasmania/King Island. Control region haplotypes from mainland Australia formed groups that showed strong correspondence with their river basin origins (Fig. 3).

As one of the key findings of this study, the North-eastern Queensland haplogroup, representing platypuses from the Burdekin, Johnstone, Barron and Mulgrave-Russell Rivers, share 11 mutations, seven of which are diagnostic for this group (Fig. S1). In addition, 15 out of the 22 specimens from this haplogroup show a single insertion ('A') in the control region.

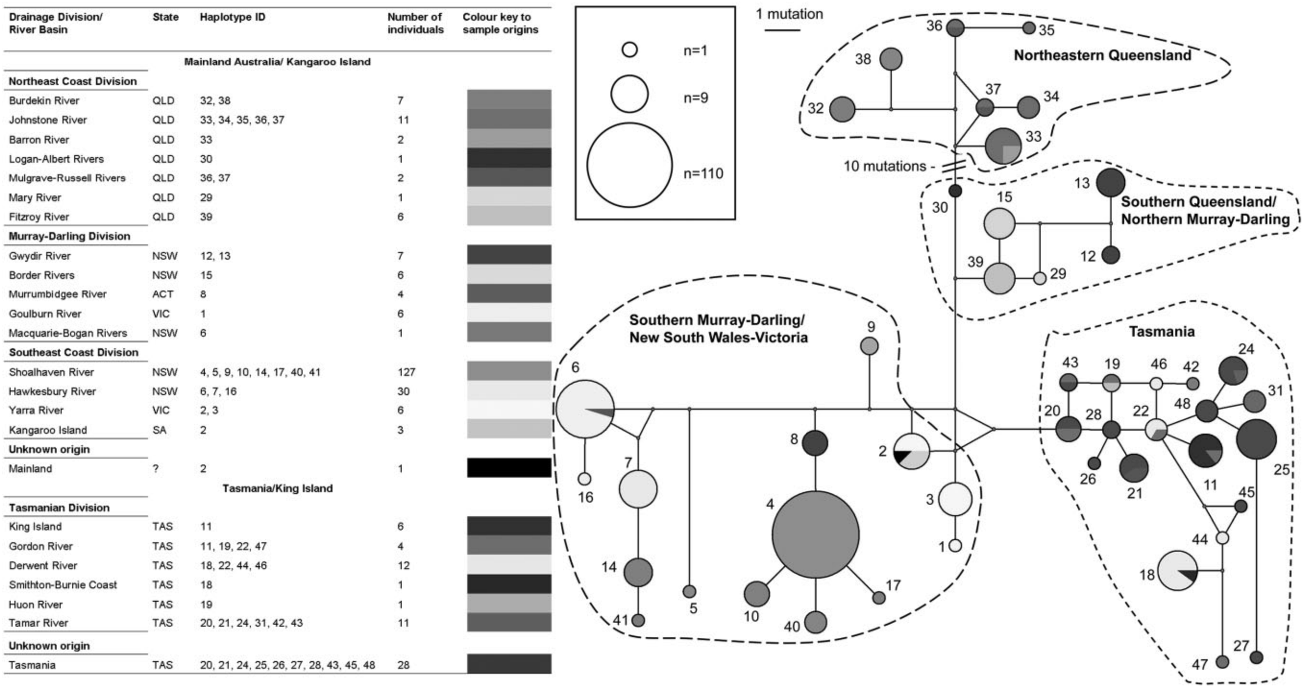
Some control region haplotypes were shared by samples from more than one river basin. Haplotypes 33, 36 and 37 occurred in the Johnstone and Barron Rivers, and haplotypes 36 and 37 in the Johnstone and Mulgrave-Russell Rivers in Queensland (North-eastern Australia). Haplotype 6 was found in the Macquarie-Bogan and Hawkesbury rivers in New South Wales and haplotype 2 in the Yarra River in Victoria and the Rocky River on Kangaroo Island. This last haplotype was also found in captive platypuses from the Warrawong Wildlife Sanctuary, which is consistent with records of their origins. The Kangaroo Island platypus population is almost certainly descended from eight pairs transferred from the Healesville area of Victoria to the Rocky (five pairs) and Breakneck (three pairs) rivers in the early 1940s (Anonymous, 1941, 1946; Fleay, 1941). Two males and a female were transferred from the Wynyard area of Tasmania to the Rocky River in 1929 (Anonymous, 1929), but we did not detect their descendants in our samples.

Among river basins within Tasmania, five shared haplotypes (numbers 11, 18, 19, 22 and 24) were observed (Fig. 3). Platypuses from King Island and those from the Gordon River in Tasmania shared haplotype number 11. This suggests a genetic link between these populations before they were isolated by the last separation of Tasmania and King Island by sea–level rises ~12 000–13 000 years ago (http://sahultime.monash.edu.au/explore.html). Ten haplotypes representing 28 sequences from unknown specific sampling location were grouped with the Tasmanian material, consistent with the overall geographic origin information for those samples.

Genetic structure of platypuses

AMOVA results (Table 1) show that there is significant divergence between river basins compared with variation within populations ($F_{ST} = 0.892$, $P < 5 \times 10^{-5}$). Seventy-eight percent of the variation occurred between the river basins, 10% among populations (sampling locations) within river basins and 10% within populations. When the river basin populations were clustered at the drainage division level, there was still a significantly high level of divergence observed ($F_{ST} = 0.902$, $P < 5 \times 10^{-5}$). However, only 40% of the variation occurred between these drainage divisions, 49% among river basins within the divisions and 9% within river basins. The level of differentiation increased to 56% when the river basins were grouped into broader geographical areas ($F_{ST} = 0.912$, $P < 5 \times 10^{-5}$) (Table 1).

Our mtDNA study and a previous analysis of nuclear LINE2/Mon-1 elements (Warren *et al.*, 2008) show concordance in their clustering within Australian mainland or Tasmanian platypus groups (Table S3). However, further comparison within mainland Australia was not possible because of the genetic admixture described above. In contrast,



Drainage Division/ River Basin	State	Haplotype ID	Number of individuals	Colour key to sample origins
		Mainland Australia/ Kangaroo Island		
Northeast Coast Division				
Burdekin River	QLD	32, 38	7	▨
Johnstone River	QLD	33, 34, 35, 36, 37	11	▨
Barron River	QLD	33	2	▨
Logan-Albert Rivers	QLD	30	1	▨
Mulgrave-Russell Rivers	QLD	36, 37	2	▨
Mary River	QLD	29	1	▨
Fitzroy River	QLD	39	6	▨
Murray-Darling Division				
Gwydir River	NSW	12, 13	7	▨
Border Rivers	NSW	15	6	▨
Murrumbidgee River	ACT	8	4	▨
Goulburn River	VIC	1	6	▨
Macquarie-Bogan Rivers	NSW	6	1	▨
Southeast Coast Division				
Shoalhaven River	NSW	4, 5, 9, 10, 14, 17, 40, 41	127	▨
Hawkesbury River	NSW	6, 7, 16	30	▨
Yarra River	VIC	2, 3	6	▨
Kangaroo Island	SA	2	3	▨
Unknown origin				
Mainland	?	2	1	■
		Tasmania/King Island		
Tasmanian Division				
King Island	TAS	11	6	▨
Gordon River	TAS	11, 19, 22, 47	4	▨
Derwent River	TAS	18, 22, 44, 46	12	▨
Smithton-Burnie Coast	TAS	18	1	▨
Huon River	TAS	19	1	▨
Tamar River	TAS	20, 21, 24, 31, 42, 43	11	▨
Unknown origin				
Tasmania	TAS	20, 21, 24, 25, 26, 27, 28, 43, 45, 48	28	▨

Figure 3 Median-joining network of mtDNA control region haplotypes from platypuses from 37 locations representing 22 river basins and four broad geographical areas (dashed lines which also denote the four proposed haplogroups). River basin and drainage divisions were assigned to the sampling locations according to map resources available at the Geoscience Australia website (http://www.bom.gov.au/hydro/wr/basins/basin-hi_grid.jpg), and details are provided in Supplementary Material Tables S1 and S2. The first two columns of the table indicate the overall geographic origin of the specimens used. The third column (Haplotype ID) provides the haplotype number and frequencies of specimens used from each river basin (Supplementary Material Table S1). The haplotype number is shown next to nodes. Each node size is proportional to the frequency of the mtDNA control region haplotype. Branch lengths between haplotypes are proportional to the number of mutations except for the branch between haplotype numbers 30 and 33, which has been shortened as indicated by the strokes. Some sequences are labeled as unknown because the sampling location information was incomplete (although it was known whether they came from the Tasmania or the mainland). As Kangaroo Island specimens originated from an introduced group from Victoria, they were grouped within the Southern Murray-Darling, New South Wales and Victoria rather than forming a separate geographical division.

the platypus population differentiation calculated using microsatellite loci between adjacent Shoalhaven and Hawkesbury river systems (Kolomyjec *et al*., 2009) is consistent with the sorting of mtDNA haplotypes from these two systems within the Southern Murray-Darling/New South Wales-Victoria cluster proposed in the current study. Basin-specific haplotypes that are similar to others from a different but nearby river basin (e.g. numbers 14 and 41 from the Shoalhaven River are similar to number 7 from the Hawkesbury River) may indicate relatively recent common ancestry, or possibly dispersal events (as indicated by microsatellite data – Kolomyjec *et al*., 2009).

Discussion

Higher-level phylogeographic structure and divergence time

Here we have demonstrated the usefulness of cytochrome *b* and control region sequences in studying the phylogeography of the platypus. The basal split between Australia and Tasmania/King Island populations is consistent with published data from microsatellites (Akiyama, 1998; Furlan *et al*., 2010) and LINE2/Mon-1 elements (Warren *et al*., 2008). Our analyses reveal that the split between Tasmanian and mainland Australian platypuses could have occurred about ~0.7–0.94 Ma. Based on these estimates, and considering the unrealistic estimate of the mutation rate that results from calibration using the last land bridge between mainland Australia and Tasmania (~13 500–14 000 years) (Appendix S5), given the oldest date for the presence of fossils of modern platypuses in Tasmania (~29 000 years) (Marshall, 1992) and the species' early presence on the mainland (>100 000 years) (Musser, 1998), we propose that the divergence of the two major clades of platypuses occurred on mainland Australia some time during the Early and Middle Pleistocene before they colonized Tasmania. However, other possible scenarios cannot be excluded, for example that those lineages could have been in those two regions since ~0.7–0.94 Ma. Under a mainland divergence scenario, additional questions remain, including those concerning the

Table 1 Analysis of molecular variance based on the mitochondrial control region sequence of platypuses from 37 sampling locations across Australia

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among 22 river basins					
Among groups	21	818.023	3.93720*	78.93	F_{CT}:0.78934
Among populations within groups	16	28.162	0.51337*	10.29	F_{ST}:0.89226
Within populations	217	116.619	0.53741*	10.77	F_{SC}:0.48856
Total	254	962.804	4.98799		
Among 4 drainage divisions[a]					
Among groups	3	455.073	2.26089*	40.97	F_{CT}:0.40973
Among populations within groups	34	391.112	2.71971*	49.29	F_{ST}:0.90261
Within populations	217	116.619	0.537415*	9.74	F_{SC}:0.83500
Total	254	962.804	5.51802		
Among 4 overall geographic areas[b]					
Among groups	3	527.049	3.47820*	56.79	F_{CT}:0.56789
Among populations within groups	34	319.136	2.10918*	34.44	F_{ST}:0.91226
Within populations	217	116.619	0.53741*	8.77	F_{SC}:0.79694
Total	254	962.804	6.12480		

*$P < 5 \times 10^{-5}$.

[a] Groups: North-east Coast, Murray-Darling, Southeast Coast and Tasmanian Divisions.

[b] Groups: North-eastern Queensland, Southern Queensland/Northern Murray-Darling, Southern Murray-Darling/New South Wales-Victoria and Tasmania.

absence of shared (ancestral) haplotypes between Tasmania and the mainland. Due to various factors described in Appendix S6, it is possible that the ancestral Tasmanian mtDNA haplotype was originally widely distributed including in southern Australia and Tasmania. However, perhaps owing to isolation, merging and interbreeding of populations, it was erased from the mainland. However, a definitive answer to this will require further surveys of river basins in this part of Australia, as well as analysis of ancient DNA from across the platypus' range to identify any links to the Tasmanian genetic profiles in southern mainland Australia.

Genetic structure and shared haplotypes

Platypuses show a genetic structure that is generally concordant with the river basins, drainage division/broad geographical areas based on AMOVA using mtDNA control region haplotypes. However, the fact that Bayesian and MJN analyses show that the individuals from some drainage divisions from southern Australia show paraphyletic relationships (e.g. the Murray-Darling and the North-east coast Divisions) or do not segregate clearly as separate clades or haplogroups (e.g. Murray-Darling and South-east coast Divisions) suggest that these particular types of hierarchical geographic divisions do not necessarily reflect overall discrete units for the platypus distribution. In contrast, the four broad geographical areas and the river basins level appear to be more useful for recognizing overall divisions and discrete units, respectively. Observations from microsatellites indicate possible isolation by distance between systems, but limited dispersal of platypuses overland may have played a role in differentiation of populations at the river basin level (Kolomyjec *et al*., 2009). The fact that most of the river basins contain specific mtDNA haplotypes, and that there is a limited number of shared haplotypes between those systems, reveals that this level of phylogeographic structure might be useful in elucidating platypus origins and dispersal.

The North-eastern Queensland haplogroup appears to be a distinct evolutionary unit given its genetic differentiation from the rest of the haplogroups. Interestingly, this haplogroup is geographically separated by a discrete gap from the rest of the platypus' range (Grant, 2007). Although it is not clear what particular geological, geographical or climatic events could have influenced this, modelling studies of distribution of platypuses based on habitat suitability since the Last Glacial Maximum (S. Kolomyjec, unpubl. data), as well as our divergence time estimates, suggest that the Northeastern Queensland clade has been isolated for a long period of time. In this scenario and based on the current phylogenetic analyses, it is possible that Northern Queensland and Tasmanian populations became sufficiently isolated that they may not have made a significant genetic contribution to the southern Australian mainland populations.

The interpretation of shared haplotypes presents some challenges, as it is unclear whether they are the result of gene flow, convergence and/or retention of ancestral haplotypes. If gene flow is the cause, either geographical proximity or environmental conditions that facilitate dispersal might be expected. In most of the cases of shared haplotypes (e.g. haplotype 6 found in the Macquarie-Bogan and Hawkesbury Rivers), the river basins are adjacent. Platypus, and especially juveniles, are known to disperse widely (Grant &

Temple-Smith, 1998; Grant, 2004, 2007), thus creating the potential for gene flow. Overland dispersal by platypuses is believed to be extremely limited due to the species' poor tolerance of high ambient temperatures (Grant & Dawson, 1978), the higher metabolic cost and slow speed of movement on land (Bethge, Munks & Nicol, 2001; Fish *et al.*, 2001; Grant, 2007), and higher predation risks (Rakick *et al.*, 2001). Microsatellite studies, however, have suggested overland movement of migrants between river systems (Kolomyjec *et al.*, 2009). This would explain the observed shared alleles between populations from adjacent river systems, and, in particular, for the Hawkesbury and Shoalhaven river basins (Kolomyjec *et al.*, 2009). However, the time frame of such dispersal and extent of resultant gene flow is still unknown. Field studies involving mark–recapture, tracking and genetic parentage analysis, along and between adjacent or adjoining rivers, may in future allow resolution of these points. Based on the current study, we propose that shared mtDNA haplotypes, at least for those adjacent river systems in the mainland, could have been result of gene flow. However, alternative explanations, such as the retention of ancestral mtDNA haplotypes and incomplete sorting among populations, cannot be excluded.

Conclusions and recommendations for future work

Here we have contributed to an understanding of genetic differentiation between Australian mainland and Tasmanian/King Island platypuses and of the genetic structure at the river basin level on mainland Australia. This provides background information to assist in defining taxonomic and/or evolutionary units of relevance for conservation and management of this species. Of particular interest is that a divergent subgroup from the North-eastern Queensland region has been identified using mtDNA. This genetic structure and published morphological and behavioural variation suggest that the platypus has been subject to different selection pressures in various parts of its current distribution. Further studies of nuclear DNA, chromosomal, reproductive, ecological and morphological data will be required in refining evolutionary and management units in the species. Finally, the mtDNA sequences from the present study provide an alternative framework for museums and zoos to assess platypus specimens of unknown origin with relevance for DNA barcoding and captive breeding programs.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Variable nucleotide sites of concatenated mtDNA sequences (1808 bp) presented for 74 platypuses.

Figure S2. Maximum-clade-credibility trees obtained by Bayesian phylogenetic analysis of separate cytochrome *b* and control region sequences.

Table S1. Summary of the mtDNA control region haplotypes found among 284 platypuses.

Table S2. Individual details of the mtDNA control region haplotypes found among 284 platypuses.

Table S3. Consistent patterns of clustering between the current mtDNA control region study and published literature of samples used in this and other studies.

Appendix S1. PCR conditions.

Appendix S2. Sequencing primers and sequencing quality control.

Appendix S3. Selection of an appropriate demographic model.

Appendix S4. Considerations on divergence time estimates.

Appendix S5. Estimating the mtDNA mutation rate using the last period of separation between mainland Australia and Tasmania.

Appendix S6. Some considerations related to the proposed scenarios for the absence of shared (ancestral) haplotypes between Tasmania and the mainland.

Appendix S7. Acknowledgements.

Appendix S8. Supplementary references.

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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 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 subunits of larger evolutionarily significant units, which represent regions of divergence due to historical isolation (Moritz 1995, 1999; Palsbøll *et al*. 2007).

The platypus is a prototherian mammal endemic to the fresh waters 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 (Lunney *et al*. 2008). However, their inherent vulnerability cannot be overlooked. At both the global and national level, riverine systems are under constant threat of anthropogenic flow alteration and pollution (Kingsford 2000; Goudie 2006). At the local scale, both range reductions and expansions of platypuses have been reported (Grant 1998; Lintermans 1998; Lunney *et al*. 1998; Serena *et al*. 1998; Rohweder and Baverstock 1999; Otley 2001).

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 (Gemmell *et al*. 1992; Gemmell 1994; Akiyama *et al*. 1996; Akiyama 1998), 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; Tasmanian populations were 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

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population structuring and gene flow in platypus populations from two river catchments in the southern tablelands of New South Wales. For comparison, we also include data and analyses based on small samples from three river systems elsewhere in New South Wales. 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 (Richards 1986; Fish *et al*. 1997, 2000, 2001; Bethge *et al*. 2001; Rakick *et al*. 2001; Grant 2007; 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 (Fig. 1). These catchments were selected because of the large number of samples available (collected during other field studies) (Grant 2004, 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 the Australian Capital Territory). These three additional river systems, however, lack the sample

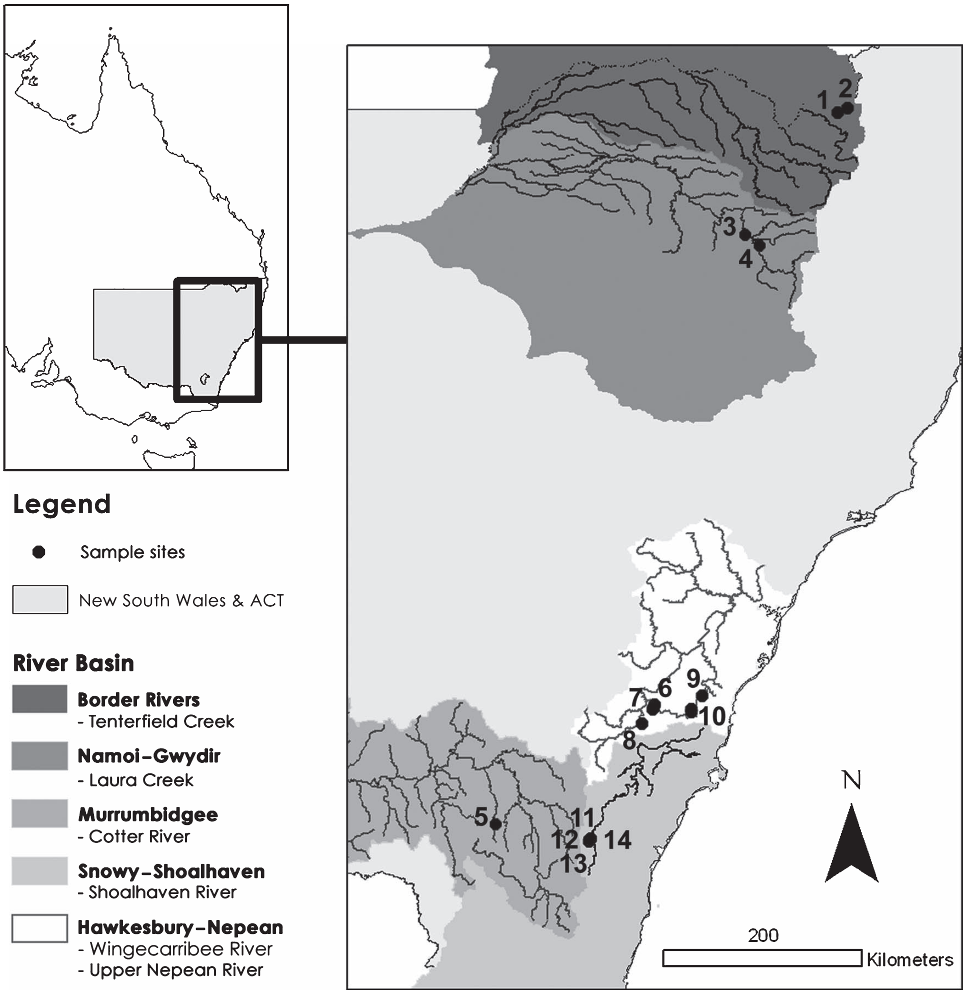

Fig. 1. Location of sample sites used in this study. River systems and their basins are shown to provide geographical context. Study sites: **1**, 1 specimen (–29.0250° 151.8667°; standard international datum: WGS 84); **2**, 1 specimen (–28.9852° 151.9504°); **3**, 2 specimens (–30.1373° 151.0250°); **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°).

sizes needed for detailed analysis but are useful in adding context to the main analysis.

Sampling and genotyping

DNA was extracted from toe-web biopsies (2 × 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 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 ver. 1.0 for this set of markers suggests a highly robust nature (Johnson and Haydon 2007*a*, 2007*b*).

Genetic diversity and data quality

Exact probabilities for Hardy–Weinberg (HWE) proportions were calculated in Genepop ver. 3.4 (option 1: dememorisation = 1000; batches = 100; iterations = 1000) while Cervus ver. 3.0.3 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 ver. 2.000 (1000 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 simply indicates the level of genetically distinct individuals undergoing sexual reproduction, 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). Misassignments 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 ver. 2.2 (admixture model with 100 000 burn-in period followed by 100 000 Markov Chain Monte Carlo repetitions), we tested whether animals from the different river systems grouped into distinct population clusters (Pritchard *et al*. 2000). Structure ver. 2.2 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 ver. 3.16 (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 HWE due to homozygote excess at several loci across populations (Table 2). Null alleles may be a possible cause of this deviation as Cervus ver. 3.0.3 did find possible evidence of null alleles, particularly in the Hawkesbury–Nepean population (Table 2). To investigate the influence of these null alleles on the

Table 1. Additional microsatellite loci from *Ornithorhynchus anatinus*

Reagent mixture for 15 µL PCR reaction: 5 p.m. primer (forward and reverse), 5 ng template DNA, 1.7 mM $MgCl^{2+}$, 400 µM dNTPs, 1.5 µL 10× 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′)[A]	Chromosome on which locus located	Repeat 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

[A] Forward primers were 5′-end-labelled with fluorochrome indicated.

Table 2. Detailed population genetics data

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 equilibrium (significant results in bold); Null allele frequency = calculated rate of null alleles. Monomorphic indicates that there was 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	N_a	H_o	H_e	F_{is}	HWE *P*-value	Null allele frequency
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	OA.01.TGTA	3	4	1.000	0.722	–0.385	0.609	ND
Cotter, ACT	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

(*continued on next page*)

Table 2. (*continued*)

Population	Locus	N	N_a	H_o	H_e	F_{is}	HWE *P*-value	Null allele frequency
	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

outcome and patterns detected, all analyses carried out on the dataset 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.

No globally significant linkage disequilibrium was detected between loci after sequential Bonferroni correction (Rice 1989) (Table 3). Expected heterozygosity (H_e) ranged from 0.413 to 0.723 across the sampled populations (mean = 0.575) (Table 2).

Pairwise F_{st} values between different river systems ranged from 0.018 to 0.152 (Table 4). The F_{st} calculated for comparison

Table 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	0.3000										
OA.03.GTAG	0.0546	0.6447									
OA.05.GTAGG	0.0856	0.3212	0.1939								
OA.06.TAAC	0.4546	0.7265	0.4296	0.8849							
OA.06.TATATC	0.1174	0.4750	0.0273	0.3500	0.1265						
OA.07.GTTA	0.3159	0.0106	0.3992	0.8008	0.0280	0.0159					
OA.11.CCA	0.6106	0.7765	0.9038	0.2189	0.0015	0.0046	0.6053				
OA.12.TCCAT	0.3902	0.8008	0.1583	0.1318	0.5500	0.0121	0.0576	0.5099			
Platy04	0.8046	0.0614	0.0796	0.2053	0.9886	0.1561	0.9197	0.2394	0.3546		
Platy08	0.6682	0.5258	0.0356	0.0265	0.7909	0.8144	0.0099	0.9962	0.1629	0.7296	
Platy19	0.4546	0.6220	0.1530	0.2682	0.0379	0.4652	0.0030	0.4121	0.5091	0.3621	0.6280

Table 4. Pairwise F_{st} values at microsatellite loci among sample sites
F_{st} values significant at the $\alpha = 0.05$ level are shown in bold

River system	A[A]	B[A]	C	D	E[A]
A – Cotter (ACT)[A]	0.00000				
B – Gwydir[A]	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[A]	0.15173	0.03710	0.03259	**0.09744**	0.00000

[A] Very low sample sizes, values added for context only.

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 5).

Population structure and gene flow

The results of the AMOVA tests (Table 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 subcatchments, with some additional variance between individual sample sites (Table 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 proximity of the individual sample sites (Fig. 1).

Table 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		Shared alleles	Total alleles
	Hawkesbury–Nepean	Shoalhaven		
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 assignment (Fig. 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 ($\chi^2_{d.f.=1} = 0.405$, $P > 0.05$).

The most appropriate number of population clusters (K) based on the likelihood values as indicated by STRUCTURE was three (Fig. 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 (Fig. 3*B*).

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 ($r = 0.6115$, $P = 0.002$) and within the Hawkesbury–Nepean systems ($r = 0.7315$, $P = 0.02$). No significant pattern of isolation-by-distance was detected within the Shoalhaven river system ($r = 0.0633$, $P = 0.590$), but this may be because distance between sample sites was very small (Fig. 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

Table 6. Analysis of molecular variance
Results of two- and three-level AMOVA; significant *P*-values are shown in bold

	Molecular variance			*P*-value
	Among-river systems	Among-sample sites	Within-sample sites	
Hawkesbury–Nepean (five sample sites) versus Shoalhaven (four sample sites)	6%	3%	91%	**0.000**
Hawkesbury–Nepean versus Shoalhaven (no subdivision)	7%	–	93%	**0.001**
Hawkesbury–Nepean (two subcatchments[A], 5 sample sites)	8%	3%	89%	**0.001**
Hawkesbury–Nepean (two subcatchments)	10%	–	90%	**0.001**
Hawkesbury–Nepean (five sample sites)	–	7%	93%	**0.000**
Shoalhaven (four sample sites)	–	1%	99%	0.425

[A] Subcatchment A = sample sites 6, 7, and 8; subcatchment B = sample sites 9 and 10 (Fig. 1).

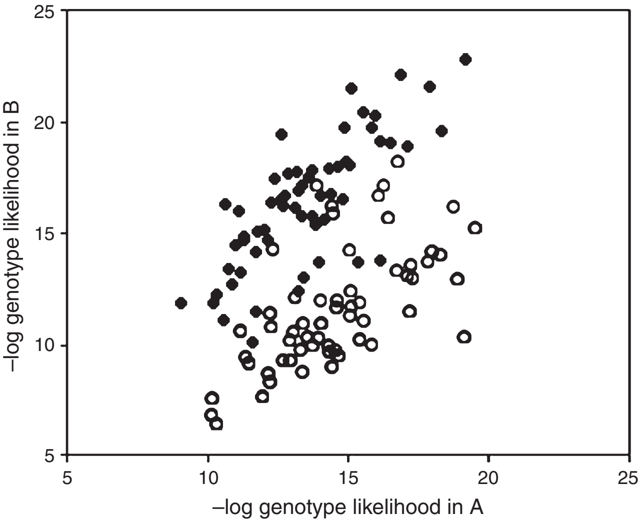

Fig. 2. Assignment plot of genotype likelihood values between the Hawkesbury–Nepean and Shoalhaven river systems. Each river system forms a distinct group with a narrow area of overlap, suggesting that the systems form distinct populations with some gene flow between them. Closed circles represent Hawkesbury–Nepean individuals and open circles represent individuals from the Shoalhaven river system.

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 substructuring, as indicated by the STRUCTURE analysis (Stow *et al*. 2006; Neves *et al*. 2008). 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 that 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 (Slatkin 1987; Bossart and Prowell 1998).

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 rapidly 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

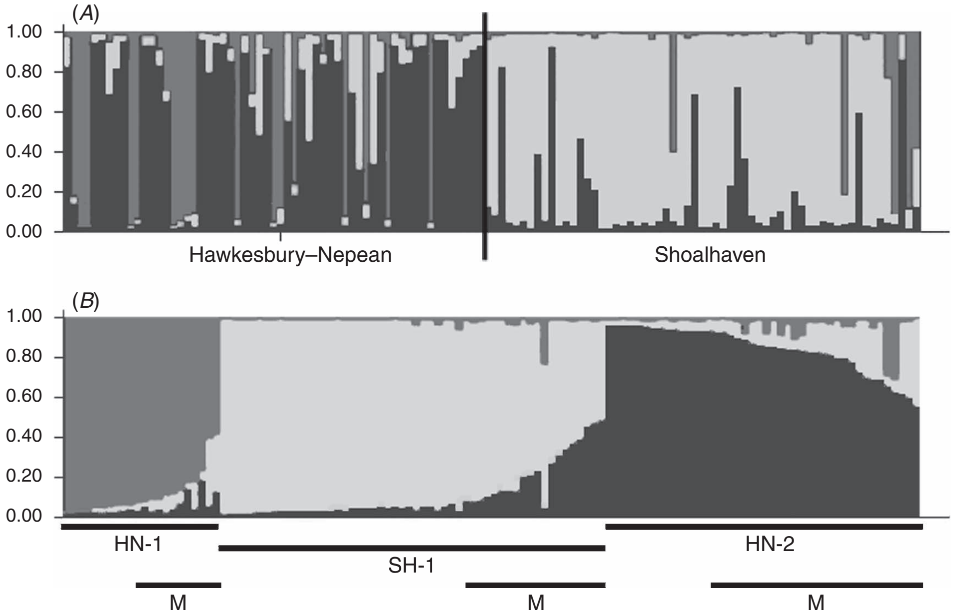

Fig. 3. STRUCTURE results. (*A*) Three population clusters (K) distributed across the two river systems. Each vertical bar represents an individual. The amount of each colour represents the proportion of each population cluster responsible for that individual's genotype. (*B*) The results from (*A*) sorted by population cluster, demonstrating the individual clusters and the individuals with mixed ancestry (M).

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; S. Kolomyjec, unpubl. data). 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 ($n_{\mathrm{m}} = 13$, $n = 120$) 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 ~1.5 km apart during normal conditions. These distances would, however, be increased during dry or drought conditions, decreasing the chance of intercatchment 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 1978*a*, 1978*b*), only being known to feed in the water (Grant 2007), the higher metabolic cost and slower overland movement (Fish *et al*. 1997, 2000, 2001; Bethge *et al*. 2001; Grant 2007) and higher predation risks (Richards 1986; Rakick *et al*. 2001; Grant 2007; 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 overland dispersal between catchments can occur and that it plays an important role in determining the population structure of the platypus.

Overland 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 (Fig. 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 that the AMOVA detected a significant amount of genetic variance between the subcatchments in the Hawkesbury–Nepean river system. However, the population clusters as detected do not correlate to the individuals taken from each subcatchment. Instead, their existence can logically be reconciled if bimodal dispersal via water and land-based movements is taken into account. The population clusters may represent subpopulations generated by the physical characteristics of the subcatchments 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 (Chaput-Bardy *et al*. 2008).

The results of our study supported our hypothesis. While able to disperse both along rivers and across land, adaptations for a semiaquatic life lead to greater gene flow along watercourses so that major catchments form the basis of regional population structuring, although further substructuring is possible depending on local geography. Despite higher than anticipated intercatchment 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 varying from the catchment to subcatchment levels. This means that to preserve the genetic diversity and evolutionary health within each management unit, entire catchments or subcatchments (depending on local geography) must be preserved in a condition usable by the platypus (Moritz 1995, 1999). To optimally preserve the local evolutionarily significant unit, 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 evolutionarily significant units known to exist in north and central Queensland (S. H. Kolomyjec, unpubl. data), the condition and topography (both natural and anthropogenic) of the land between catchments should also be considered.

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Conservation of the platypus, *Ornithorhynchus anatinus*: Threats and challenges

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The platypus (Ornithorhynchus anatinus) *is an Australian icon. It is an integral part of the biodiversity of many eastern Australian freshwater ecosystems and is protected by legislation in all States in which it occurs. Its conservation is of considerable importance not only because of its unique features, status and niche but also because it is the only living representative of a significant lineage of platypus-like animals with a 60 million year fossil history. As a result of its specific habitat requirements it is affected by many of the widely recognised threatening processes operating in Australian limnological systems. In spite of these threatening processes, the species has continued to inhabit and reproduce in considerably degraded environments. The present overall distribution of the platypus appears to be little different from pre-European times. There are, however, now almost certainly no naturally occurring populations in South Australia, where it once occurred, and its distribution has apparently shrunk in the lower reaches of the Murray and Murrumbidgee River systems in Victoria and New South Wales. Despite being considered common throughout its current distribution its abundance is not readily measured and therefore its future conservation status is not easily predicted. Several studies have reported fragmentation of platypus distribution within individual river systems. This has been attributed to poor land management practices associated with stream bank erosion, loss of riparian vegetation and channel sedimentation. There is currently also evidence for adverse effects of river regulation and impoundments, introduced species, poor water quality, fisheries by-catch mortality and disease on platypus populations, but none of these has been well studied. Investigations of these aspects of the species' biology and interaction with human activities are research priorities, while management priorities include the development and implementation of strategies aimed at reducing the effects of these human activities on the platypus and its habitat.*

Keywords: distribution, abundance, research, management

Introduction

The egg-laying mammals belonging to the Order Monotremata have a distribution restricted to the Australian mainland, Tasmania and Papua-New Guinea. The platypus, *Ornithorhynchus anatinus*, inhabits eastern river systems in Australia from around Cooktown in the north to Victoria in south and most, if not, all rivers in the southern island state of Tasmania. Although not found in the west-flowing rivers of northern Queensland, it occurs in the upper reaches of rivers flowing west from the Great Dividing Ranges in southern Queensland and in New South Wales and the streams draining the northerly slopes of these ranges in Victoria (Figure 1; Grant and Temple-Smith, 1998). The platypus is one of only two semi-aquatic mammals

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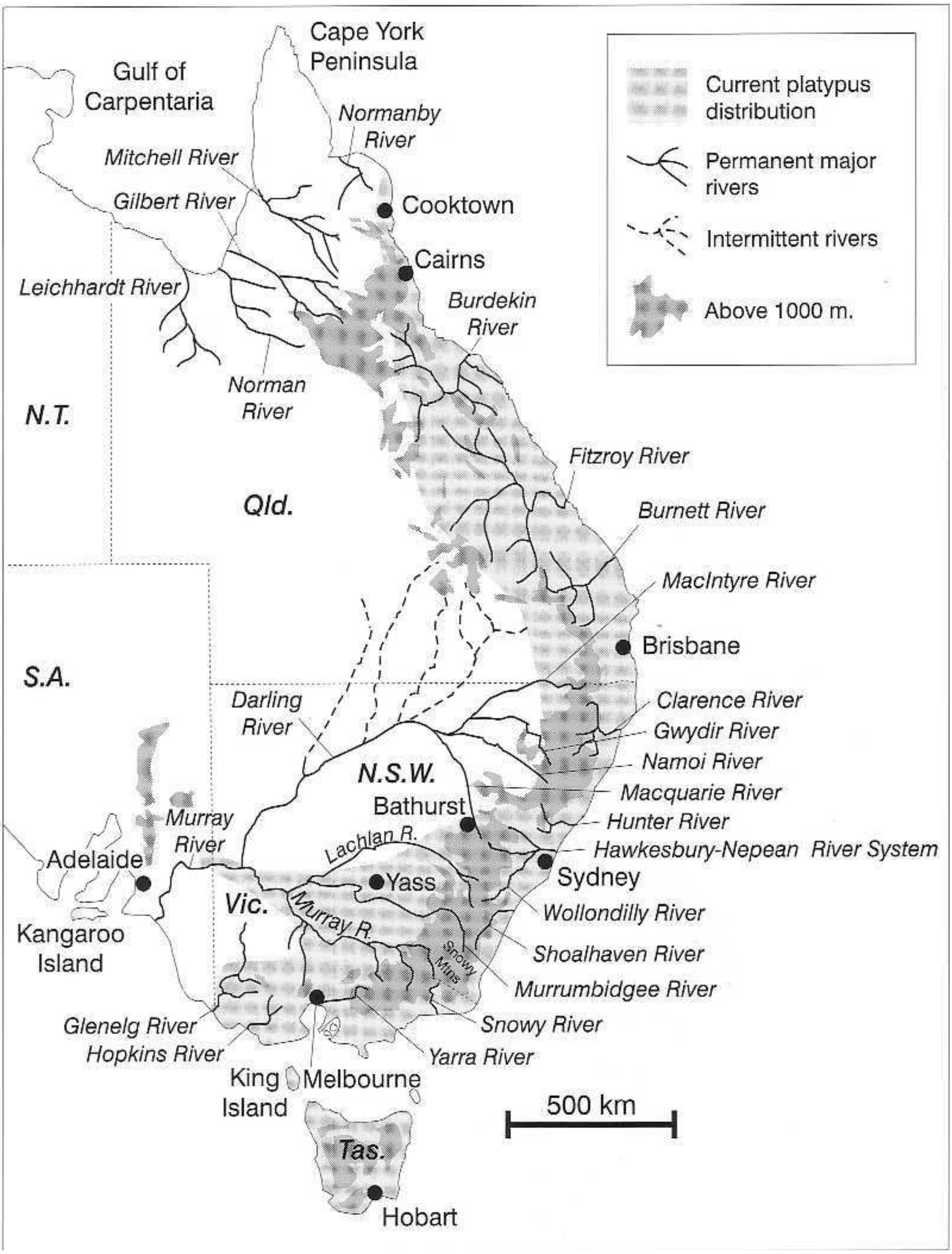

Figure 1. Distribution of the platypus in Australia (from Grant and Temple-Smith, 1998).

found in Australian freshwater systems, the other being the native eutherian water rat, *Hydromys chrysogaster*. The platypus appears to be the survivor of a more diverse platypus-like fauna within Australia and South America, with a fossil history dating back to around 62 million years (four species attributed to the Family Orithorhynchidae; Musser, 1999).

The platypus feeds predominantly on benthic invertebrates, appearing to be quite non-selective in its choice of food items (Grant and Temple-Smith, 1998).

It also requires the banks of water bodies for construction of its resting and nesting shelters (mainly burrows in earth banks) and so is dependent on both the riparian and aquatic environments of freshwater systems (Grant, 1995). As a result of this dual dependency the platypus is exposed to a range of threatening processes affecting Australian river systems (e.g. State of the Environment Advisory Council, 1996; NSW EPA, 2000). However, the species is currently not specifically categorized in terms of conservation status, nor is listed as *common* in State legislation, but is protected by legislation in all Australian States in which it occurs.

In Australia, poor land use management, especially in agriculture, urban development and forestry, directly impacts on the integrity of stream and riparian habitats on which the survival of the platypus depends (State of the Environment Advisory Council, 1996; Boulton and Brock, 1999; NSW EPA, 2000). These impacts are certainly not new to the Australian environment, being reported a short time after the first European settlement of the continent in 1788. The botanist George Cayley, traveling in a rural area of the colony of New South Wales in 1802, documented the erosion of banks and deposition of sediment in the river system, and attributed this to the unrestricted presence of cattle. In 1803 a General Order from Governor King in the colony of New South Wales stated that "from the improvident method taken by the first settlers on the sides of the Hawkesbury [River] and creeks in cutting down timber and cultivating the banks, many acres of grounds have been removed". As a result of this the Governor "hereby directed" that these practices should cease (Rosen, 1995). Unfortunately the Governor's order was ignored at that time and continued to be ignored during the establishment of towns and cities and as more land was cleared and subjected to a range of land use practices from then until the present day.

In spite of human-induced changes in stream and riparian systems it appears that the overall distribution of the platypus has remained, with few exceptions, much as it was in pre-European times (Grant, 1991, 1992a; Grant and Denny, 1991; Menkhorst, 1995; Turnbull, 1998; Grant et al., 2000). The available evidence suggests, however, that in the lower reaches of the Murrumbidgee and Murray River systems and in South Australia, where the species was probably never common, the platypus is now either very uncommon or extinct. The numbers of platypuses reported by early naturalists or captured by fur trappers up to the turn of the 20th Century suggest that the species was more abundant then (Grant and Denny, 1991; Grant and Temple-Smith, 1998; Moyal, 2001), however, there are no reliable quantitative data to substantiate this suggestion. Since that time populations are reported to have increased in number (Grant and Denny, 1991; Grant and Temple-Smith, 1998) but, again, the lack of quantitative baseline data makes such suggestions difficult to interpret. Only a few platypus population estimates have been published (Grant and Carrick, 1978; Serena, 1994; Serena and Williams, 1997), and Grant (1992b) has discussed the difficulties of making accurate population estimates using mark and recapture methods for the species, which appears to exhibit considerable mobility and differential catchability.

The platypus still occurs within the metropolitan areas of Hobart, Melbourne, Sydney and Brisbane but it is largely restricted to the less developed peripheral metropolitan areas (Stone, 1983; Grant and Denny, 1991; Grant, 1992a, 1998; Serena, 1994, 1996; Serena and Williams, 1998; Pettigrove, 2000; Grant, 2002a). The decline of platypus distribution and numbers around major metropolitan areas cannot be attributed to any particular cause but is likely to have been the result of a combination of the effects of human occupation, including sedimentation and pollution of streams, degradation of their banks, construction of dams and weirs, flow regulation and exotic species. Although not an exhaustive review, this paper identifies and discusses the key threatening process affecting the platypus, and suggests a range of research priorities and management activities that should reduce impacts on the platypus and its habitat and facilitate the conservation of the species.

Threatening processes

Few studies have been published on the direct effects of human activities on platypus populations. Possible impacts must be inferred from an understanding of the relationships between the habitat requirements of the animal and factors that affect these requirements, especially impacts on food and shelter. The collective understanding of the biology and habitat requirements of the platypus has been summarised in a number of recent publications. This knowledge, obtained from a range of reference material, including published and unpublished work, covers a spectrum from the observations of early naturalists to recent detailed analyses of habitat variables (Grant, 1995; Grant and Bishop, 1998; Grant and Temple-Smith, 1998; Williams and Serena, 1999).

Forestry

Platypuses are frequently recorded in forested areas, including those subjected to logging and associated activities. The data on the effects of these activities are limited to a few studies, the findings of which, in some instances, may be equivocal due to the multiplicity of environmental factors involved (Campbell and Doeg, 1989; Growns and Davis, 1991, 1994; State of the Environment Advisory Council, 1996). However, the main environmental effects attributed to forestry operations which are likely to affect the platypus are those which result in damage to riparian habitat and reduction in the abundance and diversity of benthic macroinvertebrate species in streams. These effects include increased stream flows, filling of interstitial spaces in stream substrates, sedimentation, elevated suspended solids, bank damage as a result of the removal or destruction of riparian vegetation and the use of pesticides and herbicides (Boulton and Brock, 1999). Because these effects may be short-term, and probably decline during the long periods needed to regenerate forests, the impact of forestry operations on aquatic ecosystems may be less in the long-term than other types of land uses, particularly agriculture (Brooks and Brierley, 1997; Boulton and Brock, 1999). Of 141 benthic invertebrate sites monitored in New South Wales State Forests or forestry reserves, using the AusRivAS system (Australian Rivers Assessment; a rapid assessment methodology comparing potentially impacted sites with more pristine reference sites), 65% were considered to be in “good health,” only 9% were considered “poor” or “very poor” and the rest were listed as “fair”(NSW EPA, 2000).

Grant (1991) reported sightings of platypuses from State Forests in New South Wales within every type of forest management from reserves to selective logging and clear felling. Turnbull (1998) recorded the presence of platypuses in most streams in the Bombala area of New South Wales, despite logging in their headwaters for over 100 years. The authors are unaware of any published studies that have directly assessed the status of platypus populations downstream of comparable logged and unlogged sites but a study currently in progress in northern Tasmania (Sarah Munks, pers. comm.) may provide the first insights.

Research priorities:

- Studies of the occurrence of platypuses in comparable logged and unlogged catchments.
- Long-term studies of platypus populations during logging operations and in the period of regeneration of the forest.

Management priority:

- Preparation, implementation and quality assurance assessment of stream and riparian protection plans during logging operations in both natural and plantation forestry.

Agriculture

Agricultural land use in Australia has resulted in the hydrological effects of the construction and operation of impoundment-based irrigation systems, increased surface runoff and the erosion of catchments and stream banks. These have caused hydrological changes in streams, increased sedimentation, elevation of suspended solids (turbidity) and the influx of salt, pesticides and nutrients into streams. Water quality parameters, fish and benthic macroinvertebrate diversity and abundance, the occurrence of feral plant and animal species, and to some extent the abundance and diversity of water-associated vertebrate species, have been variously studied as indicators or symptoms of the declining river health of river systems of Australia (Bowmer, 1998). The interactions between the various activities associated with agricultural practice in Australia, their individual or combined effects on river and riparian systems and the resultant changes in various measures of river health are summarised in Figure 2.

Platypuses are frequently reported in agricultural areas. In three separate surveys in New South Wales 52–76% of recorded platypus sightings were from agricultural land (Grant, 1991; Lunney, et al., 1998; Rohweder and Baverstock, 1999). However, it would be a mistake to be complacent about these observations and to regard agricultural use of the land as benign with respect to platypus conservation. Despite the common occurrence of platypuses in agricultural areas, there are indications that distribution fragmentation and/or reduced numbers of platypuses have occurred in streams of the Eden area (Lunney et al., 1998) and in the Bega (Brooks and Brierley, 1997), Thredbo (Goldney, 1998) and Richmond (Rohweder and Baverstock, 1999) rivers of New South Wales and in the Wimmera River system in Victoria (Australian Platypus Conservancy, 1999a, 2000, 2001). In each of these instances the changes have been attributed mainly to the effects of agricultural practices. Lunney et al. (1998) attributed fragmentation of platypus populations in the Eden area of New South Wales, including the Bega River, to the

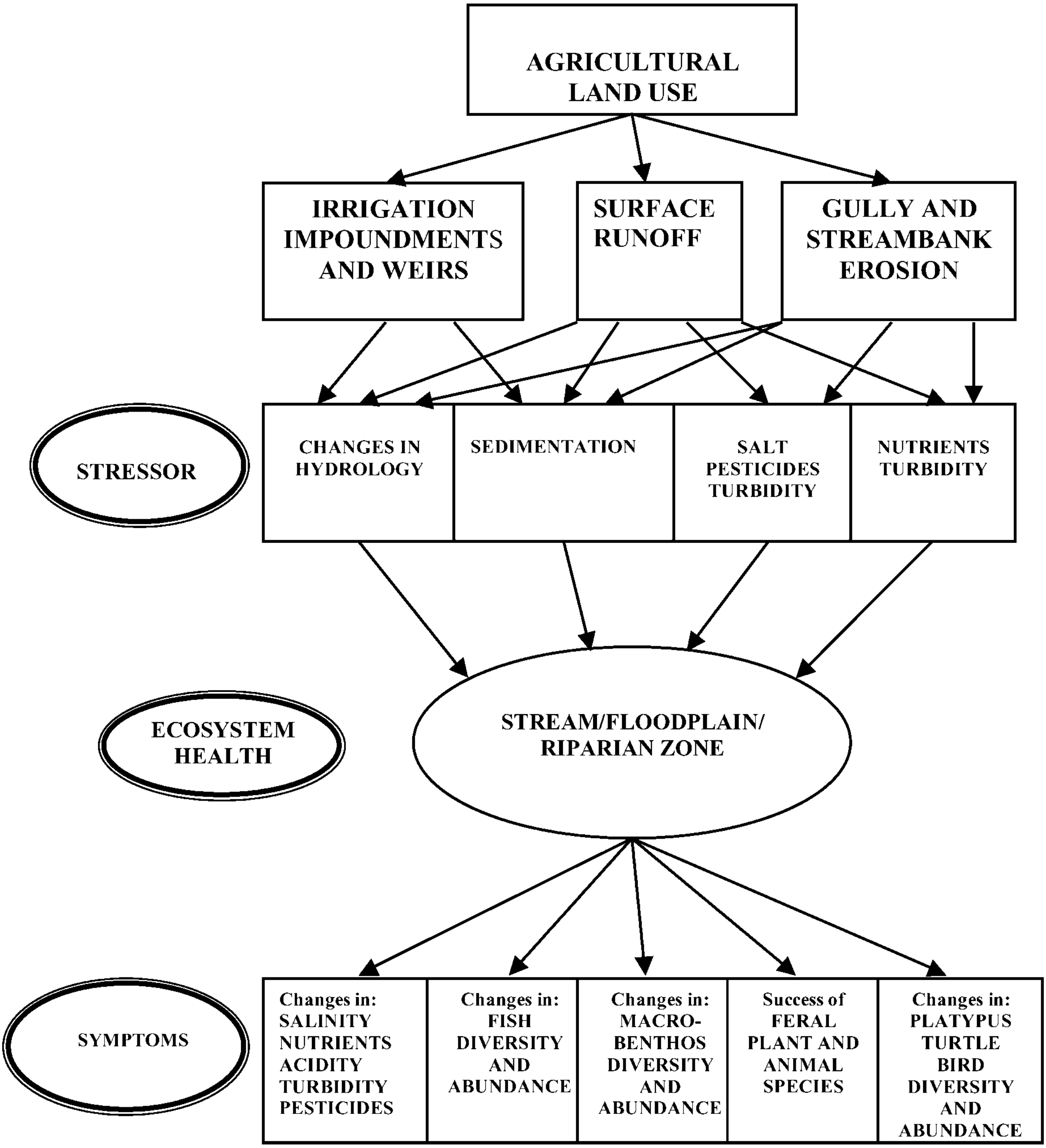

Figure 2. Impact of agricultural land use on aquatic ecosystems (Modified from Bowmer, 1998).

effects of farming, particularly cattle grazing. Turnbull (1998) showed that platypuses occur in most rivers of the nearby Bombala area of New South Wales, in spite of this area having been used for both cattle and sheep grazing for the past 160 years. However, Brooks and Brierley (1997) and Brierley et al. (1999) provide evidence that early agriculture practices in the Bega River valley of New South Wales were almost certainly responsible for the irreversible changes to that river system.

The NSW Environment Protection Authority (NSW EPA, 2000) ranked agricultural practices as the first (cropping), second (cropping and grazing) and fifth (grazing alone) out of eight categories of land uses that impact significantly on benthic invertebrates (using the AusRivAS monitoring system) in New South Wales streams. Likewise, Boulton and Brock (1999) indicate a severe or permanent impact of cropping and grazing on sedimentation of wetlands in Australia. Fortunately, many government and community programs and initiatives are now attempting to control catchment erosion and to rehabilitate streams and riparian zones in rural areas. Little is known of the effects of agricultural chemicals in the streams inhabited by platypuses. Persistent organochlorine residues were found in the fatty tissues of all the platypuses studied in Tasmania by Munday et al. (1998a) and these were correlated to the past use of the chemicals in agricultural areas (Munday, pers. comm.). There are no published studies on any clinical effects in platypuses of exposure to farm effluent released into streams.

Research priorities:

- Investigations into the occurrence of platypuses in similar stream types in different land use systems.
- Investigation of platypus populations in comparable streams in various states of degradation due to past or present agricultural practices.
- Investigation of the effects of rehabilitation of catchments, streams and riparian zones on platypus populations at local and catchment levels.
- Investigation of clinical effects in platypuses of exposure to animal effluent or agricultural chemicals released into streams and other water bodies.

Management priorities:

- Continued education of and cooperation between rural communities and government instrumentalities to improve stream protection.
- Legislative protection of streams and their riparian zones, particularly with regard to management of stock access.

Human sewage effluent

Platypuses appear to consume benthic invertebrate prey species in proportion to the occurrence of each species in an area (Grant and Temple-Smith, 1998) and should be little affected by the influx of non-toxic organic pollution from farms, meat works, dairy factories and sewage treatment plants. Organic pollution tends to result in an increase in the biomass of tolerant species (e.g. chironomid larvae and tubifex worms) but normally leads to decline in community diversity (Williams, 1983). Platypuses have been observed foraging in streams unsuitable for human primary contact [swimming] (Grant, personal observation in the Wingecarribee and Nattai Rivers in New South Wales). Grant (1991) also reported platypuses within the zone of probable influence downstream of 26 separate sewage outfalls discharging primary, secondary or tertiary treated effluent from country towns in New South Wales. There are no published studies on any effects of clinical toxicity or disease in the species arising from exposure to treated human sewage effluent.

Research priority:

- Investigation of possible toxic or disease effects in platypuses of exposure to treated human sewage effluent.

Management priority:

- Reduction of septic tank seepage into streams and ensuring that all sewage treatment plants discharging to streams have effluent quality which does not adversely impact on the platypus or its habitat and food supplies.

Dams, weirs and culverts

Weirs are often less than five metres high and provide pools that are of suitable depths for foraging by platypuses. Individual platypuses have been reported in weir pools (Grant, 1995) and farm dams (Grant, unpublished). Weirs may present a barrier to movement and perhaps increase the risk of individuals to injury and predation as they detour around these structures. Culverts (stream crossings) with entrances more than 20 cm above the stream bed may prevent platypuses from accessing and using the culvert. This has the effect of forcing them to move overland, increasing the danger of predation, or being killed by vehicles in areas where the culverts pass under main roads (Tyson, 1980; Serena et al., 1999; Mooney and Spencer, 2000). Recent research indicates that platypuses regularly use culverts (Serena et al., 1999; Mooney and Spencer, 2000), but there are no estimates of flow velocities which could prevent them from swimming against a current within such structures. Swimming against a stream velocity of 1–1.2 $\mathrm{m{\cdot}s^{-1}}$ has, however, been observed in one individual moving through an artificial fishway (Bishop, pers. comm.) and in another within a natural riffle (Grant and Bishop, unpublished) indicating that platypuses could move through culverts where such flows occur.

The downstream effects of river regulation in Australia, as reviewed by Boulton and Brock (1999), include changes in temperature, flow and sediment content, which often result in altered benthic communities and reduced foraging areas below large dams. Lowered water temperatures, characteristic of water released from below the thermocline in most impoundments in Australia, place an energetic demand on platypuses living downstream from large dams. The species is physiologically well adapted to living under cold conditions in winter over much of its current distribution (Grant and Dawson, 1978a,b; Bethge et al., 2001) but raised metabolic demand, coupled with changes to benthic food availability, must impose additional stress on animals inhabiting waters downstream of large dams. Gust and Handasyde (1995) investigated the movements of radio-tracked platypuses during operational releases for irrigation from Eildon Weir on the Goulburn River in Victoria, finding that animals tended to forage in slower-flowing backwaters during higher flows.

Upstream increases in water levels, associated with dam construction and operation, that change relatively shallow and productive lotic stream and river environments into deep, less productive lentic ones may cause the main impact on platypus populations. Platypuses appear to be unable to forage successfully for small food items at depths greater than about 5–10 metres and are only occasionally reported from deep areas of water storage impoundments. Grant (1991) reported only four records of platypuses from 32 deep (>10 metres) impoundments in New South Wales, while reporting 30 records from the shallower headwaters of these storages. Increased proximity of burrows to the water or the flooding of burrows by rising water levels are also potential impacts which have not yet been studied in impoundments or in regulated streams.

Planning and building of large dams is currently more common in Queensland, in areas where the platypus is found, than in the other States (O'Connor, 2001) where the construction of large dams has declined in the last two decades. Throughout Australia, much more consideration is now being given to the provision of environmental flows and the implementation of operational procedures for existing structures to reduce their downstream impact (e.g. New South Wales Department of Land and Water Conservation, 1997; Webster, 1998; Whittington and Hillman, 1999; Erskine et al., 1999) and even the removal of dams and weirs (Blanch, 2001).

Research priorities:

- Investigation of the effects of changed water levels and flows on the construction and use of burrows by platypuses in regulated river systems.
- Studies to determine the environmental flow strategies necessary for the maintenance of platypus populations in regulated rivers.

Management priorities:

- Development of operational procedures for storage and release of impounded water to minimise adverse effects on biotic communities downstream, including effects on the platypus and its habitat.
- Development of alternative strategies for water supply and management which avoid the construction of dams or weirs.
- Construction and maintenance of stream crossings and culverts designed to allow the free movement of platypuses.

Salinity

In Australia, increasing salinity of inland waters is a major threat to the health of aquatic ecosystems and water resources in many catchments (Ball et al., 2001). Platypuses seem to be largely restricted in their distribution to the upper sections of estuaries, within a few kilometres of the limit of tidal influence (Grant, 1999). These sections of estuaries are essentially fresh water (<0.5 ppt; 735 μS·cm^{-1}) and are classified as "fair" water quality for agricultural uses in New South Wales (0.34–1.02 ppt or 500–1500 μS·cm^{-1}; NSW EPA, 2000). The reasons for such distribution of platypuses is not known, but is probably related to a number and/or combination of biotic and abiotic factors influenced by tidal cycles in the estuarine environment, rather than to the species' physiological intolerance to a specific level of salinity. However, there are a few instances of platypuses being recorded from much more brackish conditions near the sea, especially in Tasmania (Grant, 1991, 1999; Rakick et al., 2001).

Of 11 inland rivers recorded in New South Wales as having only "fair" water quality in terms of salinity, platypuses have been recorded from close to the salinity sampling points in seven of these rivers. While not providing numerical values, Pettigrew et al. (1998) have suggested that the distances over which platypuses can locate prey, by sensing their electric fields, will decline with lowered impedance (increased conductivity) in water. These authors suggest that this may be a reason for the unexplained absence of the species from most of the streams flowing through the plains areas at the foot of the Great Dividing Ranges on the mainland of eastern Australia (Figure 1). This seems unlikely considering that platypuses are found in rivers with elevated ("fair") salinity levels and are absent from reaches of these west-flowing streams in New South Wales which do not have elevated salinities (NSW EPA, 2000). However, the authors are not aware of any published studies investigating the effects of salinity on either the foraging behaviour or osmoregulatory physiology of the platypus.

Research priority:

- Research into possible osmoregulatory or electrophysiological effects on the platypus of the range of salinities predicted for waters currently occupied by the species.

Management priority:

- Continued efforts and co-operation between the community and government agencies to manage and control rising salinity in streams and rivers.

Introduced species

Willows

Many Australians who have observed platypuses in the wild mention willows (*Salix spp.*) in their description of the habitat of the area in which they made their observations (Grant, 1991). Grant (1983) also found that 76% of the burrow areas used by radio-tracked platypuses in the upper Shoalhaven River in New South Wales were among the roots of one or more willow trees. However, a radio-tracking study in a small stream in Victoria showed that platypus foraging activity was positively correlated to the presence of native vegetation and introduced poplar trees but negatively correlated to the presence of willows. In this study there were no significant differences in the availability of benthic invertebrates between the areas dominated by willows compared to native vegetation and the authors were prompted to speculate regarding possible reasons for the apparent avoidance by platypuses of stream reaches dominated by willows (Serena et al., 2001).

Species and varieties of the genus *Salix* in Australia are known to be involved in increased local flooding, reduced summer flows, restricted fish passage, loss of habitat for aquatic macroinvertebrates and low dissolved oxygen levels (Schulze and Walker, 1997; Bobbi, 1999; Read and Barmuta, 1999; Rutherford and Abernethy, 1999; Bishop, 2000). These effects are most pronounced in small streams, particularly where willows occur in high densities. The management of willows is the subject of considerable debate (Schulze and Walker, 1997; Read and Barmuta, 1999; Rutherford et al., 1999).

In two reported instances in which willows were removed and replaced by native species of vegetation, some resident platypuses that were monitored using radio-tracking showed no apparent adverse effects from the river management activities (Australian Platypus Conservancy, 1997, 1999b). Grant (2002b) also found no difference in the numbers of platypuses observed at a study site on the Wingecarribee River in New South Wales before and after willows had been stripped from about 1.75 kilometres of the stream. However, the literature suggests that willow removal should be planned and executed very carefully, that native plantings should normally be established before the willows are completely removed and that the frequent proliferation of weed species (as a result of increased light availability) needs to be diligently managed (Bobbi, 1999; Rutherford and Abernethy, 1999; Rutherford et al., 1999). Research shows that increased light and temperature from changes to riparian vegetation can result in dramatic alteration of the distribution and abundance of aquatic invertebrate species and in-stream productivity (Bunn et al., 1999). There are currently no published studies of which the authors are aware which have quantitatively investigated the effects of removal of willows on platypus populations, their habitat or the availability of their food.

Research priority:

- Long-term studies of the effects of removal of willow trees and revegetation on platypus populations, their riparian and aquatic habitat requirements and the abundance of aquatic prey species.

Management priority:

- Careful planning of willow removal, replanting and weed management in river bank rehabilitation.

Fish

Carp (*Cyprinus carpio*) were probably first introduced into Australia around 1850 but did not spread until after the introduction of another strain in the 1960s. Ecological effects of high densities of carp are poorly understood, but increased bank damage, disturbance of aquatic macrophytes and elevated turbidity are all possible consequences. Food competition between this species and native planktonic- and benthic-feeding fish and the platypus is also possible. The overall disruption of riverine food webs by the large biomass of carp may be very complex and detrimental to the freshwater systems in which the species occurs (Schiller and Harris, 2001). Platypuses and carp certainly overlap in many areas of their current distribution. For example, they are found together in many streams of the Murray-Darling River System, as well as in many coastal streams of Queensland, New South Wales, Victoria and in restricted areas of Tasmania (Boulton and Brock, 1999).

Two species of salmonids, the brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*), may also compete to some extent with the platypus for food (Grant, 1995). These species frequently overlap the platypus in their distribution and have done so for over 100 years (Allen et al., 2002). Interestingly, some individual platypuses were reported consuming trout eggs during winter in the Thredbo River, New South Wales (Grant, 1995).

Over the range of the platypus, introduced redfin perch (*Perca fluviatilis*) are found mainly in the backwaters of rivers and in impoundments in Tasmania and Victoria. This introduced species also has a restricted overlapping distribution with the platypus in Queensland (Allen et al., 2002) and is found in some waterbodies occupied by platypuses in New South Wales (e.g. Wingecarribee and Belubula Rivers; Grant, unpublished). Although mainly feeding on smaller fish (Schiller and Harris, 2001), redfin perch may overlap slightly with the platypus in dietary requirements, consuming some crustaceans and molluscs (Allen et al., 2002).

The mosquito fish (*Gambusia holbrooki*) was introduced into all states in the 1920s and 1930s as a biological control agent of mosquitoes. It is extremely widespread over much of the distribution of the platypus (Allen et al., 2002) and the two species may compete for food. Grant et al. (1977) reported some individual platypuses feeding on mosquito fish in captivity but there is as yet no evidence that this feral species is consumed by platypuses in the wild.

The Mozambique cichlid (“Tilapia”), *Oreochromis mossambicus*, co-exists with the platypus at several sites in north Queensland (Hogan, pers. comm.; Grant, unpublished). This cichlid feeds mainly on algae, zooplankton and detritus but is known to take some benthic macroinvertebrates, creating the possibility of some dietary overlap between tilapia and the platypus.

During normal foraging platypuses may consume 15–28% of their body weight in food per day (150–280 g for an individual weighing 1 kilogram; Krueger et al., 1992; Munks et al., 2000). Recent captive studies of platypuses have estimated food consumption at a surprising level of 90–100% of the body weight in females during the later stages of lactation (Holland and Jackson, 2002; Temple-Smith and Grant, 2001). Intuitively, the insertion of one or more competing feral fish species into a riverine food web containing a breeding platypus population would be expected to result in an adverse impact, especially when carp and/or salmonids often constitute a large part of the total vertebrate biomass in some rivers and at some times of the year (e.g. during the winter spawning runs of salmonids). However, the co-existence of these species and the lack of any signs of lowered body condition in platypuses in areas of overlap with feral fish (Grant, unpublished) is an apparent anomaly requiring further investigation.

Research priority:

- Investigation of populations, breeding success, dietary overlap and body condition in platypuses in areas where their distribution overlaps with those of individual or different combinations of feral fish species.

Management priorities:

- Programs for the reduction and/or removal of feral species from streams inhabited by platypuses (see fisheries by-catch below).

Urban development

The platypus still occurs within the metropolitan areas of Hobart, Melbourne, Sydney and Brisbane, although it appears that both the numbers and distribution of platypuses in these areas have been significantly reduced (Stone, 1983; Grant and Denny, 1991; Grant, 1992a, 1998; Serena, 1994, 1996; Serena and Williams, 1998; Pettigrove, 2000; Grant, 2002a). There are still occasional reports of sightings from the outer suburbs of Sydney but the platypus is now considered very uncommon or extinct in the badly degraded streams of the Sydney metropolitan area (Grant, 1992a, 1998, 2002a). The species is often reported close to even quite large rural population centres, including Canberra, where apparently reduced numbers have been attributed to the effect of sedimentation from housing developments (e.g. Hogg and Norris, 1986; Lintermans, 1998).

Research priority:

- Investigation of the detailed distribution of platypuses within metropolitan areas, including analysis of the viability of populations.

Management priorities:

- Including in the environmental impact assessment process the study, management and conservation of suitable platypus habitat within urban areas where proposed developments could be likely to affect remnant platypus populations.
- Formulation of population conservation or recovery plans for remnant populations of platypuses in metropolitan areas.

Fisheries by-catch

An historical assessment of inland commercial fishing in New South Wales showed that the early fishery almost certainly caused significant platypus mortality when small mesh net sizes were used (Grant, 1991, 1993; Grant and Denny, 1991). No commercial or recreational fishery using nets or traps to capture native fish species or salmonids now exists in Queensland, New South Wales or Tasmania. Introduced pest species such as common carp (*Cyprinus carpio*) are targeted in New South Wales and Victoria with a variety of gear, including gill nets and electrofishing equipment. There is also a small commercial eel fishery, based on the use of fyke nets in limited numbers and sections of streams, that operates in Victoria and Tasmania and a restricted fishery for other indigenous fish species also occurs in Victoria. In Queensland, eels are commercially captured in baited traps only in farm dams and a few impoundments and in New South Wales, commercial capture of the two species of eels (*Anguilla australis* and *A. reinhardtii*) is restricted to estuaries, farm dams and some impoundments. By-catch mortality of air-breathing vertebrates has been recognised as a significant risk from these fisheries in all States where platypuses occur. This has resulted in a diverse range of regulations and gear modifications by fishers (e.g. Leadbitter, 2001), and also development of some local codes of practice aimed at reducing by-catch of non-target species. However, little research or monitoring has been done to assess the effectiveness of these regulations and initiatives.

For example, a recent study examined the effectiveness of by-catch reduction devices (BRDs) of various sizes in excluding platypuses from traps (Pease, Grant and Walford, unpublished data collected for the New South Wales Fisheries). This showed that a BRD of 70 mm diameter provided some deterrence from entry but that all platypuses tested at two sites in eastern New South Wales could pass through a 70 mm diameter BRD and smaller individuals could squeeze through an entrance of 60 mm. These experiments showed that the BDRs currently specified by some State regulations and other initiatives would be ineffective in preventing platypuses entering traps and nets used to capture eels and yabbies. Grant (1993) stated that "yabby [freshwater crayfish] fishing poses little threat to platypuses". This conclusion is now known to have been incorrect. Anecdotal reports from a number of States now suggest that yabby traps, particularly the folding so-called "Opera House" traps used by both commercial and recreational fishers, may be significantly impacting on some local platypus populations. These traps have also been implicated in mortality of other non-target species, especially freshwater turtles. The drowning of several platypuses in a single trap has been reported on a number of occasions but their attraction to these traps is not fully understood. However, platypuses are known to locate their prey by sensing the electrical fields generated by muscular activity of the prey species, especially large food items such as yabbies (Pettigrew et al., 1998). A trap containing live yabbies may therefore attract platypuses during their normal foraging activities. This unexpected by-catch in yabby traps has recently been brought to the attention of fisheries authorities in several States and the present regulations or proposed regulations restrict the use of such traps to waters where platypuses do not normally occur. It is obvious that the by-catch issue requires further research and that effective regulations and monitoring need to be implemented in the various States where platypus distribution overlaps with either commercial or recreational fishing activities.

Research priority:

- The development and rigorous testing of fishing equipment used to capture both indigenous and pest species of fish and crustaceans.

Management priorities:

- Development of appropriate regulations within both recreational and commercial fisheries to minimise the impact of these fisheries on non-target species, including the platypus.
- Effective law enforcement against illegal fishing activities to reduce the use of equipment impacting on non-target species, including the platypus.

Disease

Platypuses are known to carry a number of parasitic animals in the wild, including their own unique species of tick, *Ixodes ornithorhynchi*. They are also subject to a number of viral and bacterial infections (Whittington, 1992; Munday et al., 1998b), but appear to show few clinical symptoms of these infections. However, certain platypus populations in Tasmania suffer from a fungal infection (*Mucor amphibiorum*) which has resulted in substantial mortality in some populations (Connolly et al., 1998; Munday et al., 1998b) but has fortunately not yet been recorded in any mainland platypus populations (Whittington et al., 2002). This disease is the subject of a recently completed but as yet unpublished doctoral thesis from the University of Tasmania (Stewart and Munday, pers. comm.).

Research priority:

- Investigation of the role of disease in platypus morbidity and mortality, with particular reference to human and livestock faecal contamination of streams.

Management priority:

- Control of human and animal faecal contamination of streams.

Discussion

The present overall distribution of the platypus appears to be little different from pre-European times except that now there are almost certainly no naturally occurring populations in South Australia, where it once occurred, and its distribution has apparently shrunk in the lower reaches of the Murray and Murrumbidgee River systems in Victoria and New South Wales. With the exception of the deep waters of large storage dams, the platypus has continued to occupy aquatic systems and habitats throughout the remainder of its historical distribution, including ecosystems where various threatening processes are having major impacts on aquatic and riparian habitats. Unlike the many native fish species showing substantial declines in distribution and abundance (Schiller et al., 1997; Schiller and Harris, 2001), the platypus has continued to inhabit and reproduce in considerably degraded ecosystems, although differences in abundance before and since these systems were modified are largely unknown.

In the authors' study area in the upper Shoalhaven River, New South Wales, deep pools with a mixture of sand, woody debris and cobble/gravel substrates have changed over a period of only 20 years to small, shallow pools linked by meandering sandy channels. The disappearance of deep pools has been of great concern, due not only to the assumed reduction in productivity of benthic substrates, but also to the loss of refuge habitat for the platypus and its benthic prey organisms in periods of extended drought. During the severe drought of 1979–1983 when the upper Shoalhaven River ceased to flow, platypuses continued to occupy and breed in the large deep pools remaining in the river. The loss of deep refuge pools during future extended droughts is expected to impact more significantly on the resident platypus population. However, there was no evidence of significant loss of body condition of individuals captured during the short but relatively severe drought of 1994–95, during which there was also considerable recruitment of young platypuses to this population (unpublished personal observations). These observations highlight a poor understanding of the biology of the platypus and the environmental processes that affect it.

The material presented in this review suggests that poor land management practices are the most immediate threat to platypus populations and yet individuals in the upper Shoalhaven River population seemed little affected by quite dramatic changes in their habitat. Clearly more research effort is required to understand the reasons for such apparent anomalies. The concern is that, although many populations appear to be largely unaffected at present, they may show rapid and severe changes in response to degrading processes in the future. The fragmentation of platypus populations found in some river systems suggests the possibility that apparently secure local platypus populations may quickly become threatened or locally extinct due to the effects of one or more threatening process arising from human activities. Adequate monitoring of these populations is now required to ensure that any future population declines are recognised and arrested by the implementation of appropriate management strategies. Fortunately it appears that there is probably time for well-planned studies to be carried out to more accurately assess and predict the effect of threatening processes on populations of this unique species.

The NSW Fisheries Act of 1902 prohibited netting in many rivers of that state after the introduction of salmonids and inadvertently provided protection for platypus populations in NSW from that time. In the same way, the platypus should derive benefit from most government and community initiatives aimed at improving land management practices and at halting or reversing stream degradation in efforts to conserve other vertebrate species, particularly indigenous fish.

Inclusion of the platypus in the research, planning and execution of such initiatives is essential, not only to ensure the conservation of the species but also to facilitate more precise characterisation of its role in freshwater ecosystems in eastern Australia. The future conservation of the platypus will ultimately be determined by the halting or reversal of many of the threatening processes discussed, especially those resulting from poor land management practices, construction and operation of large water storage dams and the regulation of fisheries that may take the species as by-catch.

The occurrence of the platypus in considerably degraded streams argues against its use as an indicator of river health. However, the platypus is a national icon and is widely regarded as an integral part of most south-eastern Australian freshwater ecosystems. Caughley and Gunn (1996) indicated that "the signal of a conservation *problem* is the sustained decline rather than the final stage of low numbers [of a species]." The common occurrence of the platypus throughout much of its historical range is not a sign that we should be complacent. Targeted research and careful management, of the type recommended in this review, will be important in ensuring that *Ornithorhynchus anatinus* does not become a conservation *problem* but continues to be the surviving member of the family Ornithorhynchidae in Australia.

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