CSIRO PUBLISHING
www.publish.csiro.au/journals/wr *Wildlife Research*, 2011, **38**, 271–289

Platypus (*Ornithorhynchus anatinus*) body size, condition and population structure in Tasmanian river catchments: variability and potential mucormycosis impacts

Nick Gust[A,C] *and Josh Griffiths*[A,B]

[A]Department of Primary Industries, Parks, Water and Environment, GPO Box 44, Hobart, Tas. 7001, Australia.
[B]CESAR Consultants, Suite 102, 55 Flemington Road, North Melbourne, Vic. 3051, Australia.
[C]Corresponding author. Email: platypus.gust@gmail.com

Abstract

Context. Despite widespread interest in platypus (*Ornithorhynchus anatinus*) conservation, it is unclear how their fundamental morphometric and demographic characteristics differ over a range of scales. This hampers impact assessments and understanding of platypus ecology. Although the ulcerative fungal disease mucormycosis has infected platypuses in Tasmania for three decades, its population level impacts and conservation significance remain unknown.

Aims. This study examined morphometric and demographic patterns in Tasmanian platypuses to provide a basis for investigating impacts of mucormycosis or other anthropogenic disturbances. It also sought to identify important spatial scales of natural variability and the magnitude of seasonal variation in platypus body size, condition and population structure. The hypothesis of higher mucormycosis prevalence and mortality in adult males was also investigated.

Methods. Extensive live-trapping surveys were conducted from January 2008 to July 2009 in 75 streams and 18 river catchments across Tasmania including King Island. The sex, age, body size, tail volume index, health and moult condition of 195 individuals were assessed, and population age and sex structures characterised. Sampling focussed on assessing variability within and between river catchments and compared populations in river catchments with contrasting disease status.

Key results. Differences in platypus morphometrics within and between catchments and seasonal moulting patterns were detected. Adult males had higher fat stores than adult females, especially during winter. This study also provided the first evidence of population level consequences of disease in platypuses. The demographic group most commonly affected by mucormycosis was confirmed to be adult males. Differences in male age structure among catchments of varying disease status were consistent with the hypothesis of higher adult male mortality rates and turnover in currently affected catchments.

Conclusions. More than 25 years after mucormycosis was first detected in Tasmanian platypuses, the disease continues to play a low-level, ongoing role in affected populations.

Implications. The present study provides the first systematic multi-scale spatial investigation of platypus mucormycosis, which contributes to unravelling the epidemiology of the disease and detecting its impacts. By identifying the magnitude and important scales of morphometric and demographic differences in Tasmanian platypuses the study also assists researchers choose comparable demographic groups and spatial scales for meaningful comparisons in future impact studies.

Additional keywords: age structure, disease effects, impact assessment, natural variability, spatial scale.

Introduction

Emerging infectious diseases of free-living wild animals pose a substantial threat to the conservation of global biodiversity (Daszak *et al*. 2000). Novel pathogens are increasingly emerging in human-altered environments (Dionne *et al*. 2009), and some have disastrous impacts for hundreds of species worldwide (Pounds *et al*. 2006; Skerratt *et al*. 2007). Wildlife epidemiological investigations represent an important first step in examining the potential significance of emerging diseases and their impacts. However, obtaining useful epidemiological information about species in the wild is logistically challenging (Piggott and Taylor 2003), and consequently the role of disease in regulating wildlife populations often goes uninvestigated (Whittington 1992). Determining the impacts of disease (or other anthropogenic disturbances) on wild populations of nocturnal, elusive, rare or endangered species is particularly challenging. It is often difficult to choose an appropriate technique to detect and monitor such species (Vine *et al*. 2009), estimating disease associated mortality in free-ranging wildlife is problematic (Murray *et al*. 2009) and small sample

10.1071/WR10162 1035-3712/11/040271

sizes from wild populations often limit epidemiological inference.

Changes in morphometric and demographic parameters are commonly used to infer disease or anthropogenic impacts on wildlife (Skalski *et al*. 2005). However, individuals and populations living in different habitats may display natural differences in morphometric and demographic parameters, and these may vary considerably over wide geographic ranges. Therefore, to reliably detect disease impacts, fundamental information is required on the magnitude of natural variability in the size and condition of individuals, the sex and age composition of populations, and the key scales at which they differ. Here we used live-trapping surveys across Tasmania to quantify patterns of natural spatial and seasonal variation in the morphometric and demographic characteristics of platypuses (*Ornithorhynchus anatinus*) and explore potential impacts of the fungal disease mucormycosis.

The platypus is an enigmatic, elusive, predominantly nocturnal, egg-laying mammal and one of Australia's most widely recognised fauna icons. Given that platypus abundance is difficult to determine (Grant and Temple-Smith 2003), population trends are largely unknown and specific disease or environmental impacts on the species have been suggested and implicated, but rarely demonstrated (Gust and Griffiths 2009). Platypuses exist in the majority (44 of 48) of Tasmanian river catchments (Gust and Griffiths 2010), where they occupy diverse freshwater habitats including rivers, streams and creeks, lakes, tarns and farm dams from sea level to an altitude of 1200 m (Rounsevell *et al*. 1991). Differences in the mean size of adult platypuses have been described among some Tasmanian rivers and lakes (Koch *et al*. 2006). However, it is currently unclear how variable Tasmanian platypus morphometrics and demographics are over their heterogeneous environment, or what the key spatial scales are for differences. Quantifying this variability facilitates impact assessment and has conservation implications, as significant spatial variation in the demographics of wildlife populations may confer variable resilience to disease or anthropogenic stresses and require different management regimes over a species' geographic range (Skalski *et al*. 2005).

Ecologically important patterns and processes occur at different scales, and the spatial scale at which a system is explored will determine which patterns are detected and which are missed (Sale 1998). Accordingly, sampling designs that incorporate multiple scales are particularly valuable (Quinn and Keough 2002), but are rarely achieved for platypus studies due to the logistical and time constraints associated with live-trapping. Past research indicates that platypus morphometrics, specifically body size, can systematically differ over at least two spatial scales. At large spatial scales (spanning thousands of kilometres and the latitudinal range of the species) platypus body size differs markedly. Tasmanian platypuses are up to three times heavier than individuals of the same age and sex in north Queensland (Grant 2007). At broad scales (hundreds of kilometres within states) platypus body size can also vary considerably. For instance, adult males in the west-flowing Murrumbidgee River in New South Wales are 20% heavier than individuals in the state's east-flowing Shoalhaven River (Grant 2007).

Differences in body size over fine scales (from tens to hundreds of kilometres within and between catchments) are also possible. It seems likely that platypus size and body condition will vary across Tasmania in response to energetic considerations and habitat quality. Factors such as prey quality or abundance, water temperatures, or current velocity often vary among habitats and potentially influence the energetics of foraging, growth and condition of individuals. Even small differences in these conditions may be important, as Tasmanian platypuses are known to spend more than 12 hours a day foraging for prey (Bethge *et al*. 2003; Bethge *et al*. 2009). Initial evidence suggests platypuses using upper headwater streams in Tasmania may be smaller than those in lower catchment reaches (Koch *et al*. 2006; Olsson Herrin 2009), and smaller individuals have been reported from smaller streams on the mainland (Handasyde *et al*. 1992). The spatial scale of platypus breeding populations in Tasmania is currently unclear (Gust *et al*. 2009), although high levels of genetic differentiation among some Tasmanian river catchments (Furlan *et al*. 2010) suggest catchments may be reasonable proxies for local platypus populations, and form sensible management units. Nevertheless, some researchers have suggested that individual management units for platypuses on mainland Australia may be smaller, and vary from catchment to sub-catchment scales (Kolomyjec *et al*. 2009). Accordingly, two spatial scales may be particularly important for platypus studies: between river catchments (at scales of tens or hundreds of kilometres), and within river catchments (at scales of kilometres to tens of kilometres). We investigated both.

Mucormycosis is the only disease thought to cause significant morbidity and mortality in wild platypuses (Whittington 1992), and is only known to affect platypuses in Tasmania (Connolly *et al*. 1998; Whittington *et al*. 2002). Insufficient information currently exists on the impacts of platypus mucormycosis to rigorously assess its conservation significance (Gust and Griffiths 2009). The disease was first detected in platypuses in 1982 (Munday and Peel 1983), and is caused by the fungal pathogen *Mucor amphibiorum* (Obendorf *et al*. 1993). This pathogen can cause mortality in a variety of amphibian species (Frank 1975; Speare *et al*. 1994), and may have been introduced to Tasmania via infected frogs from mainland Australia (Munday *et al*. 1998). Though several mechanisms have been suggested, it is currently unclear what the route of individual infection is, or how mucormycosis is spread among platypuses or locations (reviewed by Gust and Griffiths 2009). Severely ulcerated platypuses suffering from mucormycosis are thought to die from secondary bacterial infections or via impaired thermoregulation and mobility (Munday and Peel 1983; Obendorf *et al*. 1993; Connolly *et al*. 1998). Mucormycosis has been speculated to cause significant platypus mortality in affected populations (e.g. Munday and Peel 1983; Whittington 1992; Connolly *et al*. 1998), although quantitative data is currently absent to test this assertion.

Mucormycosis has spread across at least 11 Tasmanian river catchments, and currently affects individuals in at least four catchments (Gust *et al*. 2009). Some catchments have been affected by mucormycosis for over 20 years, and the size, condition and health of individuals in long-term affected catchments may differ from those in unaffected catchments.

Broad demographic differences may also exist among affected and unaffected catchments if disease mortality strongly affects individuals of a certain age or sex (Skalski *et al*. 2005). Previous research in the mid-1990s suggested adult males could be ulcerated more frequently than other age and sex classes, and may be particularly vulnerable to the disease (Connolly *et al*. 1998; Stewart 2001). However, in both cases these trends were not statistically significant and it remains unclear whether there is a sex or age bias in the proportion of animals showing clinical signs of infection. If mucormycosis is more prevalent in adult males, and mortality rates reflect prevalence, we hypothesise that affected populations may become relatively female-biased, and/or contain fewer old adult males than unaffected populations.

This study used live-trapping surveys to explore natural spatial and seasonal differences in the morphometrics and demographics of Tasmanian platypuses. Specifically we quantified:

(1) the influences of demographics, seasonality and the spatial scale of investigation on platypus body length and weight;
(2) the influence of sex and seasonality on platypus tail volume index and moult condition;
(3) the sex ratios and age structures of Tasmanian platypuses;
(4) the influence of catchment disease status on platypus morphometrics and demographics; and
(5) the proportions of adult males suffering clinical signs of mucormycosis and the associated hypothesis of a demographic shift in disease-affected populations.

Materials and methods

Study area and spatial scales

A multi-scale, live-trapping platypus survey was conducted across mainland Tasmania from January 2008 to July 2009. King Island in Bass Strait was also surveyed in January 2009 because its small, isolated platypus population may be particularly vulnerable to mucormycosis due to low genetic diversity (M. Lillie, E. Miller, A. Lane, J. Griffiths, N. Gust and K. Belov, unpubl. data). Sampling was restricted to flowing waters (rivers, streams and creeks) to avoid potential differences between populations in riverine and lake habitats. We defined the primary spatial scale for assessment as individual river catchments, as they are discrete management and conservation units, and contain a network of interconnecting water bodies where platypuses can interact and breed. Sampling included catchments that were historically affected, possibly affected, and outside the known distribution of mucormycosis (for further details see Gust *et al*. 2009). Sampling spanned 18 of Tasmania's 48 river catchments (Fig. 1), with catchment boundaries defined by Land Information Service Tasmania at the Department of Primary Industries, Parks, Water and Environment (DPIPWE). Sampling was conducted using fyke and gill nets using the methods described in Gust *et al*. (2009). Sampling effort within catchments was spread across multiple waterways, with nets in waterways typically set at sites kilometres apart to seek a widely representative sample from catchments. Netting aimed to maximise captures of individuals, rather than recaptures, and avoided more than four consecutive nights of trapping at individual sites. The latitude and longitude of sampling sites is published online (at www.naturalvaluesatlas.dpiw.tas.gov.au), or can be made available on request. The individuals captured were assumed to be random samples from populations.

Free-ranging platypuses were primarily captured at night using purpose-built fyke nets that accounted for >90% of captures. Small and large fyke nets (with either 5 or 20-m-long wings and 0.6 or 1.2-m-high entrances respectively) were set according to the size of rivers and prevailing flow so that the wings completely covered the river width. Fyke nets were typically set in pairs, back-to-back facing up and downstream, with cod ends suspended well above water level. They were typically set two hours before sunset and were checked regularly for captures through the night. Unweighted and slightly weighted gill nets (50-m long and 3-m deep) were also used at Brumbys Creek and the Kermandie River following the techniques described by Grant and Carrick (1974, 1978). The location of each net was recorded using a handheld GPS along with the time of setting and retrieving each net, its size, the time and identity of any platypuses captured and by-catch species trapped. We quantify sampling effort in net-hours (one net-hour = a fyke net, or 50-m gill net deployed for an hour). Sampling sites were recorded using a handheld GPS and mapped using ArcGIS 9.3 (ESRI). Each sampling site was classified by river catchment and stream order (Strahler 1957) identified from the Conservation of Freshwater Ecosystem Values (CFEV) database v1.0 (2005) at the DPIPWE.

Within catchments we sampled from multiple water bodies to obtain a representative sample (Fig. 1, Table 1) and investigated the potential influences of position within catchments on platypus demographics and morphometrics. We used the Strahler classification system (Strahler 1957) to define position within catchments based on a hierarchy of tributaries. We considered Strahler stream orders one, two and three as upper catchment reaches, orders four and five as mid-catchment reaches, and orders six and seven as lower catchment reaches. To obtain representative samples within stream orders, netting sites were chosen where obstructions could not snag the net and the net could effective block the width of the river. Sites were typically spaced hundreds of metres to kilometres apart (range 100 m–10 km). Netting protocols aimed to maximise the total number of individuals captured rather than revisiting locations to facilitate recaptures. We only consider data here from the first capture of each individual.

Catchment disease status

The mucormycosis disease status of each sampled catchment was previously determined on the basis of historic and contemporary evidence of mucormycosis from public observations and live-trapping (Gust *et al*. 2009). To investigate potential disease impacts at the catchment level, we defined the four catchment disease status categories as follows:

(1) currently affected: catchments where one or more platypuses captured and examined by researchers in 2008 or 2009 had clinical signs of mucormycosis;
(2) historically affected: catchments where one or more platypuses examined by researchers between 1982 and

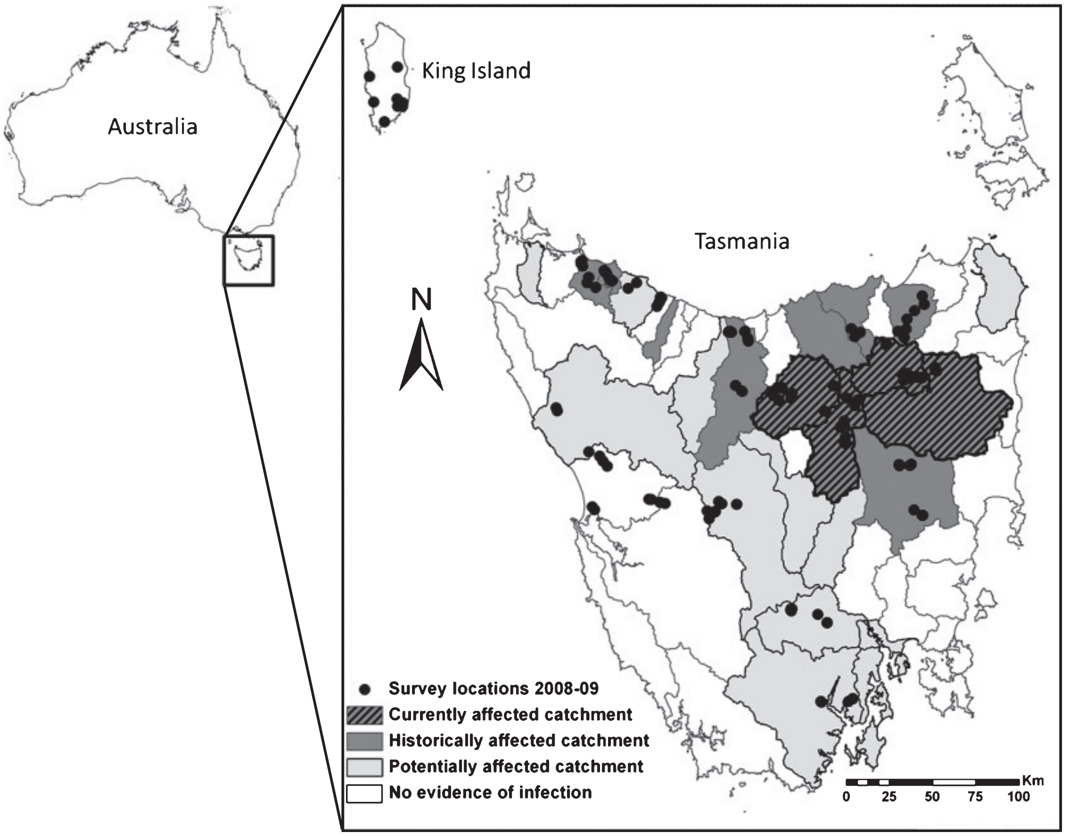

Fig. 1. Live-trapping locations sampled for platypuses across Tasmania and King Island. Trapping was conducted between January 2008 and July 2009 at 75 water bodies (circles) across 18 river catchments. River catchments are categorised by mucormycosis disease status following Gust *et al*. (2009). Shaded catchments without sampling locations indicate areas where platypuses were obtained for autopsy from public collections.

Table 1. Sampling effort across river catchments, disease status, water bodies, seasons and stream orders between January 2008 and July 2009
Mucormycosis disease status defined in Materials and methods. Water bodies were identified according to 1 : 50 000 Tasmanian topographic maps. Calendar seasons: Sp, Spring; S, Summer; A, Autumn; W, Winter. Stream orders identified from GIS layers in the conservation of freshwater ecosystem values program. The number of individuals captured (*n*) is indicated along with total netting sampling effort (soak hours)

River catchments	Disease status	Seasons	Stream orders	Water bodies	Soak hours	*n*
Cam	No evidence	A, W	1, 3, 4	2	205	4
Gordon-Franklin	No evidence	A, S	4, 5	2	161	1
King Island	No evidence	S	2, 4, 5	8	1034	19
King-Henty	No evidence	A, S	3, 4, 5	7	277	1
Pieman	Possibly	S	2, 3, 4	3	100	0
Huon	Possibly	A, S	4, 5	2	302	8
Inglis	Possibly	A	4, 6	1	54	2
Lower Derwent	Possibly	A, S	3, 5, 6	5	964	37
Upper Derwent	Possibly	A	2, 3, 4, 5, 6	8	678	6
Black-Detention	Historically	A, W	1, 3, 4, 5, 6	5	430	8
Great Forester-Brid	Historically	S	2, 3, 4, 5, 6	6	546	13
Mersey	Historically	A	4, 5, 7	4	618	11
Pipers	Historically	A	4, 5	2	182	4
Brumbys-Lake	Currently	A, Sp, S	2, 3, 4, 5, 7	3	1062	11
Meander	Currently	A, Sp	1, 4, 5, 6, 7	6	594	12
North Esk	Currently	A, Sp, W	1, 2, 3, 4, 5, 6	8	1331	29
South Esk	Currently	A, Sp	2, 5, 7	1	935	17
Macquarie	Currently	A, Sp	5, 6	2	650	0
Totals				75	10123	183

2007 had clinical signs of mucormycosis, but public observations and live-trapping surveys between 2008 and 2009 detected no affected individuals;

(3) possibly affected: catchments where there had been no confirmed records of mucormycosis in either captured or dead animals, but where the public reported apparently ulcerated platypuses between 1982 and 2009;

(4) no evidence: catchments where there were no records of clinical signs of mucormycosis, and only healthy platypuses had been reported by the public.

Sampling effort

Netting was conducted across at least four replicate catchments in each of the four disease status categories and was conducted over four calendar seasons. The distribution of sampling effort differed among combinations of river catchments, water bodies, stream orders and seasons, and is summarised in Table 1. Twelve additional platypus carcasses were collected in response to public sightings of animals that died in seven catchments between January 2008 and July 2009 (Table 2). The morphometric details were collected and necropsies were performed on fresh specimens. Stream orders could not be confirmed for six of the carcasses obtained from the public, and were not included in analyses. This sampling program provided a framework for a series of multidisciplinary investigations into platypus health and mucormycosis. These included assessments of contemporary mucormycosis distribution and prevalence (Gust *et al*. 2009), platypus relative abundance and capture techniques (J. Griffiths and N. Gust, unpubl. data), individual health and blood parameters (Geraghty *et al*. 2011), population genetics (Furlan *et al*. 2010), *MHC* gene (M. Lillie, E. Miller, A. Lane, J. Griffiths, N. Gust and K. Belov, unpubl. data) and pathogen exposure (N. Stewart, M. Kaur, R. Latham, J. Griffiths and N. Gust, unpubl. data).

Study animals

Once removed from nets, individuals were checked for and individually marked with a passive integrated transponder tag (Trovan or Allflex brands) inserted subcutaneously between the scapulae (Grant and Whittington 1991). The sex and age of captured platypuses were determined on the basis of the presence and morphology of the spur and spur sheath (Temple-Smith 1973), using the photographic key provided by Grant (2007). For the majority of analyses females were simply classified as juveniles (if spur buds were present or total weight was less than 900 g) or adults (if spur buds had disappeared and weight exceeded 900 g), while males were identified as juveniles (spur class one or two), subadults (spur class three), or adults (spur class four and five). However, when investigating patterns of adult male longevity, we considered males with blunt class five spurs to be ‘old adults’.

A tail volume index (TVI) (Grant and Carrick 1978) was adopted to provide a qualitative estimate of an animal’s stored fat and overall body condition. Platypuses store around 40% of fat in their tails (Hulbert and Grant 1983) and individuals’ tail volume and turgidity are widely used as a qualitative index of their body condition. Tail volume index values range from one (animal in excellent condition with a turgid, convex tail) to five (emaciated individual with tail vertebrae visible). Animals that could not be distinguished between consecutive TVI categories were scored with an intermediate value. Fur moult status was also assigned to one of five qualitative categories (none, very light, light, moderate or heavy) using the criteria of Grant and Carrick (1978). Values for TVI and moult status were determined by the authors with close reference to definitions carried on a laminated card on each fieldtrip.

Total body length was measured dorsally to the nearest 0.5 cm following the contours of the animal with a cotton tape measure while it was gently held flat on a foam pad. Individuals were weighed with a spring balance (Salter, Melbourne) to the nearest 25 g. All mean weights and lengths reported for demographic groups include ±1 standard error unless otherwise indicated. Visual health assessments were made of captured individuals for injuries or wounds and to detect clinical signs of mucormycosis (ulcers, lesions and granulomas) following the criteria of Munday and Peel (1983), Obendorf *et al*. (1993) and Connolly *et al*. (2000). Individuals were also searched for ticks (*Ixodes ornithorhychi*) and their numbers classified on a log scale as absent (0), low (1–10), medium (10–100) or heavy (100+). Individuals were considered to be healthy when no external injuries were observed, tick loads were not heavy and there was no evidence of disease or emaciation. Blood, skin and ticks, faeces swabs and ulcer biopsy samples were also taken for aligned investigations; methodological details are available in the related publications. The captured individuals were released at the capture location immediately after processing.

Table 2. Platypuses sampled from public collections between January 2008 and July 2009

The number of individuals sampled (*n*) is indicated and catchment disease status is defined in the Materials and methods. Calendar seasons: A, Autumn; W, Winter. Stream orders and water body names identified from GIS layers in the conservation of freshwater ecosystem values database (ver. 1.0, 2005) available from the Department of Primary Industries, Parks, Water and Environment

Catchments	Disease status	Seasons	Stream orders	*n*
Jordan	No evidence	A	6	1
Duck	No evidence	A	Unknown	1
Derwent Estuary-Bruny	Possibly	2A, 3W	4, unknown	5
Huon	Possibly	A	5, unknown	2
Upper Derwent	Possibly	W	1	1
Mersey	Historically	A	7	1
South Esk	Currently	A	7	1
Total				12

Environmental conditions

The study period and preceding 18 months were characterised by a prolonged drought in Tasmania. From June 2006 to July 2009 Tasmania experienced serious rainfall deficiencies, with levels reduced to between 70 and 80% of the long-term average across much of the state. The north-east of the state recorded the lowest rainfall since records began in 1900 (Australian Bureau of

Meteorology, http://www.bom.gov.au accessed November 2010).

Statistical analysis

The 183 live-captured and 12 dead animals collected by the public were considered random samples from stream populations and were pooled for analyses. Investigations focussed on detecting demographic, seasonal or spatial factors that influence platypus body size or condition in Tasmania, to facilitate future monitoring for impacts on the species. An exploratory approach was used to investigate patterns, with small sample sizes for some combinations of sex, age or sampling scales requiring pooling of data. With the available data and multiple factors of interest, it was not possible to conduct a single analysis to simultaneously investigate the influence of each factor on body size or condition. Instead, we used existing published literature and contemporary understanding of platypus biology to guide sequential investigations into the influence of demographic, seasonal and spatial factors of interest. The approach taken was to identify key factors of influence, remove data associated with differences and then test the effect of the next most important factor on the reduced dataset. Three significance levels were identified (* = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$) to indicate the relative strength of trends or importance of contributing factors in the sequence of analyses. Data analysis was conducted using Statistica ver. 7.1 software (Statsoft Inc., Tulsa, OK).

Body size

A hierarchical approach was used to sequentially investigate a series of factors that may influence the length and weight of Tasmanian and King Island platypuses. Given that the sequence of testing is important, we started with existing factors known to influence platypus body size. Platypuses are known to be sexually dimorphic in size (Grant 2007), adults are larger than juveniles and limited sampling of King Island individuals suggests they are smaller than Tasmanian platypuses (Stewart 2001). Accordingly, the hierarchical sequence of one-way analysis of variance (ANOVA) tests initially investigated the individual effects of sex, age and island origin on body size. If significant differences were revealed at each level, the treatment group with the smaller number of replicates was removed from subsequent analyses to retain the maximum comparable dataset following an approach recommended by McPherson (2001). For instance, we initially tested the effect of sex on body length, excluded the significantly smaller females and restricted subsequent comparison to males. Given that there was also an effect of age, we restricted comparisons of body length to adult males when investigating the influence of the next factor and so on. This approach attempted to retain the largest sample size possible for investigating scale dependence in platypus body size while identifying and removing variation in morphometrics associated with different demographic groups.

We subsequently investigated patterns in body size for the largest comparable group (adult males from Tasmania) according to increasing spatial scales: across catchment reaches, individual catchments and then catchments pooled by their mucormycosis disease status. The bodyweight and total length of platypuses were compared between catchment reaches (upper, mid and lower reaches representing Strahler stream orders 1–3, 4–5 and 6–7 respectively), all catchments where more than two adult males were captured and disease status categories using one-way ANOVAs. Where significant differences existed post hoc pair-wise comparisons of treatment means were conducted using Tukey's HSD (honestly significant difference) tests to control family-wise type 1 error rates (Quinn and Keough 2002). Prior to all ANOVA calculations the assumption of homogeneity of variances was inspected via box plots, and $\log(x+1)$ transformations were used to remove heterogeneity of variances as required.

Body condition and moult status

Platypus tail volume index may differ between established adults and juveniles. On the mainland, TVI has been shown to vary between the sexes and on a seasonal basis (Grant 2007; McLachlan-Troup 2007). Accordingly, we ordered the sequence of hierarchical investigations into factors influencing TVI to investigate the influences of age, then sex and season. As there is a potential interaction between sex and season we used a two-way factorial ANOVA specifically for this test. On the most comparable remaining dataset (adult males in all seasons other than winter), we then investigated the potential influence of increasing spatial scale on TVI using a series of one-way ANOVAs. Similarly we used a hierarchical approach to sequentially investigate a series of factors that potentially influence moulting patterns in Tasmanian and King Island platypuses. Given that age, sex and season may each play a role in platypus moult patterns we investigated their individual influences using one-way ANOVAs. We then investigated the potential influence of increasing spatial scale on patterns of moult condition using a series of one-way ANOVAs.

Demographics of disease

The demographics of animals showing clinical signs of mucormycosis were investigated to determine trends in the patterns of disease expression. In catchments currently affected by mucormycosis 70 individuals were sampled and the proportions of ulcerated and apparently healthy adult males and females were compared. Similarly, the frequencies of ulcerated adults and juveniles were compared using Chi-square tests. Binomial exact methods (http://statpages.org/confint.htmL accessed in February 2010) were used to calculate 95% confidence intervals around the estimates. To investigate a larger demographic dataset, data from currently affected catchments in the present study were pooled with data reported from affected catchments sampled during historic investigations into platypus mucormycosis in Tasmania (Connolly *et al.* 1998; Stewart 2001). Connolly *et al.* (1998) reported that 10 of 23 adult males and 3 of 13 adult females sampled from Brumbys Creek in 1994 were ulcerated, while Stewart (2001) reported 10 of 33 males and 2 of 16 females were ulcerated in affected northern Tasmania catchments over a 4-year study period from 1997 to 2000. The number of both clinically affected and unaffected juveniles caught was not made explicit in either study, with the only available data for juveniles gleaned from the present study.

Demographic structures (sex ratios, population and male age structures)

Two aspects of platypus demographic structure were examined: sex ratios and age structures. Examination of sex ratios can provide insight into the effects of disease on sex-specific survival rates (Skalski *et al*. 2005). Sex ratios were initially examined for both adults and juveniles across Tasmania. Subsequent analyses focussed on adults due to their larger sample size and to facilitate comparison with other studies. Adult sex ratios were investigated across seasons, catchment reaches and catchments pooled by disease status. Chi-square tests were used to determine whether sex ratios deviated from parity. The age structure of sampled platypuses were also characterised across the entire sample. Subsequently, analysis focussed on males to investigate the hypothesis of differential adult male mortality, and reduced proportions of old adult males in catchments currently affected by mucormycosis. Chi-square tests were used to determine whether the proportion of old males deviated from expected on the basis of catchment disease status. Binomial exact methods were used to calculate 95% confidence intervals around both sex ratios and the proportion of old males (http://statpages.org/confint.htmL accessed in January 2010). A review of adult platypus sex ratios was conducted to compare values in the present study with those reported by researchers around Australia. Values were gleaned from five published papers and four postgraduate studies, and Chi-square tests conducted on the raw data.

Results

Sample size and sampling effort

More than 10 000 h of live-trapping captured 183 platypuses from 75 water bodies across 18 catchments, seven Strahler stream orders and four seasons (Table 1). Samples included 69 individuals from four currently affected catchments, 36 from four historically affected catchments, 53 from five possibly affected catchments and 25 from four catchments outside the known distribution of mucormycosis, including King Island (Table 1). An additional 12 individuals were sampled from public collections made in autumn and winter across eight catchments and four catchment disease statuses (Table 2). A total of 195 platypuses were sampled. Numbers were not equally distributed among all possible combinations of seasons, stream orders and catchment disease status. The majority of animals (134 individuals representing 71% of the sample) were sampled from middle catchment reaches (Table 3). Smaller numbers were sampled from upper catchment reaches (25, 13%) and lower catchment reaches (30, 16%) (Table 3). Between 55 and 65 individuals were sampled in each of spring, autumn and summer, with relatively few (15, 8%) collected in winter (Table 3). Multiple individuals were sampled in 14 of the 16 possible combinations of season and catchment disease status (Table 4).

Body size

Sampled Tasmanian platypuses ranged in length from 32 to 64 cm and weighed between 460 and 3225 g (a juvenile female and an old male respectively). Strong sexual dimorphism was evident in adult Tasmanian platypuses (Table 5). In Tasmania, mean adult female body size was ~10 cm shorter and 920 g lighter than adult males, making females ~82% of the length of males and 57% of the weight. On King Island, adult females were on average 6.5 cm shorter and 625 g lighter than adult males, or 87% of the length and 64% of the weight of males. King Island platypuses were significantly smaller than Tasmanian individuals (Tables 5, 6). King Island adult males averaged ~89% of the length and 81% of the weight of their Tasmanian counterparts. King Island adult females average 94% of the length and 90% of the weight of Tasmanian females.

Sequential one-way ANOVAs indicated that platypus body length differed significantly with sex, age and island of origin

Table 3. Distribution of all sampled platypuses among seasons and catchment reaches

Sample size (*n*) is indicated along with the percentage of the total sample. Stream orders for five animals obtained from the public in autumn and one in winter were ambiguous, and are not included in analyses

Season	Catchment reaches (Strahler stream orders)			*n* (% of total)
	Upper (1–3)	Mid (4–5)	Lower (6–7)	
Spring	6	52	1	59 (31%)
Summer	6	46	3	55 (29%)
Autumn	7	29	24	60 + 5 (33%)
Winter	6	7	2	15 + 1 (8%)
n (% of total)	25 (13%)	134 (71%)	30 (16%)	195

Table 4. Distribution of all sampled platypuses among seasons and catchment disease status

The number of sampled individuals (*n*) is indicated along with the percentage of the total

Season	Catchment disease status				*n* (% of total)
	No evidence	Possibly affected	Historically affected	Currently affected	
Spring	–	8	13	38	59 (30%)
Summer	21	27	–	7	55 (28%)
Autumn	3	20	21	21	65 (34%)
Winter	3	6	3	4	16 (8%)
n (% of total)	27 (14%)	61 (31%)	37 (19%)	70 (36%)	195

Table 5. Mean (±s.e.) body length and bodyweight of male and female platypuses sampled from mainland Tasmania and King Island

Island	Sex and age categories	Body length (cm)	Body weight (g)	*n*
Tasmania	Adult male	54.8 ± 0.4	2154 ± 33	92
Tasmania	Adult female	44.9 ± 0.4	1232 ± 23	53
King	Adult male	48.9 ± 0.8	1736 ± 35	7
King	Adult female	42.4 ± 0.4	1109 ± 23	12

Table 6. One-way ANOVAs sequentially investigating factors influencing body length in Tasmanian platypuses
Where significant differences existed we continued examining the largest subsets of remaining data. Groups with significantly longer bodies are indicated in bold as determined by Tukey's HSD post hoc test. * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$

Source		d.f.	MS	*F* ratio	*P*
1. Sex (**males** vs females)	Sex	1	4109.1	223.88	0.0000***
	Error	191	18.4		
2. Age (**adult** vs juvenile)	Age	1	796.5	53.37	0.0000***
	Error	118	14.9		
3. Island (King vs **Tasmania**)	Island	1	233.4	19.80	0.0000***
	Error	97	11.8		
4. Season (spring, summer, autumn, winter)	Season	3	2.2	0.18	0.9119
	Error	88	12.6		
5. Catchment reaches (upper, mid, **lower**)	Stream order	2	37.7	3.37	0.0389*
	Error	86	11.2		
6. Catchments (Cam, Black-Dention, Forester-Brid, Huon, Upper Derwent, Lower Derwent, Mersey, Meander, North Esk, Pipers, Brumbys-Lake	Catchments	11	7.3	0.56	0.8538
	Error	56	13.0		
7. Disease status (currently, historically, possibly, no evidence)	Disease status	3	10.0	0.84	0.4773
	Error	66	11.9		

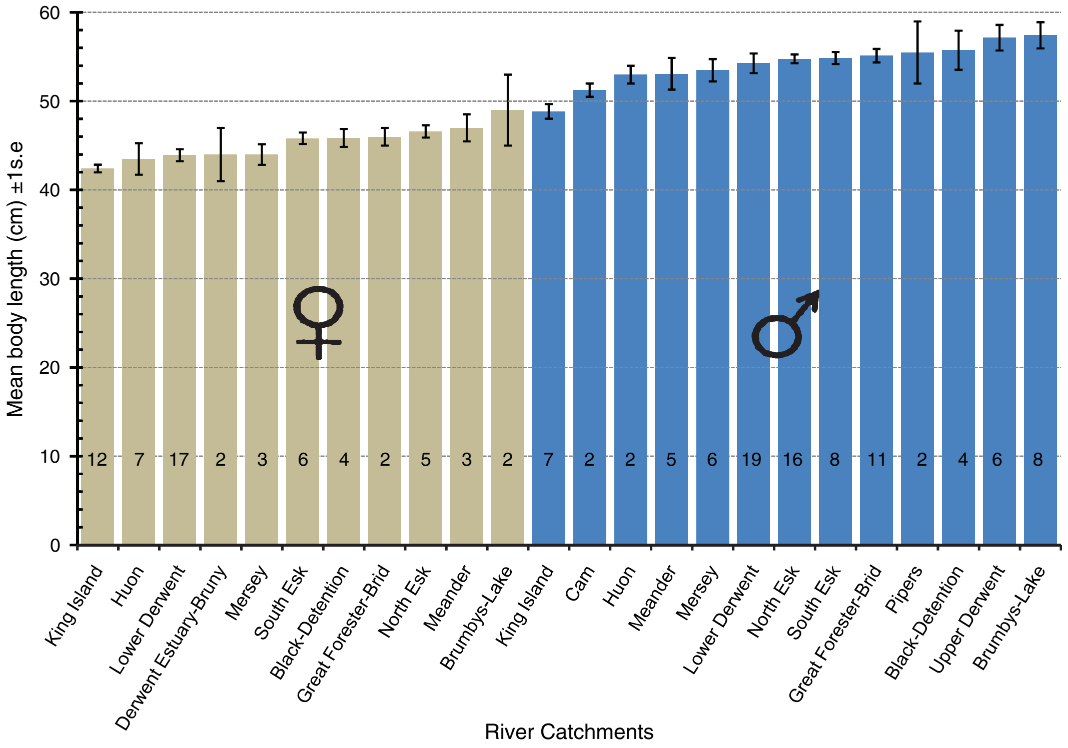

Fig. 2. Mean body lengths (±1 s.e.) for adult female (♀) and adult male (♂) platypuses across Tasmanian river catchments. Sample sizes (*n*) indicated for each.

(Table 6). Males were longer than females, adults longer than juveniles and Tasmanian animals longer than those on King Island (Table 6). Although mean adult male body length varied by up to 8.5 cm or 17% between catchments (Fig. 2), there were no statistically significant differences in their length attributable to either catchments or catchment disease status (Table 6). The order of catchments, based on increasing mean length (Fig. 2) and weight (Fig. 3), remained similar, with the exception of adult males in the Meander catchment, which were ranked second on the basis of mean weight, but tenth on the basis of mean length. There was a trend in mean length of adult males in Tasmania among catchment reaches, with individuals in lower catchment reaches being on average 2 cm longer than those in mid and upper reaches (Table 6, Fig. 4).

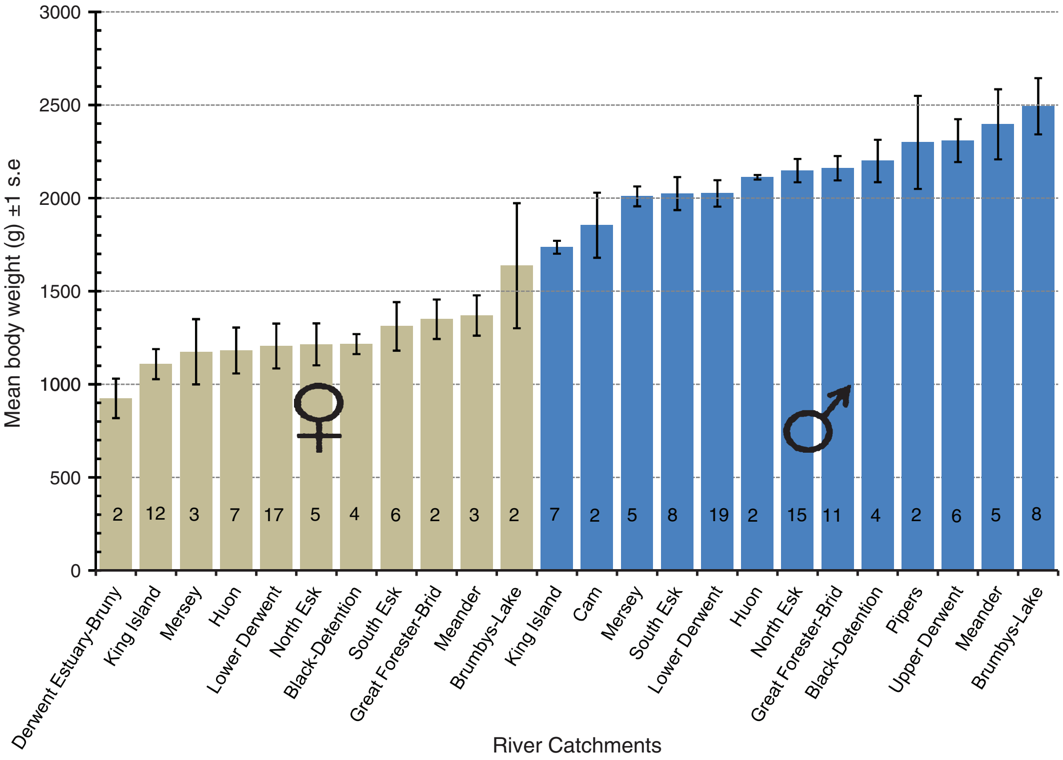

Fig. 3. Mean bodyweights (±1 s.e.) for adult female (♀) and adult male (♂) platypuses across Tasmanian river catchments. Sample sizes (*n*) indicated.

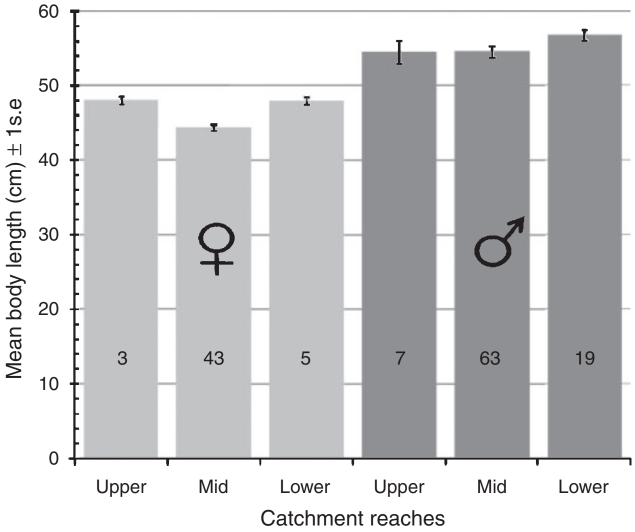

Fig. 4. Mean body lengths (±1 s.e.) for adult female (♀) and male (♂) platypuses across catchment reaches in Tasmania (excludes smaller King Island animals). Sample sizes (*n*) indicated.

Sequential one-way ANOVAs indicated that platypus bodyweight also differed significantly with sex, age and island of origin (Table 7). Males were heavier than females, adults heavier than juveniles and Tasmanian animals heavier than those on King Island (Table 7). Mean adult male weight did not differ significantly among river reaches (Fig. 5), seasons or with catchment disease status. However, there was a significant difference in mean adult male weight among catchments. Tukey's HSD tests indicated that males in the Brumbys–Lake catchment were significantly heavier than those in the other sampled catchments (Table 7). On average, adult males sampled from the Brumbys–Lake catchment were 758 g or 44% heavier than the lightest adult males from King Island (Fig. 3). Adult female bodyweight also varied considerably between catchments, with a mean weight difference of up to 712 g (77%) between individual catchments (Fig. 3); however, small sample sizes precluded detailed spatial analysis for adult females.

Body condition (TVI)

Tasmanian and King Island platypuses typically had moderate TVI values. Of the 195 animals sampled, 189 (97%) had TVI values from two to four. Only two individuals (1%) were in excellent condition (TVI less than two) and four individuals (2%) were emaciated (TVI greater than four). The mean adult TVI was 2.91 ± 0.05. Juveniles had significantly higher mean TVI values (3.28 ± 0.13), indicating lower tail fat reserves than adults. The TVI of adult males (2.74 ± 0.08) was significantly lower than adult females (3.14 ± 0.11) over the course of the study (Table 8), and this better condition was particularly notable in winter (Fig. 6). After sequential removal of sources of significantly different TVI data (juveniles, females and winter respectively), we investigated differences in TVI for adult males among catchment reaches. In upper catchment reaches mean adult male TVI (2.57 ± 0.24) was lower than in mid (2.90 ± 0.07) or lower (3.21 ± 0.14) reaches (Table 8, Fig. 7).

Table 7. One-way ANOVAs sequentially investigating factors influencing bodyweight in Tasmanian platypuses

Groups with significantly heavier bodyweights are indicated in bold as determined by Tukey's HSD post hoc tests. Where significant differences existed we continued examining the largest subsets of remaining data. * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$

Source		d.f.	MS	*F* ratio	*P*
1. Sex (**males** vs females)	Sex	1	34083833	289.45	0.0000***
	Error	188	117754		
2. Age (**adult** vs juvenile)	Age	1	6386801	62.78	0.0000***
	Error	115	101737		
3. Island (King vs **Tasmania**)	Island	1	1135495	12.32	0.0007***
	Error	95	92133		
4. Season (spring, summer, autumn, winter)	Season	3	3405	0.03	0.9916
	Error	86	101073		
5. Catchment reaches (upper, mid, lower)	Stream order	2	134123	1.47	0.2364
	Error	84	91418		
6. Catchments (Cam, Black-Dention, Forester-Brid, Huon, Upper Derwent, Lower Derwent, Mersey, Meander, North Esk, Pipers, **Brumbys-Lake**)	Catchments	11	194936	2.35	0.0152*
	Error	75	83117		
7. Disease status (currently, historically, possibly, no evidence)	Disease status	3	128965	1.67	0.1806
	Error	78	77284		

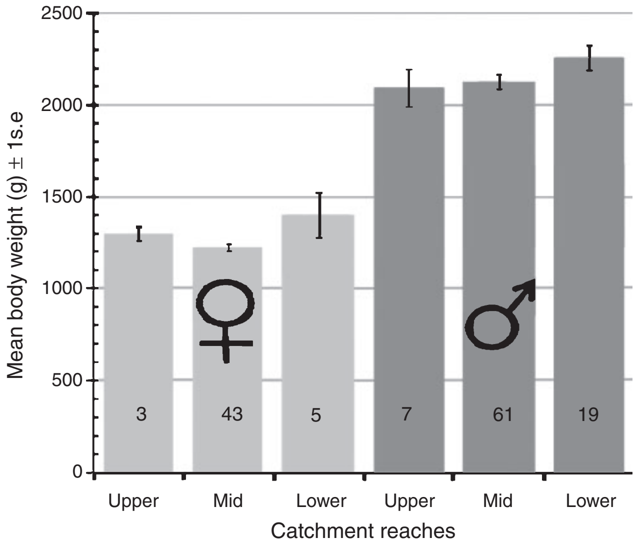

Fig. 5. Mean bodyweights (±1 s.e.) for adult female (♀) and male (♂) platypuses across catchment reaches in Tasmania (excludes smaller King Island animals). Sample sizes (*n*) indicated.

Furthermore, adult male TVI differed among catchments (Table 8), with Tukey's HSD tests indicating poorer condition and higher TVI values in the Brumbys–Lake catchment than in the other catchments investigated. There was no evidence that TVI was significantly influenced by either catchment disease status or island origin in the reduced dataset for adult males (Table 8).

Moult status

Moult status was assessed in 189 platypuses and both sexes displayed the full range of moult classes (Fig. 8). The most common moult status for both sexes was 'none', (≈30% of individuals, $n = 59$), while 'heavy moult' was the least common observation, applying to seven individuals representing less than 5% of those sampled (Fig. 8). Mean moult status did not differ significantly with age or sex (Fig. 8); however, there was a significant seasonal pattern to moulting (Table 9, Fig. 9). Although some individuals moulted throughout the year, there was a clear peak in spring, when 70% of the population showed light, moderate or heavy moult classes (Fig. 9). Moulting appeared to be largely complete by autumn, when only 13% of individuals showed light, moderate or heavy moult classes (Fig. 9). No differences in moult status were detected between catchment reaches, or with catchment disease status, although King Island individuals sampled in January displayed lower mean moult status that animals from other catchments (Table 9). There was also a significant relationship (ANOVA $F_{(4,\ 156)} = 3.9135$, $P = 0.0047$**) between adult moult status and mean TVI. Typically as moult class increased, mean tail fat reserves decreased (Fig. 10). Individuals that were not moulting typically had the highest tail fat reserves, while animals moulting heavily also had the lowest tail fat reserves (Fig. 10).

Population sex ratios

Chi-square tests over the entire sample indicated that the sex ratios for adults (1.54M : 1F, $\chi^2 = 7.42$, d.f. = 1, $P = 0.006$**) and juveniles (2.63M : 1F, $\chi^2 = 5.83$, d.f. = 1, $P = 0.016$*) were both significantly male-biased (Fig. 11*A*). Although adult sex ratios were male-biased across all catchment reaches (Fig. 11*B*), statistical divergence from parity was only detected in lower catchment reaches where males outnumbered females by almost four to one. There was a suggestion of seasonal differences in adult sex ratios, with significantly male-biased sex ratios in both spring (1.78M : 1F, $\chi^2 = 3.92$, d.f. = 3, $P = 0.048$*) and autumn (2.25M : 1F, $\chi^2 = 7.69$, d.f. = 3, $P = 0.006$**), but not in summer or winter (Fig. 11*C*). Sex ratios were significantly male-biased in both 'currently affected' catchments (2.31M : 1F, $\chi^2 = 8.32$, d.f. = 3, $P = 0.004$**) and 'historically affected' catchments (2.30M : 1F, $\chi^2 = 5.12$, d.f. = 3, $P = 0.024$*), but did not differ

Table 8. One and two-way ANOVAs sequentially investigating factors influencing body condition (tail volume index, TVI) in platypuses

Groups indicated in bold displayed significantly different TVI values from the other groups tested (as determined by Tukey's HSD post hoc tests. Where significant differences existed we continued examining the largest subsets of remaining data. * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$

Source		d.f.	MS	*F* ratio	*P*
1. Age (adult vs **juvenile**)	Age	1	3.2568	7.080	0.0084**
	Error	193	0.4600		
2. Season and sex (season, **sex**, spring vs sex, summer vs sex, autumn vs sex, **winter vs sex**)	Season	3	0.486	1.072	0.3622
	Sex	1	3.3820	8.552	0.0040**
	Sex*Season	3	2.0325	5.140	0.0020**
	Error	158	0.3954		
3. Catchment reaches (**upper**, mid, lower)	Stream order	2	1.2642	3.598	0.0315*
	Error	87	0.3514		
4. Catchments (Upper Derwent, Lower Derwent, Huon, King, Black-Denention, Mersey, South Esk, North Esk, Great Forester Brid, **Brumbys-Lake**)	Catchments	9	0.9129	3.127	0.0034**
	Error	66	0.2919		
5. Disease status (currently, historically, possibly, no evidence)	Disease status	3	0.0239	0.071	0.9751
	Error	72	0.3345		
6. Island (King vs Tasmania)	Island	1	0.6317	1.987	0.1628
	Error	74	0.3179		

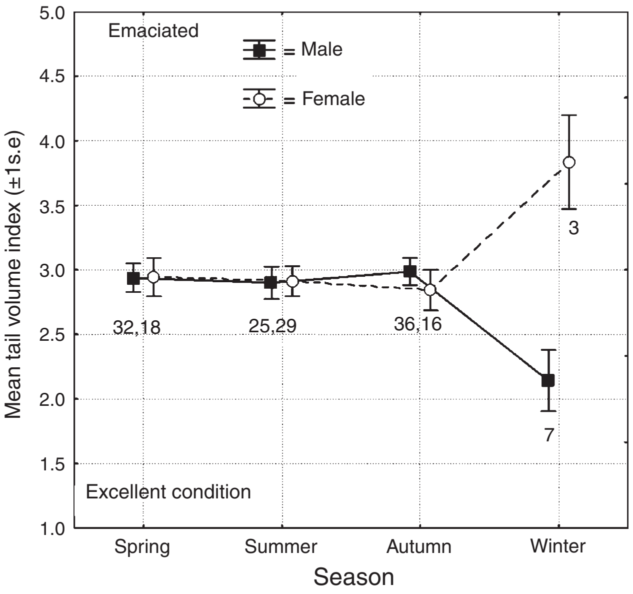

Fig. 6. Mean tail volume index (±1 s.e.) for adult males and females sampled across seasons. Sample sizes (*n*) indicated.

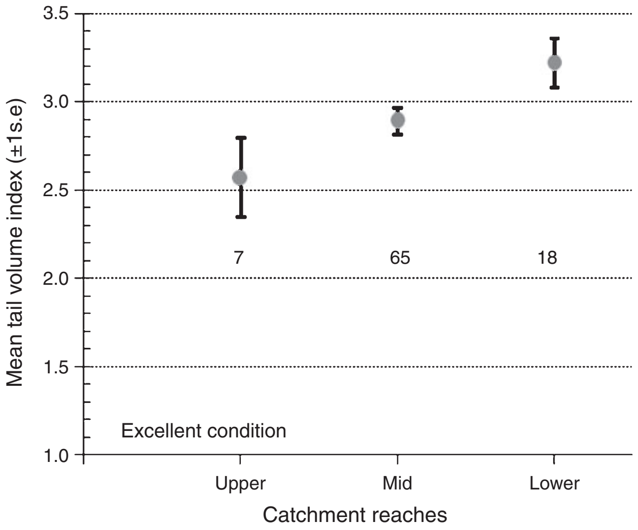

Fig. 7. Mean tail volume index (±1 s.e.) for adult males sampled across catchment reaches. Sample sizes (*n*) indicated.

significantly from parity in either 'possibly affected' or 'no evidence' catchments (Fig. 11*D*). There was no evidence to support the hypothesis that currently or historically affected catchments were relatively female-biased compared with unaffected catchments. The majority of reported sex ratios for adult platypuses across Australia do not significantly differ from parity (Table 10), with a few notable exceptions. A female-biased sex ratio (0.71M : 1F) was reported from the Shoalhaven River on mainland Australia (Grant 2004*a*). Male-biased sex ratios have been reported in Tasmania by Stewart (2001) (1.83M : 1F), Bethge (2002) (1.63M : 1F) and in the current study (1.50M : 1F). Pooled data from seven Tasmanian platypus studies suggest Tasmanian platypus populations are significantly male-biased with an overall sex ratio of 1.39M : 1F (Table 10).

Population and male age structures

Of 195 individuals sampled, 66 were adult females (34%), 8 juvenile females (4%), 13 old adult males (7%), 87 adult males (45%) and 21 juvenile males (11%). The proportion of juveniles in 'currently affected' catchments ($n = 16$, 30%) was at least twice as high as in catchments of the other three disease

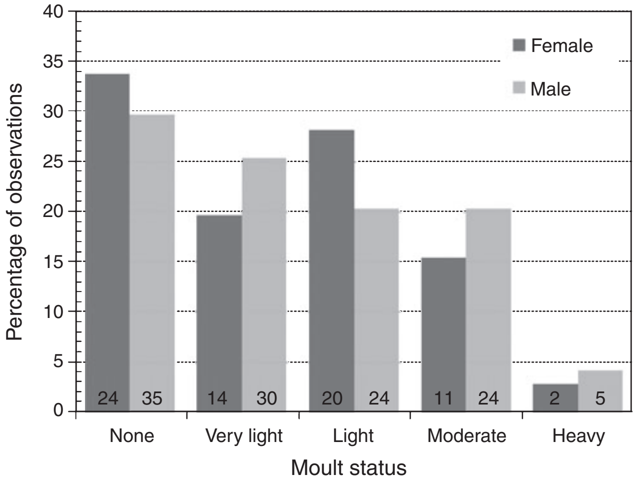

Fig. 8. Percentage of each sex across the five moult status categories. Raw frequencies of males and females in each moult class also shown.

status categories ($n = 2$, 15%; $n = 1$, 3%; and $n = 2$, 8%; Fig. 12). The proportion of old adult males in 'currently affected' catchments ($n = 2$, 4%) was at least 3-fold lower than values in the other three disease status categories ($n = 2$, 15%, $n = 4$, 13% and $n = 5$, 20%, Fig. 12). Chi-square tests indicated a significant difference in the proportions of male juveniles, adults and old adults with catchment disease status ($\chi^2 = 15.4$, d.f. = 6, $P = 0.017$*, Fig. 12).

Demographics of disease and injury

The majority (90%) of the 183 animals trapped during this study appeared to be healthy (Table 11). Three adult females inspected by a wildlife veterinarian displayed small dorsal scabs suspected to be caused by ringworm. Nine individuals had significant wounds or injuries, though none were entangled in fishing line or rubbish, or showed signs of past entanglement. Mortality in 11 of the 12 carcasses obtained from the public (92%) was attributed to trauma associated with dogs, cars, fishing nets or unknown sources. Of the total 195 animals sampled, only six adult males and one adult female showed clinical signs consistent with mucormycosis. These mucormycosis-affected animals were 6 : 1 male-biased, all affected animals were adults (Table 11), although large confidence intervals existed around the estimates and Chi-square tests of sex and age trends of disease were non-significant (Fig. 13*A*, *B*). Similar non-significant trends in the demographics of ulceration were previously reported by both Connolly *et al.* (1998) and Stewart (2001). Pooling their historically reported data with the contemporary data suggested that, over time, adult males are approximately twice as likely to be clinically affected with mucormycosis than adult females ($\chi^2 = 3.86$, d.f. = 1, $P = 0.049$*, Fig. 13*C*) and that adults are affected more frequently than juveniles ($\chi^2 = 7.21$, d.f. = 1, $P = 0.007$**, Fig. 13*D*).

Discussion

This study provides the most comprehensive and systematic multi-scale assessment of Tasmanian platypus morphometrics and demographics conducted to date. Data from extensive live-trapping surveys, augmented with necropsy information and previously reported mucormycosis studies, enabled a detailed analysis of natural spatial and seasonal variability in platypus body size, condition, population structure and mucormycosis epidemiology. The magnitude of natural variability within and between catchments was quantified, and enabled investigation of mucormycosis impacts. A sample size of 195 individuals sampled over a period of 18 months enabled comparisons of morphometric and demographic characteristics within and between river catchments, and facilitated examination of seasonal influences and disease impact hypotheses.

Body size

Platypus body size, in particularly weight and to a lesser extent length, was found to vary markedly over a range of spatial scales, but not between seasons in Tasmania. To avoid demographic confounding of patterns and remove the effects of sexual

Table 9. One-way ANOVAs sequentially investigating factors influencing moult condition in platypuses

Groups with significantly heavier moult condition are indicated in bold (as determined by Tukey's HSD post hoc tests). Where significant differences existed we continued examining the largest subsets of remaining data. * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$

Source		d.f.	MS	*F* ratio	*P*
1. Age (adult vs juvenile)	Age	1	2.2093	1.512	0.2204
	Error	187	1.4611		
2. Sex (male vs female)	Sex	1	0.4670	0.318	0.5737
	Error	187	1.4700		
3. Season (spring, summer, **autumn,** winter)	Stream order	3	21.4254	18.771	0.0000***
	Error	187	1.1414		
4. Catchment reaches (upper, mid, lower)	Reaches	2	2.4000	1.800	0.1696
	Error	122	1.3000		
5. Catchments (Lower Derwent, Derwent Estuary-Bruny, Huon, **King Island,** Black-Denention, Cam, Meander, South Esk, North Esk, Brumbys-Lake Great Forester Brid)	Catchments	10	3.3403	2.916	0.0027**
	Error	114	1.1456		
6. Disease status (currently, historically, possibly, no evidence)	Disease status	3	1.9000	1.600	0.1859
	Error	103	1.2000		

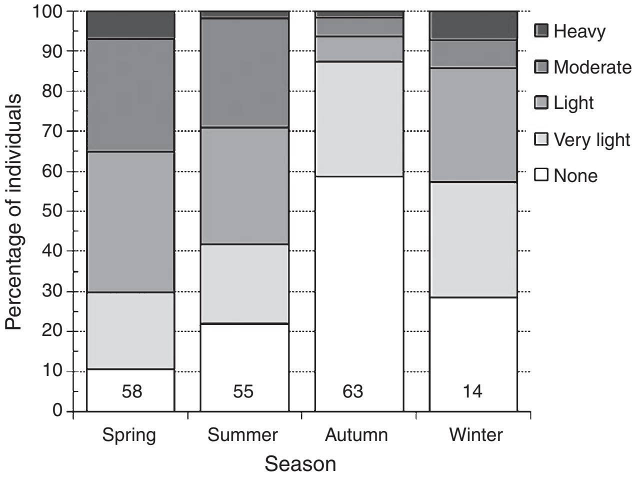

Fig. 9. Seasonal moulting pattern in Tasmanian platypuses. Sample sizes (*n*) indicated.

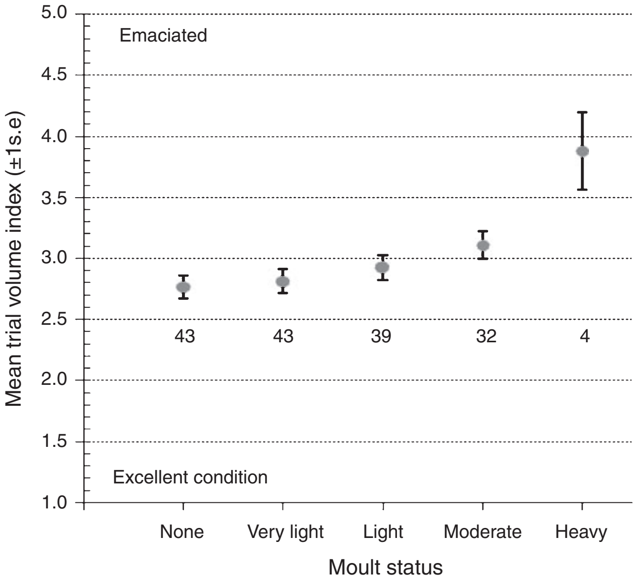

Fig. 10. Relationship between mean tail volume index (±1 s.e.) and moult status for adult platypuses. Sample sizes (*n*) indicated.

dimorphism, we examined spatial and seasonal trends in body size by focusing on adult males. At the largest scale investigated, platypus body size differed significantly between King Island and Tasmania. King Island platypuses were previously thought to be unusually small on an Australia-wide scale (Stewart 2001). However, animals sampled in 2009 were a very similar size to those reported from the Murrumbidgee River in NSW (Grant 2007), a location roughly mid-way in the species' latitudinal distribution and size range. Platypus size in Tasmania varies markedly among river catchments, as previously noted by Koch *et al.* (2006). The differences noted within Tasmania in the current study were almost twice the magnitude of differences previously documented for platypuses between eastern- and western-flowing rivers in NSW (Grant 2007). Large differences in body size between river catchments suggest differing platypus growth curves exist over scales of tens to hundreds of kilometres, potentially correlated with differences in prey abundance or benthic productivity between catchments. Although it was suggested almost thirty years ago that platypus body size differences may be due to different benthic productivities of water bodies (Grant and Temple-Smith 1983), to our knowledge this is yet to be tested. It is thus currently unclear why adult male platypuses from the Brumbys–Lake catchment were significantly heavier than those sampled from the other 17 catchments, although their exceptional mean size suggests they are the largest free-living Australian platypuses.

Within Tasmanian river catchments mean adult male platypus length increased downstream. Adult males in order six and seven streams were longer, but not necessarily heavier than those in orders four and five, or orders one, two and three. Widespread differences in body size suggest that lower catchment reaches represent optimal platypus habitat in Tasmania as hypothesised by Koch *et al.* (2006). Studies of platypus distribution within river catchments on the mainland (e. g. Turnbull 1998; Rohweder and Baverstock 1999) and in Tasmania (Koch *et al.* 2006; Olsson Herrin 2009) suggest platypuses are less abundant in first order headwater streams. Optimal platypus habitat identified in several studies consists of permanent water bodies with relatively steep, consolidated earth banks, overhanging native riparian vegetation, abundant invertebrate prey and pools with water less than 5 m deep (Ellem *et al.* 1998; Serena *et al.* 1998; Serena *et al.* 2001; Grant 2004*b*). Such habitat is predominantly found in the middle and lower parts of river catchments in Tasmania (Koch *et al.* 2006), and is reflected by a trend of increasing body size further downstream in catchments.

Condition

Pooling tail fat data over age or sex classes has the potential to mask important differences within the population, as condition can differ among demographic classes (Grant 2007). Past platypus research has often had insufficient sample sizes to assess seasonal differences in platypus body condition or moult status within equivalent age and sex classes without pooling data over multiple years. Subtle but statistically significant differences in TVI were detected in Tasmania on the basis of age, sex, season and among catchment reaches. On average, juveniles were in poorer condition than adults, which is not surprising given their smaller body size, reduced foraging experience and likely competition for resources with adults and other juveniles. Adult Tasmanian platypuses of both sexes in 2008–09 had relatively consistent tail fat stores through spring, summer and autumn. Given the high latitude and strongly seasonal climate of Tasmania this consistency was surprising. Conversely, considerable seasonal variability in TVI has been reported from studies at lower latitudes in Victoria (Gust and Handasyde 1995) and NSW (e.g. Grant and Temple-Smith 1983; Hulbert and Grant 1983).

In winter, mean tail fat stores of adult Tasmanian platypuses differed substantially between the sexes, when females lost condition and males increased fat stores. The observed decline

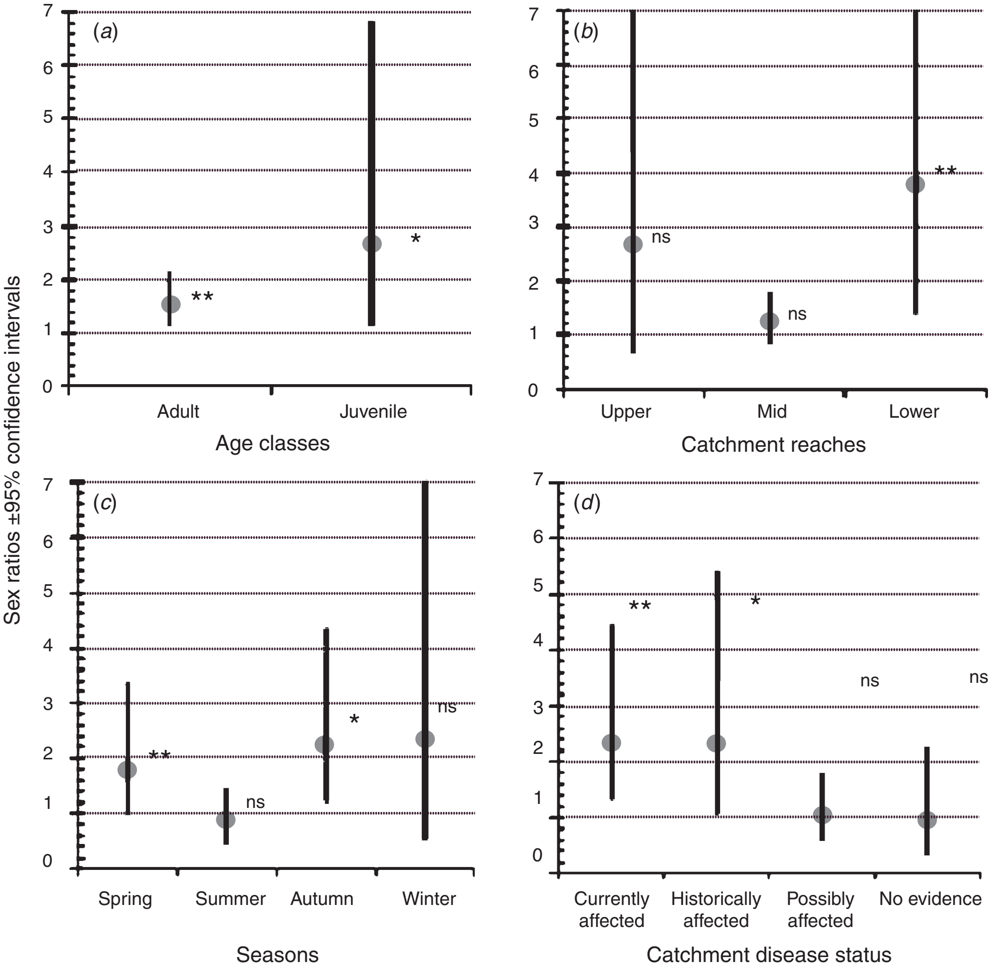

Fig. 11. Sex ratios compared across: (*a*) age classes, (*b*) adults across catchment reaches, (*c*) adults across seasons and (*d*) adults in catchments pooled by disease status. The ratio of males to females in the sample is indicated (±95% confidence intervals). Results of χ^2 tests are shown for each comparison. Sex ratios that do not significantly differ from parity (i.e. 1) are marked 'NS', while significant differences are marked * if $P < 0.05$, and ** if $P < 0.01$.

in winter for adult females probably reflects depleted energy reserves associated with the end of lactation (Grant 2007), which has very high energy costs in marsupials (Tyndale-Biscoe 2005). Previous studies on mainland Australia have described declining platypus weight and condition during winter when temperatures decrease, the photoperiod is shorter and food availability can decline (Grant and Carrick 1978; McLachlan-Troup 2007). However, seasonal trends are inconsistent between study areas and sampling years (Grant 2007). In cold Tasmanian rivers in autumn and winter females show a significant increase in energy expenditure (Munks *et al*. 2000), which may reflect an increased energetic cost of foraging and a possible reduction in food availability. Platypus food consumption in captivity can peak in winter (Krueger *et al*. 1992; McLachlan-Troup 2007), and radio-tracked Tasmanian platypuses foraged for an hour longer in winter than in summer (Bethge *et al*. 2009), presumably to acquire sufficient food. As such, it was surprising that adult males in the current study appeared to increase fat storage over winter, although this pattern was previously documented on the dam-controlled Goulburn River in Victoria (Gust and Handasyde 1995). Adult males had significantly improved tail fat stores in upper catchments than in mid or lower reaches, which is intriguing and inconsistent with the hypothesis that upper catchment reaches represent marginal habitats in Tasmania (Koch *et al*. 2006).

This study described demographic and seasonal patterns in moulting among Tasmanian platypuses for the first time. Moulting patterns did not differ among sexes or ages, and did not reflect the catchment reach or disease status of the catchments sampled, but were clearly seasonal. Moulting peaked in spring and was largely completed in autumn, so that maximum coat condition and guard hair coverage was achieved in winter. This pattern seems intuitive for a species subject to prolonged bouts of foraging in very cold water in winter. Nevertheless, moulting is a gradual process in this species and at no stage of the year is the fur changed significantly (Grant 2007). Moult condition and TVIs were related; individuals in heaviest moult typically displayed the poorest tail fat reserves. This may reflect increased heat loss or energy expenditure for thermoregulation when fur thickness is reduced.

Table 10. Sex ratios for adult platypuses reported on mainland Australia (italics) and Tasmania

Disease status is defined in the Materials and methods and is applied at the time of each study, where NE, no evidence; PA, possibly affected; HA, historically affected; CA, currently affected. The number of adult females (F) and adult males (M) are indicated along with the total sample size (*n*), sex ratio, Chi-square (χ^2) statistic and *P* value. Sources: 1, Gardner and Serena 1995; 2, Connolly *et al*. 1998; 3, Stewart 2001; 4, Bethge 2002; 5, Grant 2004*a*; 6, Koch *et al*. 2006; 7, McLachlan-Troup 2007; 8, McGregor 2008; 9, Olsson Herrin 2009; 10, present study. * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$

Study locations (state)	Disease status	M	F	*n*	M : F	χ^2	*P*	Source
Watts River, Badger Creek, Vic.	NE	17	12	29	1.42 : 1	0.862	0.353	1
Brogers Creek, Kangaroo River, NSW	NE	34	44	78	0.77 : 1	1.282	0.257	7
Wingecarribee Rivers, NSW	NE	30	29	59	1.03 : 1	0.017	0.896	5
Barnard River, NSW	NE	22	24	46	0.92 : 1	0.087	0.768	5
Various streams, NSW and ACT	NE	101	117	218	0.86 : 1	1.174	0.278	5
Thredbo River, NSW	NE	10	14	24	0.71 : 1	0.667	0.414	5
Shoalhaven River, NSW	NE	177	292	469	0.61 : 1	28.198	0.000***	5
Brumbys-Lake, Tas.	CA	22	12	34	1.83 : 1	2.941	0.086	2
Brumbys-Lake, North Esk, Mersey, Tas.	CA	32	17	49	1.88 : 1	4.592	0.032*	3
Lower Derwent, Lake Pedder, Tas.	NE	13	8	21	1.63 : 1	1.190	0.275	3
Lake Lea, Tas.	PA	30	15	45	2.00 : 1	5.000	0.025*	4
Lower Derwent, Tas.	PA	12	7	19	1.71 : 1	1.316	0.251	4
South Esk, Tas.	CA	35	34	69	1.03 : 1	0.014	0.906	6
Inglis and Emu, Tas.	PA	15	23	38	0.65 : 1	1.684	0.194	8
Lower Derwent, Tas.	PA	9	11	20	0.82 : 1	0.200	0.655	9
King Island	NE	7	12	19	0.58 : 1	1.316	0.251	10
Brumbys-Lake, Meander, North Esk, South Esk, Tas.	CA	37	16	53	2.31 : 1	8.321	0.004**	10
Black-Detention, Great Forester-Brid, Mersey, Pipers, Tas.	HA	23	10	33	2.30 : 1	5.121	0.024*	10
Derwent Estuary-Bruny, Huon, Inglis, Lower Derwent, Upper Derwent, Tas.	PA	29	28	57	1.04 : 1	0.018	0.893	10
Cam, Gordon-Franklin and King-Henty, Tas.	NE	11	12	23	0.92 : 1	0.043	0.836	10
Tasmania (18 river catchments)	All	268	193	461	1.39 : 1	12.202	0.000***	2, 3, 4, 6, 8, 9, 10

Demographic structures (sex ratio, age structures)

Sampled Tasmanian platypus populations displayed significantly male-biased sex ratios for both adults and juveniles. Although males and females are assumed to have equal probability of capture, adult sex ratios were male-biased in spring and autumn, but not summer or winter. This raises the possibility of seasonal differences in behaviour among the sexes influencing their chances of being captured. Adult females, for instance, may be less likely to be caught when they are incubating eggs in burrows, while individuals that forage over large distances are more likely to encounter, and be trapped in nets than those with small home range sizes. Nevertheless, demographic data compiled from live-trapping studies conducted widely across Tasmania over a range of seasons and the last 15 years also indicated a significantly male-biased sex ratio (average 1.39M : 1F). Together this data suggests Tasmanian platypus population structures are unusual in the Australian context, as the majority of studies report sex ratios that do not differ significantly from parity, although a study in NSW reported a female-biased sex ratio (Grant 2004*a*). Sex ratios in mammals have been attributed to a wide variety of factors including, but not limited to variation in: local population density; temperature and other climatic factors; the competitive ability of the sexes; nutritional status of the breeding female; and differences in prevailing predation pressure (Skalski *et al*. 2005). It is currently unclear whether any of these factors can explain the observed patterns, or whether male-biased sex ratios in Tasmania are simply a reflection of behavioural differences between the sexes that increase the chance of males being captured.

It was hypothesised that if mucormycosis is more prevalent in adult males, and is also reflected in higher mortality rates, then affected populations may become relatively female-biased and/or contain fewer old adult males than unaffected populations. There was no evidence to suggest that female-biased sex ratios occur in mucormycosis-affected catchments, in fact they were significantly male biased. Female-biased sex ratios now seem unlikely, as this scenario invokes the prospect of a major disease epidemic, with adult male mortality rates greatly elevated over adult females. It seems more likely that the disease, which has dropped in prevalence 4-fold since the mid-1990s (Gust *et al*. 2009), now exerts a low-level, ongoing influence on affected Tasmanian populations. Mucormycosis may now influence populations by gradually removing affected individuals, particularly adult males, resulting in a higher turnover of males in affected populations than in areas unaffected by disease. Patterns in male age structures documented in this study are consistent with this hypothesis,

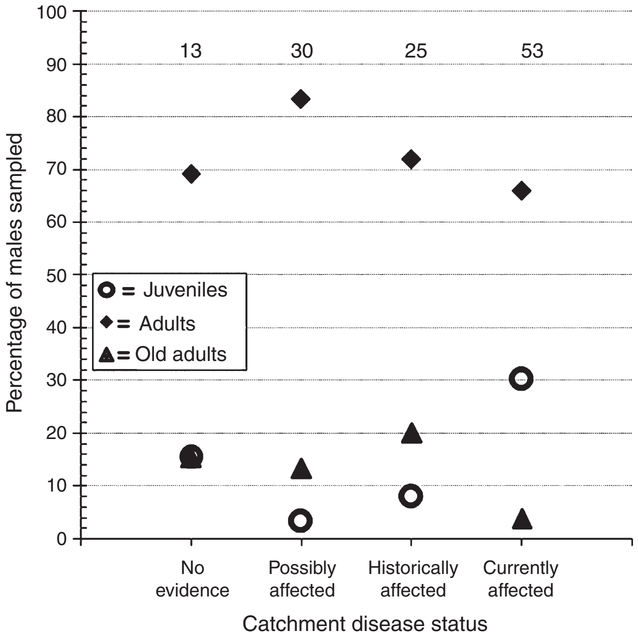

Fig. 12. Age based composition of male Tasmanian platypuses compared across catchments with differing disease status. Sample sizes (*n*) indicated.

Table 11. The health of 183 captured platypuses, and sources of mortality determined by necropsy for 12 individuals
A, Adult; J, Juvenile; M, Male; F, Female

Health status and causes of mortality	Sex and age	*n*
Apparently healthy	84 AM, 55 AF, 19 JM, 6 JF	165
Ulcerated	5 AM, 1 AF	6
Dorsal scabs – possibly ringworm	3 AF	3
Open tail wounds >2 cm long	2 AM, 1 AF	3
Open leg wounds >2 cm long	2 AF, 1 JM	3
Open bill wounds >2 cm long	2 AM	2
Missing spur	1 AM	1
Dead – unknown trauma	1 AM, 2 AF, 1 JF	4
Dead – road-kill	1 AM, 1 AF, 1 JF	3
Dead – dog attack	1 AF, 1 JM	2
Dead – drowned in fishing net	2 AM	2
Dead – clinical signs of mucormycosis	1 AM	1
Total	100 AM, 66 AF, 21 JM, 8 JF	195

where affected populations had 3-fold reductions in the proportion of old males and 4-fold higher proportions of juveniles than catchments historically or possibly affected, or those with no evidence of disease. Further investigations into the influences of habitat quality on the age structures of platypus populations, and more data on female infections would assist interpretation of these findings.

Demographics of disease and injury

Local differences in the size, age and sex structures of populations can reflect spatial differences in growth, longevity, mortality or movement between local populations (Gust 2004). Difficulty in capturing platypuses has often precluded detailed demographic assessment because large sample sizes are rarely achieved over short time frames. This study is the first to characterise platypus population structure at a range of scales across Tasmania. Demographic trends for platypuses with clinical signs of mucormycosis were investigated across affected catchments during the current study, and considered data from affected locations studied in Tasmania during mid and late 1990s (Connolly *et al.* 1998; Stewart 2001). In each of the three epidemiological studies adult males were the most commonly affected individuals, but small sample sizes limited power to detect significant patterns. Pooled data across the studies revealed that adults display clinical signs of mucormycosis more frequently than juveniles, and that adult males are affected at roughly twice the frequency of adult females. The routes of infection that enable the fungal pathogen *M. amphibiorum* to affect individual platypus are currently unclear. Contamination of skin wounds is one suggestion to provide the initial route of infection (Obendorf *et al.* 1993), although several other plausible possibilities exist (Gust and Griffiths 2009). Higher adult male infection rates could reflect a higher frequency of skin wounds in these individuals, and if *M. amphibiorum* is present in the environment (in water or soil), it could infect them via contaminating skin abrasions, spur wounds or cuts. Wider ranging in adult males may lead to greater exposure to a patchily distributed environmental pathogen, although the route of mucormycosis infection and mechanism of spread remain to be established.

Unfortunately little is known about the causes and incidence of mortality in platypuses (Grant 2004*a*). Existing examinations of mortality in Tasmanian platypuses, including the current study and Connolly *et al.* (1998), are limited by methodology, as most free-living animals that die are not recovered for autopsy. Apportioning relative mortality rates on the basis of autopsies potentially underestimates the relative importance of mucormycosis (Gust and Griffiths 2009). For instance, members of the public are more likely to find a platypus carcass if it is killed on a road, or by their dog, than one that has succumbed to mucormycosis in its burrow or somewhere along a waterway with limited human access. The frequency of reported mortality events is thus unlikely to accurately represent the relative importance of all sources. Nevertheless, if adult males are affected at twice the frequency of adult females, then significantly increased mortality in adult males may explain observed differences in male age structure among catchments of varying disease status. Specifically, mucormycosis is implicated in the reduced frequency of old males in currently affected catchments, compared with catchments with little or no evidence of mucormycosis. Similarly, the 4-fold higher proportion of juveniles in male age structures in currently affected catchments suggests a more rapid turnover of males in these locations, and raises the possibility of differing population dynamics between affected and unaffected catchments.

Results from a recent investigation into chytrid disease in Australian frogs cautioned that wild amphibian populations can face significant ongoing pressure (and mortality) from

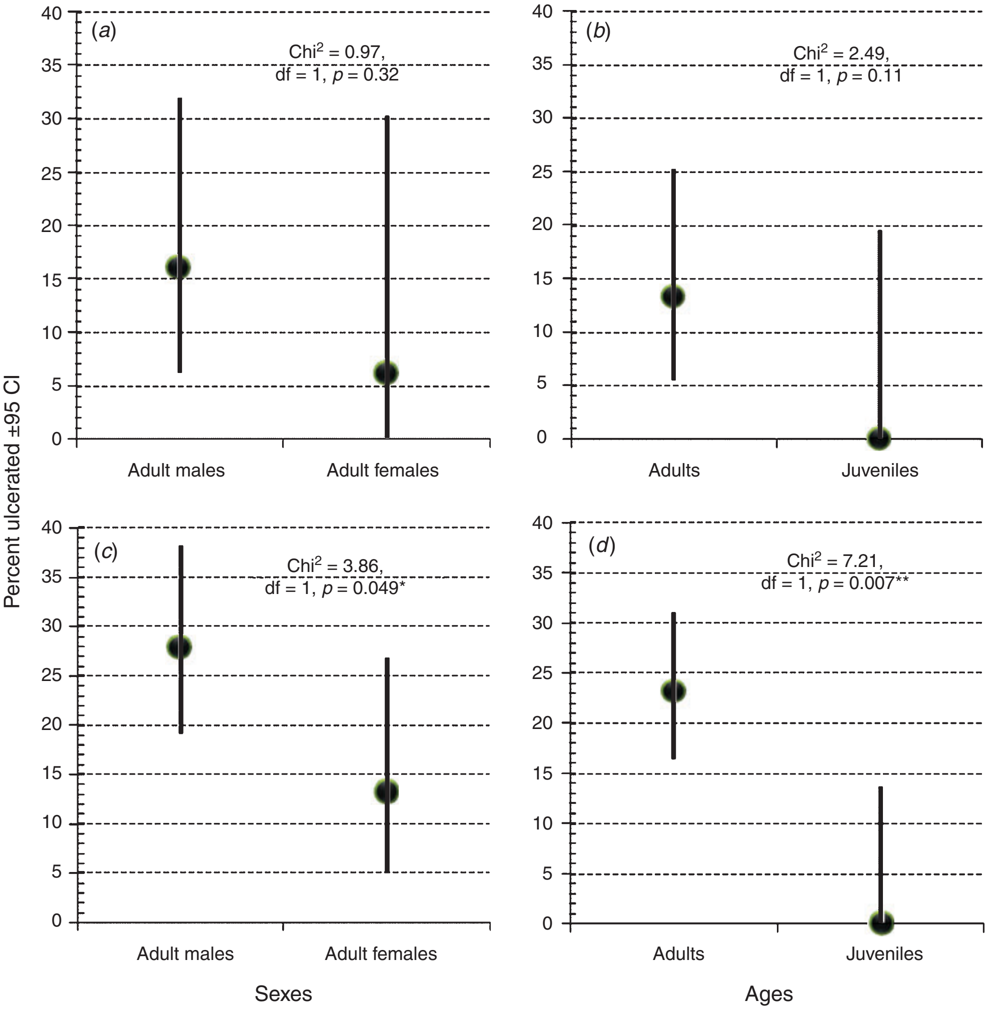

Fig. 13. Proportion of ulcerated individuals (±95% confidence intervals) in currently affected catchments comparing (*a*) adults of both sexes and (*b*) age classes. Panels (*c*) and (*d*) show data from affected catchments pooled with equivalent data reported by Connolly *et al.* (1998) and Stewart (2001).

fungal pathogens long after the epidemics associated with their initial invasion subside (Murray *et al.* 2009). While available evidence is patchy, a similar scenario may exist for mucormycosis in Tasmanian platypuses. A 4-fold decline in mucormycosis disease prevalence over the past 15 years in Tasmania (Gust *et al.* 2009) suggests that disease prevalence may have peaked, at least in some catchments. It appears that although mucormycosis has persisted in Tasmania for nearly 30 years, its contemporary influence on platypus populations is low but measurable, with no evidence of catastrophic impacts currently available. Rather it appears that the disease persists in at least four river catchments and is gradually spreading around the state (Gust *et al.* 2009), where it continues to affect and presumably kill individuals, particularly adult males, and thereby influences the age structure, longevity and turnover of affected populations. Drought conditions encountered during the current study may have influenced observed patterns. The influence of environmental variability on mucormycosis epidemiology and platypus demographics remains to be investigated.

Conclusions

Considerable natural spatial variability exists in both platypus morphometrics and demographics and is scale dependent. To maximise sensitivity to detect change, future platypus surveys and impact assessments in Tasmania should adopt designs that take into account patterns of sexual, spatial and seasonal variability documented in this study. It does not appear that platypus populations in Tasmania are currently significantly threatened by mucormycosis; however, if the disease persists and continues to spread, existing evidence suggests it may impact on the age structures of these iconic mammals. Understanding the patterns of morphometric and demographic variation described within and between catchments will help guide future impact assessments and conservation management decisions for platypuses.

Acknowledgements

This research was undertaken in accordance with Department of Primary Industries, Parks Water and Environment animal ethics permit 16/2007–08. Netting was conducted under Inland Fisheries Services exemption permit 2007/47, and DPIPWE platypus collection permits were issued to both N. Gust and J. Griffiths. The authors gratefully acknowledge the assistance of our colleagues M. Driessen and A. Phillips. S. Munks kindly loaned us several items of field equipment and assisted with fyke netting methodology at the beginning of the program. Field assistance was appreciated from both DPIPWE staff and volunteers, including M. Driessen, A. Phillips, B. Brown, M. Blytheman, E. Furlan, P. Holcombe, J. Sommerfeld, R. Olsson-Herrin, R. Miller, S. McColl, J. McGregor and S. Zulli. We thank landowners and fisheries staff across Tasmania for granting access to waterways and the Tasmanian public for reporting sightings of ulcerated platypuses. We thank M. Driessen, D. Geraghty, N. Stewart and the anonymous reviewers for their constructive comments on this manuscript. We gratefully acknowledge research funding from the Australian Government via both Natural Resource Management North and the Caring for Our Country program.

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Manuscript received 10 September 2010, accepted 16 May 2011