Breeding biology of the platypus
(Ornithorhynchus anatinus)

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Submitted in total fulfilment of the requirements of the degree of
Doctor of Philosophy

April 2018

School of Biosciences
The University of Melbourne
To Binarri, an extraordinary platypus
Abstract

This thesis examines the different behavioural stages of the reproductive cycle in the platypus, *Ornithorhynchus anatinus*, the time and energy investment of the female in breeding, and use of burrows by wild juveniles during the period after they first emerge. Many aspects of platypus reproduction are poorly understood due to their cryptic, nocturnal, semi-fossorial and semi-aquatic behaviour, which makes studies in the wild difficult. I studied a group of captive platypuses at Healesville Sanctuary and newly emerged juveniles from the wild population within Badger Creek, Victoria. My aims were to examine prey selection and seasonal energy intake, quantify and describe courtship, mating, nesting behaviour and maternal care given to nestlings, and describe how juvenile platypuses use the habitat in their natal home range.

In captivity, platypuses consumed the fewest kilojoules during the breeding season and most kilojoules during the post-breeding breeding season. They showed a preference for less-mobile prey (mealworms, earthworms and fly pupae). Crayfish formed the largest quantity of food in the diet and was highly nutritious for energy (kJ), vitamins and minerals. The platypus diet was influenced by nutritional content, the stage of the breeding season and the behaviour of the prey species.

Female platypuses controlled breeding encounters with males via three strategies; avoidance, by having lower activity levels and changing their activity pattern to partially diurnal; flight, by leaving the area immediately upon encountering the male; and resistance, terminating breeding encounters with the male and using a non-contact courtship behaviour prior to contact courtship behaviours. These strategies are likely to protect females from injury and coercion. After mating, females invested $8 \pm 1.5$ hours over 3 nights collecting wet vegetation for their nesting burrow. The morphology of burrows varied each year, but contained the same structural features: narrow tunnels, dead ends, ‘pugs’ of backfilled earth and multiple entrances that lead to a nesting chamber containing a spherical vegetation nest.
The female’s energy intake increased to twice that of a non-lactating female in the final month of lactation, indicating the high cost of milk production. The length of lactation dependence for platypus nestlings was 128 ± 1 days. Females spent less time in the nest with twins compared with a single nestling. I developed an infra-red camera technique which allowed platypus nestling behaviour, growth and development to be observed in their burrow. Weaning occurred as an instantaneous event when the nestlings emerged into the water.

Newly emerged wild juvenile platypuses each used multiple burrows for single or multiple nights within their natal home range. There was no significant correlation with vegetation communities along the bank at burrow sites, indicating burrow site selection was not driven by vegetation structure. No juveniles dispersed, suggesting they persist in the natal home range until the sub-adult stage which may assist their survival as they develop their skills and complete their growth in high quality habitat.

My study demonstrates that female platypuses invest a high amount of time and energy in breeding, from avoiding the male platypus, through courtship and mating, creating the nesting burrow, maternal care during lactation, and while juveniles persist in her home range after weaning. I have provided captive management recommendations based on my research to advance the animal welfare and captive breeding success of the platypus.
Declaration

This is to certify that

i. the thesis comprises only my original work towards the PhD except where indicated in the Preface;

ii. due acknowledgment has been made in the text to all other material used;

iii. the thesis is less than 100,000 words in length, exclusive of tables, amps, bibliographies and appendices.

[Signature]  18/4/2018

Signature  Date
Preface

The only data used in this thesis that I did not collect myself were food remains in the platypus tanks and activity data that were collected by keepers Mason Hill and Sarah Boyd at Healesville Sanctuary on my weekends and annual leave days.

I collected data on breeding events and activity levels in breeding platypuses between June 2008 and March 2012 as part of routine animal monitoring as part of my employment as platypus keeper at Healesville Sanctuary.

Chapter 3 is accepted for publication in the *Australian Journal of Zoology*

Chapter 4 is published in *Behaviour* 155 (2018): 27 - 53

Chapter 5 is accepted for publication in the *Australian Journal of Zoology*

This research was conducted under approval from Animal Ethics Committee ZV12014, Research permit under the Wildlife Act 1975 10006550 and Fisheries permit RP1183.
Acknowledgements

Firstly, I am profoundly grateful to my supervisors Dr Marissa Parrott, Dr Kath Handasyde and Assoc. Prof. Peter Temple-Smith. Doing this part-time, it has been a long journey for us, but in many ways it feels like it has flown by. You made this study so enjoyable because you were also all friends. I knew I had it pretty good when the most challenging part of our meetings was keeping you all focused. I’ve learnt so much from all of you, including how easy it is to bait you all with a little competition to get my drafts back. Marissa, thank you for all of your support and encouragement, the coffees and the cookies and also for getting this project off the ground with Zoos Victoria. Often my biggest struggle was balancing work and study and your assistance was exactly what I needed. Kath, you have taught me so much, particularly about writing papers and I am honoured to be your last PhD student before you retire. Peter, my ever-willing field assistant. You always made yourself available at the drop of a hat to come up to Healesville Sanctuary to attach transmitters, drill into burrows or go trapping in the field with your squelchy waders. You imparted so much about this amazing species to me and I hope to continue studying them as long as you have.

To my round partners and team mates Sarah Boyd and Mason Hill, not many people get to, go to work every day with their best mates but to also have them alongside every step of the way through a PhD. Through the good times and the bad, one thing that was never in question was the support I had from you both. While picking up worms came with constant complaining from one of you and never a complaint from the other, you helped me collect the data so thoroughly because of the respect you had for my study. Mason, you fixed so many problems with your ‘MacGyver’ skills. I couldn’t have done this without you both.

Dr Jenny Gray, Glen Holland, Russel Traher and Dr Rupert Baker, as CEO of Zoos Victoria, director and general curators of Healesville Sanctuary, you were under no obligation to support this study when I first asked. It is not without your support, encouragement and dedication to providing the highest standard of care possible, that I have been able to complete this. I am so proud to work for Zoos
Victoria and am confident that my findings will be of enormous benefit to animals in our care. Very few people get to study and work at their dream job and I am so privileged for this opportunity.

All the keepers who have worked on Team Aquatic during the past six years, but specifically Nay Emerson, Katy Weller, Guni Hudson and Jason Bell. Thank you for collecting all those worms with tweezers. To my first round partner Ian Elton, you certainly showed me what an amazing species we got to work with and it was on my first day that you introduced me to Millsom, the platypus who first drew my fascination. To Fisk and Norm, you both taught me so much about platypus breeding from the past ‘100 years’ and even the detail records you kept from your time on the section, gave me the confidence to work through each problem as it arose as it was usually something you had seen before.

I would like to thank my team and my manager, Sue Jaensch for organising time and staffing to allow me to do my work. I know it wasn’t easy at times to find cover for me when I needed it and to my team mates who came over to help, I really appreciate it. To the rest of the staff at Healesville Sanctuary, thank you for attempting to be quiet in the office.

Many researchers have helped me along the way. Josh Griffiths, thank you letting me be an ‘unpaid employee’ and for teaching me how to trap platypuses using fyke nets. Melody Serena, I really appreciate the support you have given me. It has been so helpful to compare my work with yours and how you generous you were with your time, just for a chat. Jenny Martin, thank you for being on my panel. You provided supportive words when I needed it.

To all of my field volunteers who came out trapping overnight - Jason, Karina, Amie, Sarah, Elise and Meg. I was so fortunate to be able to have such a skilled group of volunteers and friends come out with me. It made staying awake all night easy.

Judy Robertson, my ‘work mum’ and Megan Croucher, thank you for all the grammar, spelling and proof reading support along the way. You also got my research out into the general community and provided me with so much encouragement and friendship.
My friends Meg Lane, Kim Hollis and Jemima Amery-Gale. I am grateful for the friendship, support, encouragement, fun times and also if I needed it, just a whinge. I would also like to thank my friends at the morning tea table at the bistro. You joked about getting an acknowledgment in my thesis, but here it is. The light-hearted conversation, jokes and complaining you all provided each morning, especially during write-up, encouraged me to take a break and was a welcome relief and what I needed to clear my head.

To my family; my mum, Lynne and dad, Bruce, my brother Adam, sister in-law Kristin, and my nieces Audrey and Claudia. Thank you for your ongoing support of my education and encouraging me to, if I didn’t know something, to work it out. It is how this PhD started, to answer the questions that no one could give me the answers to.

My sincere thanks to Bill Holsworth and also to the Winifred Violet Scott Trust for their generous funding support for this project through six years.

Ultimately my deepest thanks goes to the non-human individuals who gave me my initial and ongoing inspiration about every aspect of their being, the platypuses at Healesville Sanctuary. From my first day on the job, they each showed me their unique personalities and behaviours that drew me in and continue to fascinate me. Getting to care for them every day is what has made this study and going to work so enjoyable and it has become my passion.

Finally, I would like to dedicate this thesis to one individual, Binarri, an extraordinary platypus. Out of all the animals that have been in my care over the last 10 years, she is probably the least well known. She isn’t in front of the display for visitors to see, or in the daily presentations where people connect to the individuals there and know their names. When we monitor her in the breeding area on camera, while the others show behavioural quirks that allow us to tell them apart, she has none, she’s ‘the other one’. I spend the least amount of time with her, but she is also the one who has taught me the most about platypus biology. While she is simply going about her life - feeding, breeding and raising her young, she has shown me all of it. She has produced the most offspring of any platypus at any zoo and has bred so reliably. The majority of this thesis is
about her and while she is never the one in the limelight, to me, she is the one who shines the brightest – she is truly extraordinary.
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Figure 7.8: Mean (±SE) occurrence of vegetation types, both native and exotic, around each burrow type (frequent-, moderate- and single-use burrows). Black = frequent-use burrows, dark grey = moderate-use burrows, light grey = single-use burrows. Open circles = data points. ......................................................................................... 158
Chapter 1 General introduction
Chapter 1
General introduction

Reproduction is essential for the survival of all species. Studying the breeding biology and more specifically, the breeding behaviour of a species, is critical to understanding their requirements. Many species can be difficult to study in their wild habitat due to issues such as the habitat being inaccessible for humans, low population numbers or cryptic behaviour. However, for some species, captive animals can provide, often the only opportunity to easily observe and study the behaviour of a species in a controlled and measurable environment. These studies can, although under captive conditions, still answer fundamental questions about biology such as breeding behaviour and nutrition (Ferrie et al., 2014; Hogan et al., 2011). With the changing role of modern zoos towards becoming conservation organisations and positive animal welfare ambassadors (Gray, 2015), this information can improve animal welfare conditions and breeding success in captivity, and can also identify critical habitat resources required for the conservation of wild populations. Australia is known for being home to some of the most unique and diverse fauna on earth (Keast, 2013). Yet, 35% of all modern mammalian extinctions, that have occurred are endemic Australian species (Woinarski, Burbidge, & Harrison, 2015). While some Australian mammals have been well studied, such as macropods (Renfree & Shaw, 2001), others such as the platypus, *Ornithorhynchus anatinus*, have received less attention. With recent changes to the platypus’s conservation status from ‘least concern’ to ‘near threatened’ (IUCN, 2008), it is becoming increasingly important to study the biology of the platypus which, as the only extant species in this family, occupies a significant place in the world’s biodiversity (Bino, Grant, & Kingsford, 2015).

The platypus is one of two semi-aquatic mammals endemic to Australia. It is the only living species in the family Ornithorhynchidae and one of only five species in the order Monotremata, which comprises a unique group of mammals that lay eggs and suckle their young with milk. The platypus is found throughout freshwater river systems along eastern Australia from Cooktown in north
Queensland to southern Tasmania (Grant, 2007). Platypuses have been recorded in a variety of different habitats along waterways from estuaries to high altitudes, and pristine rainforests to highly degraded agricultural drainage areas (Grant & Temple-Smith, 1998b). The major threatening processes affecting platypus populations are habitat degradation, including loss of riparian vegetation, bank erosion and poor water quality; however they are also vulnerable to predation from introduced predators and entanglement in rubbish and fishing equipment (Bino et al., 2015; Grant, 2007; Grant & Temple-Smith, 2003; Serena, 1994; Serena & Pettigrove, 2005). Its nocturnal, cryptic, fossorial and aquatic nature has made field studies of the platypus extremely challenging and, as a result, little is known about platypus breeding behaviour and breeding requirements in the wild. However, captive studies can provide new knowledge to fill these gaps through the ability to closely monitor the animals. Already, much of the data on platypus reproduction has been learned/obtained from captive breeding programs which have enabled individuals to be monitored intensively with cameras. Until recently, limited research had been conducted within zoos and much of this information about breeding biology has been recorded by keepers but never rigorously studied and rarely published. My study has focussed on the gaps in our knowledge on diet and reproduction using a captive group of platypuses at Healesville Sanctuary, a Zoos Victoria property specialising in native Australian fauna. This thesis examines aspects of the breeding biology of the platypus, which includes seasonal changes in nutrition and prey selection that occur around the breeding season, courtship and mating behaviours between males and females, the structure of nesting burrows, maternal care towards nestlings and burrow use by independent juveniles in their natal home range in the wild.

Diet

Diet and nutrition are critical for survival and reproduction. Animals will adjust their diet according to changing energy requirements that occur seasonally in response to changing temperatures, food availability and for breeding (Gende, Quinn, & Willson, 2001; Knott, 1998; Krockenberger, 2003). The responses an animal shows in relation to their diet under optimal conditions can highlight which
components within habitats should be conserved. For some species, that have a varied diet or live in a habitat not easily observed by humans, it can be very challenging to study dietary selection in the field. In these situations, captive animals can provide us with a better opportunity to study feeding behaviour and food choice, as the animals are more accessible and the environment and food availability can be carefully managed to answer specific questions.

The platypus is an opportunistic carnivore that uses specialised electro and mechano-sensory systems in their bills to locate prey underwater (Proske, Gregory, & Iggo, 1997). They move their heads from side to side in a reflex response to stimuli from electric fields emanating from their prey as they forage through the benthos (Manger & Pettigrew, 1995). They are able to use electric sensors to locate prey using alternating current (AC) signals and electro-navigate around underwater objects in flowing water using direct current (DC) signals (Proske et al., 1997). Platypuses prey on a variety of aquatic invertebrates and will also opportunistically consume aquatic vertebrates such as tadpoles and fish fingerlings (Faragher, Grant, & Carrick, 1979; McLachlan-Troup, Dickman, & Grant, 2010). Food is stored in cheek pouches while foraging underwater and then masticated with grinding pads in the bill when the animal is back at the surface. Platypuses do not regularly defecate on land or when handled, making it difficult to collect faecal samples for dietary studies. The most successful method to study the diet in the wild has been to collect samples from inside the cheek pouches (Faragher et al., 1979; Marchant & Grant, 2015). Analysis of the contents can identify the families of invertebrates consumed from parts of their masticated exoskeleton. While this technique has provided us with most of the information we have on platypus diets, it has the limitations in that it will not easily detect prey without exoskeletons (i.e. oligochaete worms) and those that require the exoskeleton to be removed before consumption (e.g. crayfish) (Marchant & Grant, 2015). The overall quantity of food consumed, on a daily basis, can also not be determined using this method, as each sample represents the prey eaten in a single point in time and not the amount eaten over a day. For the platypus, studying the diet in captivity can allow us to observe prey selection and their daily energy intake across the year.

There are 12 families of invertebrates regularly consumed by platypuses, with the most commonly consumed food item from one study being Trichoptera
However, the most abundant invertebrates in a stream are also the most common in cheek pouches (Faragher et al., 1979) suggesting that platypuses are opportunistic feeders in the wild. The species richness of invertebrates in waterways and richness consumed by platypus can vary seasonally, with richness being higher in summer and lower in winter in some catchments, while other studies found a general decrease in the abundance of invertebrates over winter, but no change in overall composition (Marchant & Grant, 2015; McLachlan-Troup et al., 2010). Out of 16 Orders and 74 Families of macro-invertebrates found in one waterway, platypuses had consumed prey from all 16 Orders and 55 of the Families (McLachlan-Troup et al., 2010). Platypuses consume 15 – 30% of their body weight in food per day (Holland & Jackson, 2002; Krueger, Hunter, & Serena, 1992). The broad diversity of prey consumed and the quantity required by wild platypuses has made replicating their diet in captivity difficult. The current diet at Healesville Sanctuary is based on the most similar prey options that are commercially available, which include earthworms (Oligochaeta sp.), freshwater crayfish (Cherax albidus and C. destructor), fly pupae (Musca domestica) and mealworms (Tenebrio molitor). While this diet is readily consumed, it has never been analysed for its nutritional content.

Good body condition is often essential for breeding success in mammals, especially females, as the period of parental care during lactation is energetically costly (Oftedal, 2000). Body condition in platypuses is most commonly determined by assessing fat stores in the tail (Temple-Smith, 1973). Platypus body condition in the wild varies considerably with season (Handasyde, McDonald, & Evans, 2003). Peak physical condition in both males and females occurs over summer when invertebrates are present in high numbers (Grant & Carrick 1978; Hulbert & Grant, 1983; Temple-Smith, 1973). This is also the time of year during which females are lactating (Grant, Griffiths, & Leckie, 1983). Wild female platypuses exhibit low body condition during the breeding season, with average weights recorded almost 250 g lower and lower tail fat stores, compared to their optimal condition (Grant & Carrick 1978; Hulbert & Grant, 1983; Temple-Smith, 1973). Males also exhibit their poorest body condition during the peak of breeding season, with average weights nearly 400 g less than over summer (Temple-Smith, 1973). These changes in body condition and weight could result,
in part, from seasonal changes in the diet or activity. However, the amount of energy platypuses consume and the reason why these changes occur have not been studied.

**Breeding**

Platypuses are seasonal breeders with a north-south cline occurring over their range (Grant, 2007). Courtship and mating typically occurs in early spring, with independent juveniles emerging from burrows in late summer. This species is polygynous, with male-male competition playing a role in sexual selection (Temple-Smith, 1973). Male platypuses are highly territorial and aggressive during the breeding season, which is driven by the seasonal enlargement and activation of the androgen-secreting Leydig cells in the enlarged testes (Temple-Smith, 1973). The increasing androgens stimulate the onset of sperm production, sexual activity and development and activation of venom and scent glands (Temple-Smith, 1973; Temple-Smith & Grant, 2001). Male platypuses have venomous spurs on their hind ankles which they use as weapons against other males (Temple-Smith, 1973). Female platypuses may not breed every year in the wild (Grant, Griffiths, & Temple-Smith, 2004). In the Shoalhaven River in NSW, some females bred consecutively for three years, while others did not (Grant et al., 2004). However, as captive females have also not bred in consecutive years in the past, the availability of food may not be the only factor contributing to reproductive success.

Few researchers have observed platypus breeding behaviour in the wild due to the difficulties with observations: platypuses spend the majority of their time underground or underwater, and are also predominantly nocturnal (Gust & Handasyde, 1995; Serena, 1994). Most of our understanding is from anecdotal, and often serendipitous, observations in the wild or from the rare breeding successes in captivity. Male and female platypuses undergo a complex courtship display prior to mating. This has been described as the male biting the tail of the female, with both then engaging in a swimming pattern of twisting and turning together on the surface of the water (Fleay, 1944; Hawkins & Battaglia, 2009; Holland & Jackson, 2002). However, it is not known how this behaviour affects mate selection, or the amount of time that is invested in courtship over a breeding
season. These behaviours are easily observable in captivity using infrared cameras, yet have not been extensively studied due to the low breeding success rate that has existed until recently. While the behaviours of animals in captivity are not directly comparable to the behaviours of animals in the wild, captive studies may provide a better understanding of the time budgets involved in breeding and maternal behaviours.

As mammals, female platypuses invest a significant part of their reproductive effort in maternal care, particularly lactation. The length of gestation in a platypus has only been estimated based on female behaviour in captivity. The time from mating, to when a female does not emerge from the burrow for one night, has been used to estimate gestation of 15 – 21 days (Hawkins & Battaglia, 2009; Holland & Jackson, 2002). In echidnas, gestation is also short in duration: 22 – 24 days (Rismiller & McKelvey, 2000). The short period of gestation in the platypus means that the chances of capturing a gravid female in the wild to study, would be relatively small. During gestation, the female constructs a specialised nesting burrow where she lays her eggs and houses and protects her young during lactation. The nesting burrow can be up to eight meters in length and is complex in design, containing features such as ‘pugs’, which are sections backfilled with earth in the tunnel to block the passage (Burrell, 1927). The breeding female will carry copious amounts of wet nesting material in her tail back to the chamber. This is believed to provide a high level of humidity to protect the eggs and unfurred young from desiccation (Temple-Smith & Grant, 2001). Individual females in captivity have demonstrated penchants for nesting material available in the water including *Eucalyptus* leaves (Holland & Jackson, 2002) and stringy bark from submerged logs (Hawkins & Battaglia, 2009), with grasses and fern fronds as a secondary choice. This behaviour has been observed 5 – 9 days (Holland & Jackson, 2002), and 2 – 5 days (Hawkins & Battaglia, 2009) prior to egg laying. Nesting behaviour has been observed in captivity in years where young were successfully reared and also when no young were produced (Hawkins & Battaglia, 2009). The advantage of studying female platypuses in captivity is that they are easily located and observed while creating their burrows and nests. It is still unknown if nesting behaviour occurs through maternal recognition of pregnancy or pseudo-pregnancy, the types of materials that are
important in nest construction, the time investment that is required to build a nest, and how critical this is for successful breeding.

To date, no one has been able to view the activity inside the nesting burrow of a platypus from egg laying through to the emergence of young. Such a study would provide data on incubation lengths, behavioural development of the nestlings at known ages and how the female interacts with her nestlings. Inactivity of the female platypus has been used as the key indicator of incubation in captivity. The duration of incubation in the platypus has also been estimated at 10 days, based on the duration observed in short-beaked echidnas (Griffiths, 1978; Morrow & Nicol, 2013). While there are many similarities between platypuses and echidnas, they are likely to have different adaptations to suit the different habitats they occupy, thus there is a need for further studies on the platypus to establish the length of incubation in this species. For example, while the echidna carries their eggs in a pouch, platypuses leave their eggs in a nest during short feeding bouts (Temple-Smith & Grant, 2001). At hatching, platypus nestlings are unfurred and ~15 mm in length (Manger, Hall, & Pettigrew, 1998). Platypuses hatch with one milk tooth on the upper and one on the lower jaw to assist them with breaking through the egg shell (Green, 1937). While Manger et al. (1998) has described the physical development of platypus nestlings at different ages, the exact ages of those specimens were unknown. These specimens were collected by Burrell (1927) from wild nesting burrows and assigned ages based on his estimate.

Female platypuses, like other monotremes, have mammary glands with areola, rather than teats, through which they lactate. The young are nourished completely with milk from the female for three to four months, until they emerge from the nesting burrow as independent juveniles (Grant et al., 2004). The female will frequently attend to the young during early development until approximately 40 days after egg laying, at which point she decreased the time she spent in the nest (Holland & Jackson, 2002). The only observations of live platypus nestlings have been obtained through footage from documentary filmmakers and these have not been extensively studied or published. External examination of the female’s mammary area found it had regressed back to the non-breeding condition by 22 days after the young had emerged from the nest, indicating that
weaning was completed quickly (Holland & Jackson, 2002). However, in practise, palpating the mammary glands to confirm lactation is not reliable and an injection of oxytocin is required to confirm milk let down (Grant et al., 1983). At emergence, the juveniles are up to 70% of the adult weight and 87% of the adult length (Grant & Temple-Smith, 1998a). The behavioural changes that occur between the mother and offspring at weaning are poorly understood. It is unknown if there is maternal care after weaning or how newly emerged juveniles develop survival skills in the environment. Juveniles are thought to disperse from the natal home range within their first year (Grant, 2004), although the exact timing and mechanisms which drive dispersal are also unknown.

**History of captive breeding**

Since the first successful long-term housing of a platypus at a zoo in 1932, there has been limited success breeding them in captivity. The platypus was first bred in captivity in 1943 at Healesville Sanctuary (then, Sir Colin Mackenzie Sanctuary). They were not bred again until 1998 (Table 1.1). To date, only two institutions have been successful in breeding the platypus, Healesville Sanctuary and Taronga Zoo, despite concerted attempts from at least four other zoos. While both of these institutions have had success, their animals have also failed to breed on many occasions, even when maintaining the same conditions and husbandry as in successful years. Many improvements have been made to housing conditions and husbandry of platypuses in captivity, such as an improved diet, larger enclosures and the provision of environmental and behavioural enrichment. This has resulted in better welfare outcomes and improved longevity for the animals and has likely contributed to the recent successful captive breeding. However, we are still unclear about exactly which conditions are associated with successful breeding, as there have never been enough successful breeding events to rigorously study these animals.
Table 1.1: History of successful captive breeding of the platypus. Offspring produced are listed as '[male].[female].[unknown]'. This table includes only the years where offspring were produced and confirmed surviving through at least the first half of the lactation period at Healesville Sanctuary (Zoos Victoria) and Taronga Zoo.

<table>
<thead>
<tr>
<th>Year</th>
<th>Institution</th>
<th>Breeding pair</th>
<th>Origin of parents</th>
<th>Age of parents (years)</th>
<th>Offspring produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>1943</td>
<td>Healesville Sanctuary</td>
<td>Jill</td>
<td>Wild caught</td>
<td>7</td>
<td>1.0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jack</td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>Healesville Sanctuary</td>
<td>Koorina</td>
<td>Wild caught</td>
<td>8</td>
<td>2.0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>Healesville Sanctuary</td>
<td>Koorina</td>
<td>Wild caught</td>
<td>9</td>
<td>1.0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>Taronga Zoo</td>
<td>Maryanne</td>
<td>Wild caught</td>
<td>9</td>
<td>0.2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abby</td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>Taronga Zoo</td>
<td>Maryanne</td>
<td>Wild caught</td>
<td>10</td>
<td>1.1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abby</td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>Taronga Zoo</td>
<td>Maryanne</td>
<td>Wild caught</td>
<td>11</td>
<td>0.1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abby</td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>Healesville Sanctuary</td>
<td>Binirri</td>
<td>Captive born</td>
<td>5</td>
<td>1.1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Barak</td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>Healesville Sanctuary</td>
<td>Binirri</td>
<td>Captive born</td>
<td>6</td>
<td>0.1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Barak</td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>Healesville Sanctuary</td>
<td>Binirri</td>
<td>Captive born</td>
<td>7</td>
<td>1.0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Barak</td>
<td></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Healesville Sanctuary</td>
<td>Binirri</td>
<td>Captive born</td>
<td>9</td>
<td>1.1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tarrabi</td>
<td>Wild caught</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>Healesville Sanctuary</td>
<td>Binirri</td>
<td>Captive born</td>
<td>10</td>
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<tr>
<td></td>
<td></td>
<td>Tarrabi</td>
<td>Wild caught</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>Healesville Sanctuary</td>
<td>Binirri</td>
<td>Captive born</td>
<td>12</td>
<td>0.0.1</td>
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<tr>
<td></td>
<td></td>
<td>Tarrabi</td>
<td>Wild caught</td>
<td>4</td>
<td></td>
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<tr>
<td>2015</td>
<td>Healesville Sanctuary</td>
<td>Binirri</td>
<td>Captive born</td>
<td>13</td>
<td>2.0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tarrabi</td>
<td>Wild caught</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>2016</td>
<td>Healesville Sanctuary</td>
<td>Binirri</td>
<td>Captive born</td>
<td>14</td>
<td>2.0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tarrabi</td>
<td>Wild caught</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
There are many gaps in our knowledge on the fundamental reproductive biology of the platypus, making captive breeding attempts difficult. By studying breeding biology in captive colonies we can develop more successful captive breeding programs, as well as provide confidence for programs in the future should this species become endangered and require release of captive bred animals back into the wild. Thus, in this study, my aims were to:

- Determine if captive platypuses show seasonal prey selection in relation to the breeding season
- Describe and quantify breeding behaviours between male and females
- Determine the structure and morphology of the nesting burrow
- Describe the maternal care provided to dependant nestlings and their behavioural development in the nesting burrow
- Determine how newly independent juveniles in a wild population use burrows in their natal home range

**Thesis Outline**

This thesis examines the breeding biology of the platypus. In Chapter 2 (General Methods), I describe the structure and characteristics of the captive breeding facility at Healesville Sanctuary and also the study site, Badger Creek, where wild juveniles were monitored. In Chapter 3, I examine the dietary preferences of captive platypuses in relation to the breeding season. In Chapter 4, I determine the behavioural interactions of courtship and mating between male and female platypuses during the breeding season. In Chapter 5, I describe the structure and morphology of nesting burrows used by breeding female platypuses to lay their eggs and rear their young. In Chapter 6, I describe the behavioural development of platypus nestlings and the maternal care provided by the female. In Chapter 7, I examine burrow use by juvenile platypuses, after emergence, in a wild population in Badger Creek. Finally, in Chapter 8, I discuss the significance of the findings of my research and their practical application to captive breeding programs and conservation of wild platypus populations.
References


Chapter 2 General methods
Chapter 2

General methods

Animals

This research was conducted at Healesville Sanctuary, a Zoos Victoria property, which is located 52 km north east of Melbourne, Victoria. Healesville Sanctuary is a captive institution located within a natural bushland setting, with the Badger Creek flowing through the grounds and an adjacent Australian National Heritage site, the Coranderrk Bushland Reserve, which is managed by Healesville Sanctuary. Eight adult platypuses varying in age, gender and origin (Table 2.1) were housed at Healesville Sanctuary between 2008 and 2017 and formed the main study population. Wild juvenile platypuses from Badger Creek were also studied.

Table 2.1: Individual platypus information held at Healesville Sanctuary. Born HS indicates animals were born at Healesville Sanctuary, Born TZ indicates animals born at Taronga Zoo.

<table>
<thead>
<tr>
<th>Animal name</th>
<th>Sex</th>
<th>Origin</th>
<th>Rearing</th>
<th>Social housing</th>
<th>Year of birth</th>
<th>Age range during study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fleay</td>
<td>Female</td>
<td>Wild</td>
<td>Parent</td>
<td>Single</td>
<td>1993</td>
<td>15 – 24 years</td>
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<tr>
<td>Millsom</td>
<td>Male</td>
<td>Wild/rehab</td>
<td>Hand reared</td>
<td>Single</td>
<td>2002</td>
<td>6 – 15 years</td>
</tr>
<tr>
<td>Binarri</td>
<td>Female</td>
<td>Captive (Born TZ)</td>
<td>Parent</td>
<td>Breeding group</td>
<td>2002</td>
<td>6 – 15 years</td>
</tr>
<tr>
<td>Waddirrang</td>
<td>Female</td>
<td>Captive (Born HS)</td>
<td>Parent</td>
<td>Breeding group</td>
<td>2007</td>
<td>1 – 9 years</td>
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<tr>
<td>Ember</td>
<td>Female</td>
<td>Captive (Born HS)</td>
<td>Parent</td>
<td>Female pair</td>
<td>2008</td>
<td>0 – 9 years</td>
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<tr>
<td>Yamacoona</td>
<td>Female</td>
<td>Wild/rehab</td>
<td>Parent</td>
<td>Female pair</td>
<td>2010</td>
<td>0 – 7 years</td>
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<td>Tarrabi</td>
<td>Male</td>
<td>Wild/rehab</td>
<td>Parent</td>
<td>Breeding group</td>
<td>2009</td>
<td>0 – 8 years</td>
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<tr>
<td>Alooka</td>
<td>Female</td>
<td>Captive (Born HS)</td>
<td>Parent</td>
<td>Female pair</td>
<td>2011</td>
<td>0 – 6 years</td>
</tr>
</tbody>
</table>
Captive husbandry

Housing

The platypus facility at Healesville Sanctuary consists of four separate enclosures within the ‘World of the Platypus’ nocturnal display and an off-limits breeding facility. In the ‘World of the Platypus’, the tank volumes range between 8,000 – 10,000 L each. They have a wooden burrow network attached above the water line, leading to a wooden nest box maintained at 18˚C, based on the study by Bethge, Munks, Otley, and Nicol (2004) of burrow temperatures. The tanks were furnished with river pebbles, rocks, logs and tree fern trunks and held at ambient temperature. Vegetation was changed once per week and consisted of *Eucalyptus* sp., tree fern fronds (*Cyathea australis* and *Dicksonia antarctica*) and ground ferns (*Calochlaena dubia* and *Pteridium esculentum*). Each enclosure has a multi-cyclone and sand filter (Waterco) which returns water at 225L/min to the tank via ‘waterfall’ or underwater jets. Tanks are manually cleaned by keepers once or twice per week and water replaced with mains tap water.

Breeding facility

The platypus breeding facility at Healesville Sanctuary comprises three large ponds (totalling 50,000 L) with flowing water (Figure 2.1A – C), three 5000 L aquaculture feed tanks (Figure 2.1D) where food is delivered, two dirt tanks filled with soil for burrowing and two temperature controlled artificial nesting rooms for resting. The water bodies were made up of rain and mains water using four sand filters and multi-cyclone units flowing at 353L per minute. The main ponds were furnished with partially submerged logs, native vegetation along the edge (including tree ferns and grasses) and areas of mulch along the perimeter. Feed tanks were cleaned twice weekly and the water replaced. The nest box rooms consist of six wooden nest boxes (500 x 400 x 400 mm) that are connected to each other via 200 mm PVC pipe lined with non-slip rubber matting (Figure 2.1E). The boxes contain leaf litter and other organic plant matter as nesting material. The dirt tanks contain 5000 L of clay rich soil and are planted with native grasses, ferns and a tree stump (Figure 2.1F), for the animals to create their own burrows. All parts of the facility were monitored using eight CCTV motion activated infrared cameras (Techview, 800TVL CMOS bullet camera) that were connected to a digital video recorder (DVR; Ganz DR16NRT Digimaster). The
footage was reviewed daily and data collected including activity levels of the animals and breeding events.

All platypuses had a behavioural enrichment program with enrichments targeted towards foraging, manipulative, environmental, sensory and social behaviours which were rotated daily on a monthly program. The key enrichments include novel foods such as shrimp and blackworms, novel natural play objects such as floating bark and logs, substrate changes, water level and flow changes and enclosure swapping.

**Diet**

Platypuses were fed *ad libitum* daily. The diet consisted of live freshwater crayfish (*Cherax destructor* and *C. albidis*), mealworms (*Tenebrio molitor*), earthworms (*Oligochaeta sp.*) and fly pupae (*Musca domestica*) with all items weighed prior to placement in the enclosure (refer to Chapter 3 for details). House crickets (*Acheta domesticus*), blackworms (*Lumbriculus variegates*) and freshwater shrimp (*Paratya australiensis*) were offered twice per month at < 50g each as part of the enrichment program.

*Freshwater crayfish*

Crayfish were farm bred in outside dams and purchased fortnightly from Otway Aquaculture, Victoria. They were raised on trout pellets in the dams and fed vegetable scraps (primarily carrot and pumpkin) until being placed in the platypus tanks. At Healesville Sanctuary, crayfish were held until use in 5000 L aquaculture tanks with sand and biological filtration which uses microbe oxidation of biological waste products in the water.
Figure 2.1: The platypus breeding facility at Healesville Sanctuary. A, B, C: Main ponds; D: Feed tank showing access platform; E: Nest box room showing tunnels of PVC pipe that connect to the nest boxes and ponds; F: Dirt tank for burrow construction by animals
**Mealworms**

Mealworms were purchased live weekly from The Amphibian Research Centre, Victoria and held at 4°C in bran and cardboard. Mealworms were then housed at room temperature and fed a gut loading diet of ‘Egg and biscuit’ mix (Wombaroo) and ‘Insectivore’ mix (Wombaroo) at a ratio of 3:1 for a minimum of 24 hours before use.

**Earthworms**

Earthworms were purchased live weekly from Worms ‘R’ Us, Brisbane and housed in sugar cane mulch under light until used. Earthworms were fed bran and sprayed with watered as required.

**Fly pupae**

Fly pupae were produced at Healesville Sanctuary by the food preparation staff. Maggots were fed a diet of sour milk and bran. Fresh pupae were washed and frozen for 24 hours to prevent them hatching into house flies, before being offered to platypuses.

**House crickets, blackworms and freshwater shrimp**

House crickets were purchased weekly from Pisces, Sydney and housed at 20°C. They were fed a diet of endive, carrot and insectivore mix. Blackworms were purchased monthly from Aquarium Industries, Victoria and fed a diet of fish flake. They were housed in a 500 L freshwater aquarium with water chilled to 12°C. Freshwater shrimp were purchased fortnightly from Otway Aquaculture, Victoria. They were housed in a 500 L freshwater aquarium and fed carrot and peas.

**Badger Creek study site**

The Badger Creek starts at the summit of Mount Donna Buang, approx. 80 km north east of Melbourne, Victoria, and flows into the Yarra River approximately 1 km downstream from Koo Wee Rup Road, Healesville. A weir built approximately half way along the creek regulates the flow through Healesville Sanctuary and the adjoining Coranderrk Bushland Reserve. My study area extended from approximately 3 km below the weir, through Healesville Sanctuary and finished at the lower reach of Coranderrk Bushland Reserve,
where Healesville Koo Wee Rup Road intersects the river (Figure 2.2). The vegetation along the creek is riparian forest with small sections of farmland. Some of this area has undergone revegetation by community Landcare groups and Healesville Sanctuary.

Figure 2.2. Map of Badger Creek, Victoria. Black triangles indicate the upper and lower boundaries of the field study site which extends for 3 km. Circle indicates Healesville Sanctuary and the Coranderrk Bushland.

**Animal capture, handling and collection of data**

Twelve fyke nets (T and L Net-making, Mooroolbark, Victoria) were set up late afternoon at intervals of approximately 600 m apart along the study site with one net facing upstream and one downstream (Figure 2.3). The face of the net was set with the water level half way up and the wings of the nets were anchored to the base of the river with rocks and secured to the bank with timber stakes. The cod end of the net was set out of the water so that air breathing animals could survive if captured.
Figure 2.3. A set of fyke nets in Badger Creek. One net faces upstream and the other downstream. The wings of each net are secured to creek bank and cod suspended out of the water.

Nets were monitored from sunset to sunrise (21:00 – 06:00 h) with no more than 4 hours between checks. All by-catch including fish, turtles and water rats were released at each check. Captured platypuses were removed from the nets, placed in a calico bag and taken to the vehicle for processing. At initial capture, each animal was given a unique microchip (Trovan Unique ID100 FDX-A) for future identification. Animals were sexed and weighed to the nearest gram (Pesola spring balance 2 kg, Switzerland). Head length, body length and bill length were measured to the nearest 0.1 mm using Vernier callipers and pelage and tail fat condition were assessed (Grant & Carrick 1978). Animals were placed into age categories according to spur classification (Figure 4; Temple-Smith, 1973). Platypuses were released within 30 minutes of the net check, back into the creek at the site of capture.
Radio-tracking

Juvenile animals (n = 7) that were captured were also fitted with a Core VHF radio-transmitter (model V2G 152C; Sirtrack, New Zealand), with a whip antenna, weighing 13 g with a battery life of approximately 6 months. A 15 x 8 mm patch of fur on the rump of the platypus was removed with scissors, then shaved down to the skin with an electric shaver and razor blade to ensure a smooth fit. The transmitter was glued on with a fast setting adhesive (Sealy’s Quick Fix Supa glue) by applying it to the skin and back of the transmitter, then held in place for 30 seconds until attached. Glue was applied to the sides of the transmitter and the surrounding fur secured flat over it for streamlining (Figure 2.4).
2.5). The glue was dry within several minutes and the animal was released back into the stream at its original site of capture.

Each animal was radio-tracked daily with a digital Ultra receiver (8MHz) with a Yagi three element antenna (Sirtrack, New Zealand), during daylight to the location of their burrow (402 fixes recorded). The burrow site was marked using a GPS (eTrex Vista HCx, Garmin). Fixes that were within 1 m of a previous point were considered to be the same burrow. Transmitters were allowed to fall off the animal naturally over time and were programmed to deliver a ‘motionless’ signal when they had not moved for 24 hours (refer to Chapter 7).

Figure 2.5. Juvenile male platypus in Badger Creek. Arrow indicates the dorsal attachment of the radio-transmitter with whip antenna to the rump of the animal (Photo credit: Amie Hindson)
References


Chapter 3 *Seasonal changes in food selection and nutrition of captive platypuses* (*Ornithorhynchus anatinus*)
Seasonal changes in food selection and nutrition of captive platypuses (*Ornithorhynchus anatinus*)

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Abstract

Anecdotal observations of captive platypuses (*Ornithorhynchus anatinus*) suggest they show a seasonal preference for particular foods, but this has never been rigorously measured. This study aimed to determine seasonal food preferences and energy consumption of captive platypuses so that better protocols for maintaining platypuses in captivity can be developed. Seven platypuses were fed an *ad libitum* diet with all food items weighed in and out of tanks. Food items were analysed for energy, fats, carbohydrates, proteins, vitamins and minerals. Platypuses preferred less mobile prey, (mealworms, earthworms and fly pupae), over highly mobile prey (crayfish). There was no significant seasonal change in preference for different dietary items, which is more likely to be driven by prey behaviour. Crayfish contributed the largest percentage (mass) consumed and was highly nutritious. While the relative percentage of items in the diet did not change seasonally, the quantity eaten did. The mean energy intake of platypuses was 921 kJ/kg/day and varied seasonally, being lowest during the breeding season (810 kJ/kg/day) and highest in the post-breeding season (1007 kJ/kg/day). These changes were associated with preparation and recovery from the breeding season. We conclude that the platypus diet is influenced by nutrition and seasonal factors as well as by prey behaviour. This knowledge will contribute to improving the husbandry and
management of platypuses, which are widely recognised as difficult to maintain in captivity.

**Introduction**

The incentive for animals to consume sufficient nutrients and energy is driven by the need to survive and reproduce. Many factors influence food selection, including seasonal availability (Gloury & Handasyde, 2016), digestibility (Varennes, Hanssen, Bonardelli, & Guillemette, 2015), food quality (Van Beest, Mysterud, Loe, & Milner, 2010), macronutrient content (Mayntz, Raubenheimer, Salomon, Toft, & Simpson, 2005) and the energetic costs of obtaining food (Graeb, Mangan, Jolley, Wahl, & Dettmers, 2006). Prey species vary in vitamin, mineral, macronutrient and energy content (Finke, 2002), and some predators target specific organs containing high concentrations of certain vitamins, fatty acids and minerals (Stahler, Smith, & Guernsey, 2006).

The platypus (*Ornithorhynchus anatinus*) is a generalist carnivore, using electrochemical and mechanoreceptor systems in their bills (Scheich, Langner, Tidemann, Coles, & Guppy, 1986) to detect and locate a variety of benthic invertebrates in fresh waterways (e.g. Marchant & Grant, 2015). While predominately preying on aquatic insects and crustaceans, they also consume some aquatic vertebrates (Faragher, Grant, & Carrick, 1979; Krueger, Hunter, & Serena, 1992). Food items are stored in cheek pouches during foraging dives, then masticated and consumed when animals surface. Unlike many species, platypus faecal samples are not useful when examining diet, because the characteristic chitinous exoskeletons used to identify invertebrate prey are expelled from the mouth during mastication. In the wild, platypuses are generally opportunistic feeders; the most locally abundant invertebrates are the most common prey items found in platypus cheek pouches (Faragher et al., 1979). While some studies have concluded that wild platypuses exhibit selective foraging (McLachlan-Troup, Dickman, & Grant, 2010), there is evidence to the contrary suggesting that they consume prey based on availability (Marchant & Grant, 2015). This generalist feeding strategy would be advantageous for a semi-aquatic species in eastern Australia, which has variable rainfall and,
consequently, a continually changing supply and taxonomic assemblage of invertebrates in freshwater systems (Boulton, 2003). However, due to the difficulties of sampling wild animals there are only limited studies on platypus diet.

The feeding strategy of platypuses makes accurate studies of their wild diet problematic. Cheek pouch sampling is the most widely used method of investigating diet in the wild (Faragher et al., 1979), but has limitations as only small prey species, and those with identifiable exoskeletons, will be sampled. Other prey, for example fish and invertebrates without exoskeletons, are very difficult to detect. Further, prey such as crayfish, are too large to be stored in cheek pouches and are “shelled” before consumption, thus are underestimated in dietary studies. Recent studies have investigated the use of stable isotope analysis of fur samples of wild platypuses to detect the types of prey that may not be present in cheek pouches, however these techniques are not able to determine the quantity of food consumed (Klamt, Davis, Thompson, Marchant, & Grant, 2016). Captive environments provide more controlled conditions to determine the preferences and energy requirements of platypuses on a seasonal basis.

There are often challenges in accurately replicating the natural diet of many species in captivity. For the platypus, there is a lack of knowledge about the wild diet, only a few aquatic invertebrates are available commercially and in limited quantities, there are considerable time constraints associated with collecting enough wild prey from the river and possibly sustainability issues for long term harvesting of wild prey. Failure to replicate the wild diets of some captive species can result in vitamin and macronutrient deficiencies which can impair reproduction and potentially cause health problems or even death (Chesney & Hedberg, 2010; Gilbert & Blair, 1975). Domestic fowl (Gallus sp.) may cease laying on a low calcium diet (Gilbert & Blair, 1975) and female numbats (Myrmecobius fasciatus) need to be weaned onto an all termite diet prior to breeding to achieve reproductive success (Power & Monaghan, 2003). Therefore, it is important to evaluate the nutritional value of captive diets to ensure that animals receive appropriate nutrition that mimics seasonal changes where possible. Platypuses are widely regarded as difficult to keep in captivity due to the difficulty in acquiring natural dietary items.
The aims of this study were to 1) determine whether captive platypuses exhibit dietary selectivity and whether their intake of different prey varies seasonally and 2) describe the nutritional value and energy content of prey consumed.

Materials and methods

Study animals and captive enclosures

We studied seven platypuses, housed at Healesville Sanctuary, Victoria, Australia, between January 2013 and December 2015. Individuals ranged from one to twenty years of age and came from various wild and captive sources (Table 3.1). Six platypuses were housed in indoor nocturnal display tanks and one in the outside breeding enclosure. Each enclosure comprised a flowing freshwater body of approximately 8000 L, tree trunks, rocks, fresh branches and a wooden burrow/tunnel system connecting the water to a nest-box containing bedding material (leaf litter and towelling).

Table 3.1: Age and sex, history and housing of platypuses used in this study at Healesville Sanctuary, Victoria, Australia, between 2013 and 2015. The development stage of all wild caught platypuses is presented in brackets under the source column. Animals A – F were housed in the nocturnal display area, while animal G was housed outside in the breeding area for the duration of the study.

<table>
<thead>
<tr>
<th>ID</th>
<th>Sex</th>
<th>Age at start of study (years)</th>
<th>Source</th>
<th>Rearing</th>
<th>Housing</th>
<th>Average body mass (g ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Female</td>
<td>20</td>
<td>Wild (juvenile)</td>
<td>Parent</td>
<td>Single</td>
<td>989 ± 8</td>
</tr>
<tr>
<td>B</td>
<td>Male</td>
<td>10</td>
<td>Wild (nestling)</td>
<td>Hand-reared</td>
<td>Single</td>
<td>1685 ± 25</td>
</tr>
<tr>
<td>C</td>
<td>Female</td>
<td>6</td>
<td>Captive born</td>
<td>Parent</td>
<td>Female pair</td>
<td>1241 ± 19</td>
</tr>
<tr>
<td>D</td>
<td>Female</td>
<td>5</td>
<td>Captive born</td>
<td>Parent</td>
<td>Female pair</td>
<td>1524 ± 21</td>
</tr>
<tr>
<td>E</td>
<td>Female</td>
<td>2</td>
<td>Wild caught/rehabilitated</td>
<td>Parent</td>
<td>Female pair</td>
<td>897 ± 9</td>
</tr>
<tr>
<td>F</td>
<td>Female</td>
<td>1</td>
<td>Captive born</td>
<td>Parent</td>
<td>Female pair</td>
<td>1348 ± 23</td>
</tr>
<tr>
<td>G</td>
<td>Male</td>
<td>2</td>
<td>Wild caught/rehabilitated (juvenile)</td>
<td>Parent</td>
<td>Breeding pair</td>
<td>1121 ± 17</td>
</tr>
</tbody>
</table>
Body mass and body condition

We weighed platypuses housed in nocturnal displays (n = 6) in a box to an accuracy of 1 gram approximately once per month using table weighing scales (Model A12, Microchips Australia) and assessed their condition, based on a tail fat index from 1 (excellent) to 5 (poor body condition) as described by Grant and Carrick (1978). We also weighed and scored the condition of an animal housed in the outside enclosure (n = 1) opportunistically (approximately once every three months) in order to minimise disturbance to breeding.

Feeding and calculation of intake

We fed platypuses daily, a weighed *ad libitum* quantity of live mealworms (*Tenebrio molitor*), earthworms (*Oligochaeta* sp.), freshwater crayfish (*Cherax albidus* and *C. destructor*) and frozen fly pupae (*Musca domestica*). All food was scattered in their tanks. On average, each animal had access to 19 ± 1 g of fly pupae, 70 ± 1 g of mealworms, 68 ± 2 g of earthworms and 476 ± 10 g of crayfish per day.

We collected, sorted and weighed all uneaten food items on table scales (Model A12, Microchips Australia) once to twice weekly to the accuracy of one gram. The fly pupae were collected with a dip net and all other items collected with tongs and tweezers. The methodical collection of food items by hand ensured minimal loss of biomass. The weights of all dry food were adjusted for the weight gain that occurred to the food remains from constant submergence in water that occurred from the time of feeding to the time of removal from the tank before analysis, using factors calculated from a trial study of 10 replicates of each food type over seven days. Dry weights of fly pupae were multiplied for a water saturation gain of 1.5, mealworms: 1.1, and earthworms: 1.1. This allowed an accurate calculation of intake via subtracting the weight of remains from the adjusted weight of fresh food provided. The weight of crayfish were not adjusted as they were constantly submerged in water prior to being fed and did not gain weight. For platypuses housed in pairs, food consumption results were halved. We then calculated monthly totals of weight (g) consumed by each individual and present the data as a daily mean ± SE.
Analyses of nutritional content of food

Samples of the foods fed to captive platypuses in this study were analysed by the National Measurement Institute, Melbourne, Australia. Three hundred grams wet mass of each food species was provided for a nutritional panel analysis of macronutrients: gross energy, fat (mojonnier extraction), protein (kjeldahl), and sugars; trace elements: Ca, P, Mg, K, Fe (ICP-OES); Zn, Cu, Mn, Se (ICP-MS) and functional components: Vitamins A, B₁, B₂, B₃, B₆, C, D, E, K₁ (HPLC); B₅ and B₁₂ (bioassay) (AOAC, 1995; Brubacher, Müller-Mulot, & Southgate, 1985; De Leenheer, Lambert, & De Ruyter, 1985).

Definition of terms

Three distinct phases of the year were identified, during which platypuses showed differences in their physiology and behaviour. The “pre-breeding season” in southern Australia occurs in the period from April to July. During this phase, testis size and testosterone production increases (New, Jabukowski, Stone, & Jones, 1998; Temple-Smith, 1973) and males show increased territorial behaviour (Gust & Handasyde, 1995), while females attempt to avoid the male by swimming to another pond or by reducing their activity levels to spend more time in the burrow (J. Thomas pers. obs.). The “breeding season” is defined as the period of courtship, mating and nest building by females and occurs from August to October. The “post-breeding season” occurs from November to March when hormone levels are at their seasonal nadir, testes and ovaries are inactive (New et al., 1998; Temple-Smith, 1973). This season is characterised by lactation and care of nestlings in those females that have bred (Grant, Griffiths, & Temple-Smith, 2004) and reproductive inactivity in non-breeding males and females. Lactating females were not included in this study.

Analyses and statistics

Prey preference was assessed using Jacob’s preference index \((D)\) (Jacobs, 1974).

\[
D = \frac{r-p}{r + p - 2rp}
\]

Where \(r\) is the proportional amount of a food type consumed and \(p\) is the proportional amount of all food types offered. The index ranges from -1 (negative
selection) to 0 (consumed in proportion to availability) to +1 (positive selection). This index was selected as it minimises bias towards foods given in lower quantities as it is independent of relative abundances between food types in the environment and corrects if an item is depleted (Jacobs, 1974).

Repeated measures Analysis of Variance (ANOVA) were used to determine whether there was any variation between seasons for the body weight of individual animals, energy consumption for all animals, the amount of each food type consumed and the amount of macronutrients. Repeated measures were selected because each animal was measured over a three year period. When ANOVA was significant, an *a posteriori* Bonferroni pairwise comparison test was used to determine which groups differed from each other. Two-sampled t-tests were used to compare the mean energy consumption between males and females. *P* values < 0.05 were deemed significant. All statistical calculations were performed using SYSTAT (Systat 13, San Jose, CA). All data are reported as means ± SE.

**Results**

**Seasonal body weight and tail fat condition**

Mean body weights within sexes were variable (Table 3.1). Five of seven animals (71%) reached maximum body weight in the pre-breeding season and six of seven animals (86%) dropped to their minimum weight during the breeding season. A comparison of mean body weight for each individual in each season and corresponding *P* values are in Table 3.2. The *post hoc* Bonferroni pairwise comparison showed that the seasons that were significantly different varied between individual animals. Two animals showed significant differences between the pre-breeding and breeding seasons (*P* = 0.04 and *P* = 0.01), one individual between the pre-breeding and post-breeding seasons (*P* < 0.001) and one individual between the breeding and post-breeding seasons (*P* = 0.05; Table 3.2). All animals were in average to excellent condition throughout the study: tail fat scores ranged between 1 and 3 with no consistent seasonal variation apparent.
### Table 3.2: Mean body mass for individual platypuses at each season in grams ± SE. P values less than 0.05 are denoted with * and ^ as significant; df=2

<table>
<thead>
<tr>
<th>ID</th>
<th>Sex</th>
<th>Pre-breeding body mass</th>
<th>Breeding body mass</th>
<th>Post-breeding body mass</th>
<th>F ratio</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Female</td>
<td>989 ± 16 (n=14)</td>
<td>983 ± 27 (n=6)</td>
<td>991 ± 8 (n=16)</td>
<td>1.75</td>
<td>0.25</td>
</tr>
<tr>
<td>B</td>
<td>Male</td>
<td>1721 ± 44 (n=13)</td>
<td>1595 ± 34 (n=7)</td>
<td>1694 ± 39 (n=17)</td>
<td>0.98</td>
<td>0.60</td>
</tr>
<tr>
<td>C</td>
<td>Female</td>
<td>1236 ± 35 (n=15)</td>
<td>1168 ± 23* (n=8)</td>
<td>1272 ± 27* (n=19)</td>
<td>2.01</td>
<td>0.05*</td>
</tr>
<tr>
<td>D</td>
<td>Female</td>
<td>1599 ± 19* (n=12)</td>
<td>1423 ± 83* (n=7)</td>
<td>1514 ± 21 (n=18)</td>
<td>5.18</td>
<td>0.01*</td>
</tr>
<tr>
<td>E</td>
<td>Female</td>
<td>929 ± 9 (n=12)</td>
<td>876 ± 13 (n=5)</td>
<td>881 ± 13 (n=17)</td>
<td>4.69</td>
<td>0.1</td>
</tr>
<tr>
<td>F</td>
<td>Female</td>
<td>1463 ± 8*^ (n=11)</td>
<td>1335 ± 52* (n=6)</td>
<td>1269 ± 29^ (n=15)</td>
<td>12.78</td>
<td>0.0001*</td>
</tr>
<tr>
<td>G</td>
<td>Male</td>
<td>1134 ± 13 (n=9)</td>
<td>1075 ± 31 (n=5)</td>
<td>1197 ± 39 (n=6)</td>
<td>0.33</td>
<td>0.72</td>
</tr>
</tbody>
</table>

### Food/energy consumption

Males consumed between 13.3 – 20.2%, and females between 13.1 – 20.7%, of their body mass each day. The mean daily energy consumption, for all individuals combined over the year was 921 ± 18 kJ/kg/day (total number of days sampled = 7665). There was no significant difference in mean annual energy consumption between males (936 ± 25 kJ/kg/day; range: 773 – 1090 kJ/kg/day; days sampled = 2190) and females (915 ± 23 kJ/kg/day; range: 785 - 1083 kJ/kg/day; days sampled = 5475; \( t = -0.61, \) d.f. = 187, \( P = 0.54; \) Figure 3.1). In both sexes, energy consumption varied significantly by season (\( F = 12.21, \) d.f. = 2, \( P < 0.001 \)). The lowest energy consumption occurred over the breeding season months of August to October (810 ± 34 kJ/kg/day) and the highest over the post-breeding season months of November to March (1007 ± 20 kJ/kg/day). In the pre-breeding season from April to July, 897 ± 38 kJ/kg/day were consumed. Post hoc Bonferroni found a significant difference between the pre-breeding and breeding seasons (\( P = 0.017 \)) and between the breeding and post-breeding seasons (\( P < 0.001 \)).
Figure 3.1: Mean daily intake of gross energy (kJ/kg body mass/day) in each month by male (n=2, open circles) and female (n=5, closed circles) platypuses; data for 2013 –2015 combined. Results presented as kJ/kg/day. Black line: females (mean); grey line: males (mean). The pre-breeding season is defined as April to July, the breeding season from August to October and the post-breeding season from November to March.

**Food intake**

There was a significant difference between seasons in the mass of mealworms \((F = 12.6, \text{d.f.} = 2, P < 0.01)\), earthworms \((F = 34.8, \text{d.f.} = 2, P < 0.01)\) and crayfish \((F = 4.6, \text{d.f.} = 2, P = 0.01)\) consumed. The results of *post hoc* comparisons indicated that both mealworms and earthworm consumption varied significantly between the pre-breeding and post-breeding seasons \((P < 0.001)\). In addition, crayfish consumption varied significantly between the pre-breeding and breeding seasons \((P = 0.01)\). Crayfish consumption peaked during the pre-breeding season, whereas mealworms and earthworm consumption peaked during the post-breeding season (Figure 3.2). There was no difference in the mass of fly pupae consumed between seasons \((F = 2.5, \text{d.f.} = 2, P = 0.08)\).
Figure 3.2:: Mean (± S.E.) weight of different foods eaten by platypuses (n = 7; female and male data combined) per day from 2013 - 2015. Pre-breeding season = April - July; Breeding season = August - October; Post-breeding season = November – March. Significance ($P < 0.01$) was found between seasons for crayfish, mealworms and earthworms. Black: pre-breeding season; light grey: breeding season; dark grey: post-breeding season.

The percentage of each food type in the diet varied between seasons. Crayfish contributed the highest percentage of the diet (39%), and mealworms lowest percentage (26%), in the pre-breeding season compared to the other seasons. The highest percentage of fly pupae (15%) was consumed in the breeding season. The highest percentage of earthworms (29%) and mealworms (29%) and lowest of fly pupae (9%) was consumed in the post-breeding season (Figure 3.3). Platypus do not consume whole crayfish due to their tough exoskeleton that needs to be removed before the flesh can be eaten. The largest component of crayfish eaten was the viscera contained in the thorax (less than 0.6g remaining per day), with the majority of remains consisting of unshelled tail meat (15g remaining per day pre-breeding, 16g breeding and 10g post-breeding).
Figure 3.3: Percentage, by wet mass, of each food type in the total diet consumed during each season (n=7, female and male data combined). Pre-breeding season = April - July; Breeding season = August - October; Post-breeding season = November – March. White = earthworms, black = mealworms, light grey = fly pupae and dark grey = crayfish.

Food preference

The combined data for males and females indicated that fly pupae, mealworms and earthworms were preferred food items when compared with crayfish (Figure 3.4). There was no variation between seasons. Food remains included 61% of the offered crayfish and 24 – 28% of the offered fly pupae, mealworms and earthworms indicating the diet was *ad libitum*.
Figure 3.4: Jacob's index for each food type by season. Values approaching -1 were negatively selected, to 0 were consumed in proportion to their availability and approaching +1 were positively selected. Black: pre-breeding season; light grey: breeding season; dark grey: post-breeding season.

**Nutritional composition of foods consumed**

Proteins formed the largest component of the diet year round, followed by fats and carbohydrates (Figure 3.5). There was a significant difference between seasons for intake (g/100g) of proteins ($F = 7.1$, d.f. = 2, $P = 0.001$), fats ($F = 8.0$, d.f. = 2, $P < 0.001$) and carbohydrates ($F = 14.5$, d.f. = 2, $P < 0.001$). The results of *post hoc* comparisons showed a significant difference between the breeding and post-breeding season for all three components ($P < 0.001$) and also between the pre-breeding and post-breeding season for carbohydrate intake ($P < 0.001$).
Figure 3.5: Mean consumption per day of macronutrients in the complete diet (± S.E.). Pre-breeding season = April - July; Breeding season = August - October; Post-breeding season = November – March. Significance (p<0.01) was found between seasons in each categories. Black: pre-breeding season; light grey: breeding season; dark grey: post-breeding season.

Energy (kilojoules) and proportions of fats, proteins and carbohydrates for each of the food items are listed in Table 3.3. Mealworms and crayfish viscera contained the highest energy and fat content and earthworms contained the lowest amount of energy. Mealworms and fly pupae contained the highest protein levels.
Table 3.3: Macronutrients in the dietary items fed to captive platypuses

<table>
<thead>
<tr>
<th></th>
<th>Fly pupae</th>
<th>Mealworms</th>
<th>Earthworms</th>
<th>Freshwater crayfish viscera</th>
<th>Freshwater crayfish tail</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy (kJ/100g)</strong></td>
<td>560</td>
<td>960</td>
<td>220</td>
<td>650</td>
<td>280</td>
</tr>
<tr>
<td><strong>Fats (g/100g)</strong></td>
<td>4.7</td>
<td>13.2</td>
<td>1.1</td>
<td>13.3</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Protein (g/100g)</strong></td>
<td>18.9</td>
<td>20.6</td>
<td>7.4</td>
<td>9.2</td>
<td>14.9</td>
</tr>
<tr>
<td><strong>Carbohydrate (g/100g)</strong></td>
<td>4</td>
<td>7</td>
<td>3</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td><strong>Saturated fatty acids (%)</strong></td>
<td>37.8</td>
<td>24.0</td>
<td>40.2</td>
<td>26.9</td>
<td>41.5</td>
</tr>
<tr>
<td><strong>Mono-unsaturated fatty acids (%)</strong></td>
<td>39.2</td>
<td>47.5</td>
<td>17.9</td>
<td>48.9</td>
<td>35.0</td>
</tr>
<tr>
<td><strong>Poly-unsaturated fatty acids (%)</strong></td>
<td>22.7</td>
<td>28.2</td>
<td>35.7</td>
<td>22.6</td>
<td>22.6</td>
</tr>
<tr>
<td><strong>Moisture (g/100g)</strong></td>
<td>70.2</td>
<td>56.6</td>
<td>87.7</td>
<td>74.9</td>
<td>79.2</td>
</tr>
</tbody>
</table>

**Fatty acids**

Crayfish tails and earthworms contained the highest proportion of total saturated fats (41% and 40% respectively). Crayfish viscera and mealworms contained the highest proportion of mono-unsaturated fats (49% and 47% respectively), and earthworms contained the highest proportion of poly-unsaturated fats (36%).

**Vitamins, macronutrients and trace elements**

Many vitamins, macronutrients and trace elements occurred in similar amounts in the food items analysed (Table 3.4). However, fly pupae contained the highest levels of manganese, B₂, B₅ and magnesium, mealworms contained the highest levels of vitamin A and crayfish viscera contained high levels of vitamin B₁₂ and iron, and crayfish tails contained the highest levels of calcium.
Table 3.4: Vitamins, macronutrients and trace elements found in the dietary components fed to captive platypuses

<table>
<thead>
<tr>
<th></th>
<th>Fly pupae</th>
<th>Mealworms</th>
<th>Earthworms</th>
<th>Crayfish viscera</th>
<th>Crayfish tail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A (µg/100g)</td>
<td>&lt;5</td>
<td>16</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;1&lt;/sub&gt; (mg/100g)</td>
<td>0.1</td>
<td>0.26</td>
<td>0.06</td>
<td>0.07</td>
<td>0.23</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;2&lt;/sub&gt; (mg/100g)</td>
<td>1.9</td>
<td>0.42</td>
<td>1.1</td>
<td>0.27</td>
<td>0.12</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;3&lt;/sub&gt; (mg/100g)</td>
<td>5.4</td>
<td>4</td>
<td>4.3</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;5&lt;/sub&gt; (mg/100g)</td>
<td>3.8</td>
<td>1.6</td>
<td>0.1</td>
<td>0.52</td>
<td>0.48</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;6&lt;/sub&gt; (mg/100g)</td>
<td>0.03</td>
<td>0.34</td>
<td>0.03</td>
<td>0.08</td>
<td>0.03</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt; (µg/100g)</td>
<td>0.08</td>
<td>1.21</td>
<td>14.3</td>
<td>20.5</td>
<td>7.41</td>
</tr>
<tr>
<td>Vitamin C (mg/100g)</td>
<td>&lt;1</td>
<td>1.7</td>
<td>0.4</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Vitamin D (µg/100g)</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Vitamin E (mg/100g)</td>
<td>3.7</td>
<td>6.2</td>
<td>0.1</td>
<td>8.3</td>
<td>3.4</td>
</tr>
<tr>
<td>Vitamin K (µg/100g)</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>2.3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Copper (mg/kg)</td>
<td>5.2</td>
<td>6.2</td>
<td>1.1</td>
<td>8.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Iron (mg/kg)</td>
<td>71</td>
<td>56</td>
<td>28</td>
<td>140</td>
<td>30</td>
</tr>
<tr>
<td>Manganese (mg/kg)</td>
<td>69</td>
<td>9.7</td>
<td>1.9</td>
<td>8.3</td>
<td>11</td>
</tr>
<tr>
<td>Selenium (mg/kg)</td>
<td>0.18</td>
<td>0.65</td>
<td>0.45</td>
<td>0.45</td>
<td>0.27</td>
</tr>
<tr>
<td>Zinc (mg/kg)</td>
<td>63</td>
<td>41</td>
<td>12</td>
<td>62</td>
<td>12</td>
</tr>
<tr>
<td>Iodine (mg/kg)</td>
<td>0.085</td>
<td>0.11</td>
<td>0.27</td>
<td>2</td>
<td>1.9</td>
</tr>
<tr>
<td>Calcium (mg/kg)</td>
<td>1300</td>
<td>4600</td>
<td>370</td>
<td>6700</td>
<td>11000</td>
</tr>
<tr>
<td>Phosphorus (mg/kg)</td>
<td>3400</td>
<td>3000</td>
<td>860</td>
<td>2200</td>
<td>2900</td>
</tr>
<tr>
<td>Potassium (mg/kg)</td>
<td>3200</td>
<td>3800</td>
<td>1100</td>
<td>1700</td>
<td>2800</td>
</tr>
<tr>
<td>Magnesium (mg/kg)</td>
<td>1400</td>
<td>950</td>
<td>130</td>
<td>340</td>
<td>430</td>
</tr>
</tbody>
</table>
Discussion

This is the first study to demonstrate that captive platypuses exhibit seasonal variation in their preference and intake of different prey types. In the pre-breeding season the animals consumed the most crayfish viscera, a lipid rich food that is also high in many minerals, macronutrients and vitamins. During the breeding season the lowest number of kilojoules were consumed. Finally, in the post-breeding season platypuses consumed the most kilojoules and the highest amount of energy rich mealworms and low quality earthworms. These patterns are consistent with predicted changes in energy requirements for different stages of the breeding cycle (see below).

Seasonal body condition

We recorded the highest body weights in the pre-breeding season, which probably resulted from the high energy intake in the preceding post-breeding period. This is consistent with patterns observed in wild platypuses for which highest body weights occur in late summer (Grant & Carrick 1978; Hulbert & Grant, 1983; Temple-Smith, 1973), and is likely to be driven by the need to increase body reserves prior to breeding. Similar patterns occur in other aquatic mammals, e.g. phocid seals (Sparling, Speakman, & Fedak, 2006). We found that platypuses consumed 13 – 21% of their body mass daily. This is comparable to previous studies on captive platypuses that were found to consume 15 – 30% (Holland & Jackson, 2002; Krueger et al., 1992).

Seasonal energy requirements

We found that captive platypuses consumed the lowest number of kilojoules and had the lowest mean body weights during the breeding season (late winter to early spring), suggesting a decrease in foraging effort despite the majority of animals in the study being housed alone or in female pairs. Energy requirements, and therefore the amount and quality of food consumed, is known to change with factors such as season (Knott, 1998), food availability, behaviour patterns (Gende, Quinn, & Willson, 2001) and reproduction (Krockenberger, 2003). Over winter, wild platypuses swimming in colder water have been found to experience increased thermal stress, correlated with an increase in metabolic rate (Grant & Dawson, 1978). They also exhibit behavioural and physiological changes associated with their late winter/ early spring breeding pattern (Gust
Handasyde, 1995; Handasyde, McDonald, & Evans, 2003). Platypuses during this stage of the year have also been found to have higher blood concentrations of glucocorticoids, indicating they are mobilising energy reserves in their bodies (Handasyde et al., 2003). Platypus body weights in the wild also vary seasonally with lower body weights recorded during winter (Temple-Smith, 1973). These patterns are not uncommon amongst mammals. Male fallow deer (*Dama dama*) decrease feeding activity to increase their search time for females and defend territories in the breeding season (Pélabon & Komers, 1997), and male northern quolls (*Dasyurus hallucatus*) decline in body weight during the mating period (Oakwood, Bradley, & Cockburn, 2001). Surprisingly, however, the tail fat stores (indicating body condition) of captive platypuses did not vary between seasons, which is in contrast to the published literature on wild platypuses (Grant & Carrick 1978; Handasyde et al., 2003; Temple-Smith, 1973). Bethge (2002) found that wild platypuses spend more time foraging in winter than in summer, increasing their feeding rates by 68% in winter to meet their energy requirements. Their study was located in Tasmania where the breeding season occurs around three months later than in Victoria (Grant, 2007). The Tasmanian winter therefore falls between our post-breeding and pre-breeding season and Tasmanian breeding season occurs over summer; this is consistent with our findings.

While the captive animals in this study were exposed to normal seasonal temperature changes, in particular colder water temperatures in winter, they are fed ad libitum and, although active in their tanks, have a restricted foraging area which would limit their energy expenditure. This is in contrast to wild males that were recorded swimming over 1km in 30 minutes (Gust & Handasyde, 1995). Previous studies have calculated energy expenditure in platypuses to be 341 kJ/kg/day (Grant & Carrick 1978) and 684 kJ/kg/day (Bethge, 2002) which is much lower than the energy intake that we found of 921 kJ/kg/day. While energy intake is expected to be higher than expenditure for growth and reproduction (Nagy, 2005), the first listed estimate of energy expenditure is based on the assumption that the platypus spends eight hours foraging and 16 hours resting which may explain why it is lower. Wild platypuses in Tasmania were found foraging for up to 16 hours during the winter (Otley, Munks, & Hindell, 2000) and have been found to have significant seasonal variation in their activity levels with
these being higher during the breeding season (Bethge, 2002). In captivity, platypuses that were housed in breeding pairs were often active in the water for more than 24 hours at a time during the breeding season and would therefore be expected to have a higher energy expenditure than at other times of the year (J. Thomas, pers. obs.). Further study should be considered to investigate the activity patterns of non-breeding captive platypuses to determine if the changes in seasonal energy consumption are correlated with changes in seasonal energy expenditure.

Prey selection

Captive platypuses showed selection for smaller prey items rather than crayfish, despite crayfish forming close to 40% of the total diet. Crayfish and mealworms are similarly nutritious, but differ in the time and effort required to obtain, process and consume. Optimal foraging theory predicts that predators will adapt their hunting behaviour to obtain food most efficiently, and gain the most kilojoules while expending the least energy (Stephens & Krebs, 1986). One limitation to maximising efficiency is the behaviour of prey (Sih & Christensen, 2001). Crayfish are mobile and exhibit strong antipredator behavioural responses (Zucker, 1972), thus, required active hunting. They also require considerably more processing to remove their hard exoskeleton before consumption (J. Thomas pers. obs.). In contrast, fly pupae, mealworms and earthworms are soft bodied, making processing easier. Platypuses likely capture small species with less effort, because they are less mobile and have no predator avoidance response. This suggests that encounter rates with these prey are likely to be higher, reducing the energetic costs of acquisition. We suggest that these factors contribute to the preference for smaller prey items by platypuses in our study.

Wild platypuses only eat a portion of the available prey species present in their environment, although the level at which prey selection operates remains unclear (Marchant & Grant, 2015; McLachlan-Troup et al., 2010). Marchant and Grant (2015) reported 55 families of invertebrates of 74 available in their study area in the diet of platypuses, and McLachlan-Troup et al. (2010) recorded 26 families in the diet, out of 36 available. These data suggest that wild platypuses exhibit selection for prey species. However, once the data in the first study were
subjected to rank correlation, the prevailing species in the diet were positively correlated with their availability in the environment, indicating there was no selection for particular prey (Marchant & Grant, 2015). Alternately, when the latter study was analysed using Ivlev’s index of electivity, positive selection occurred for more than 60% of available prey species in each season and habitat type. We selected Jacob’s index as it is a variation of Ivlev’s electivity index that can be used where different prey types occur in different abundances in the environment (Jacobs, 1974). However, both studies sampled platypus cheek pouches to obtain dietary data. This only represents a single time point for food intake and therefore indicates only the presence of a prey species in the diet, not the amount consumed. In addition, larger prey items, such as crayfish, and also soft bodied prey items, such as oligochaete worms and larval dipterans, are underrepresented as they are not stored in cheek pouches (Klamt et al., 2016; Marchant & Grant, 2015). While Marchant and Grant (2015) quantified prey production and availability, and correlated this with prey presence in the diet, our study is the first to accurately record quantities of food consumed by platypus over time. This is only possible in a captive environment where food consumption can be directly measured against availability, though comparison with wild diet is difficult due to the differences in prey species availability and abundance.

In this study, crayfish meat made up the highest percentage of the total food eaten in each season (32 – 39%). This occurred despite Jacob’s selectivity index indicating that this prey was avoided (i.e. consumed at a lower rate than its availability). The dominance of crayfish in the diet is likely due to their large size, with each providing around 30g of meat once the exoskeleton was discarded. In contrast, one mealworm weighed around 0.5 grams. Thus, consuming one crayfish provided a higher mass of food in a single feeding event, which may explain their large contribution to the diet. Mealworms formed the second largest prey contribution to the diet (26 – 30%), followed by earthworms (23 – 29%), with fly pupae contributing the least (9 - 15%). Platypuses are predominantly benthic feeders (Faragher et al., 1979), thus the low contribution of fly pupae might be explained because they float, thus require different foraging behaviour compared to other prey types that sink to the substrate.
Nutrition of the prey

Although crayfish provide a large amount of meat, we found that platypuses often consumed the thorax and viscera, leaving the tail untouched. Tails were discarded most often during the pre- and breeding seasons which indicates the animals were mostly eating the viscera, which was also one of the most nutritious food items provided to them. Grey wolves (Canis lupus) are also known to consume the most nutritious component, the internal organs, of their prey first, then muscle, with bones and hide eaten last (Stahler et al., 2006). The internal organs are high in vitamin A, B complex, minerals and fatty acids. Similarly, crayfish viscera are more nutritious than the tail, so consuming more viscera may allow platypuses to maximise nutrient intake. The timing of this pattern of selection may facilitate efficient accumulation of nutrients in preparation for the breeding season.

We found that, although crayfish provided less kilojoules to mealworms, crayfish were consumed in larger amounts in the pre-breeding season. Crayfish contained higher levels of vitamin B₁₂, iron and calcium (20, 1.5 and 3 times higher respectively), while mealworms had higher levels of potassium, magnesium and Vitamin B₅ (2.2, 2.8 and 3 times higher respectively) and were the only source of Vitamin A in the diet. Selection for particular nutrients can also influence prey selection and the volume of food consumed. Locusts target different foods to balance either protein or carbohydrate deficiencies and to acquire required amounts of macronutrients (Raubenheimer & Tucker, 1997). Likewise, some mammalian predators are able to regulate their macronutrient intake to reach a specific intake target (Mayntz et al., 2009). Selective nutrient intake can also affect reproductive output. Predatory beetles, consumed quantities of protein and lipids that maximised egg production, while those that consumed nutrients in excess or deficit had poor egg production (Jensen et al., 2012). It is unclear why earthworms were consumed in higher proportion by our captive platypuses during the post-breeding season as they contained the lowest energy and were generally low in macronutrients, vitamins and trace elements. The timing of changes we observed in our captive platypuses diet may be due to differing nutritional and energetic demands during preparation for the breeding season.
Prey selection by captive platypuses appears to be largely influenced by the nutritional value and behaviour of the prey. Seasonal variation in the amount of food, nutrients and energy consumed correlated clearly with preparation for the breeding season, which is an intense and energetically costly period. The current captive diet for platypus was largely created based on commercially available invertebrates rather than the nutritional and energy requirements. This research provides important information on the role of each prey item in the captive diet of platypuses and also the need to provide a varying quantity of food at different times of the year rather than a constant amount year round. While our study provides important information about diet and feeding behaviour of captive platypuses, it is difficult to draw comparisons between captive and wild diets due to the availability of food in captivity. Overall our study provides novel data that will help to improve captive feeding regimes and assist with the management, welfare and breeding of platypuses in captivity.

Acknowledgments
We thank keepers from Healesville Sanctuary, particularly Sarah Boyd and Mason Hill, for meticulous collection of data, and Dr Kim Miller for statistical advice. This project was funded by Healesville Sanctuary and the Winifred Violet Scott Trust. This study was conducted under approval from the Zoos Victoria Animal Ethics Committee ZV12014.
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Chapter 4 Female control of reproductive behaviour in the platypus (Ornithorhynchus anatinus), with notes on female competition for mating
Female control of reproductive behaviour in the platypus (*Ornithorhynchus anatinus*), with notes on female competition for mating

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Received 14 November 2017; initial decision 21 December 2017; revised 30 January 2018; accepted 31 January 2018; published online 13 February 2018

Abstract

Opportunities for studying platypus courtship and mating behaviours in the wild are limited due to the nocturnal and cryptic nature of this species. We report on platypus courtship and mating behaviour from a successful breeding program at Healesville Sanctuary, Victoria, in which platypuses were held as either breeding pairs or trios over seven years. Behaviour was recorded daily on infrared cameras resulting in over 80,000 h of footage that was analysed for activity periods, and courtship and mating behaviours including non-contact and contact courtship, mating and avoidance. Our aims were to describe and quantify courtship and mating interactions between males and females, and to determine if either sex controlled the initiation and continuation of the behaviours. From our observations, we describe a new courtship behaviour, non-contact courtship, which constituted the majority of all mating season interactions between males.
and females. The time between first and last appearance of a courtship and mating behaviour was 41.0 ± 6.6 days, with the females showing behavioural receptivity for 29.6 ± 5.1 days. Female platypuses used three evasive strategies in relation to approaches by males: avoidance, flight and resistance. Females controlled the duration of 79% of encounters using resistance. For the first time, two females were seen competing with each other over access to the male platypus in their enclosure and for nesting material. Time investment in courtship and mating behaviours was a poor indicator of receptivity and breeding success, and we suggest that breeding failure is more likely to be associated with failure of fertilisation, nest building, embryonic development or incubation. We describe how female platypuses demonstrate evasiveness and control of courtship and mating behaviours, and the importance of providing these opportunities in captivity to promote successful breeding.

Keywords
platypus, captive breeding, mating, courtship, seasonality, female competition.

Introduction
Animals select mates based on a range of often species-specific attributes to allow them to maximise their fitness and reproductive success (Clutton-Brock & McAuliffe, 2009). The innate courtship behaviours performed prior to mating can assist individuals to alleviate hostility between pairs in territorial species and synchronise reproductive physiology of pairs (reviewed in Fusani, 2008). Courtship behaviour is a process of sexual selection allowing animals to either select or reject mates based on performance and/or other physical, behavioural and genetic characteristics. Throughout the animal kingdom these species-specific behaviours present in different ways, for example, vocalisations by tungara frogs (Engystomops pustulosus; Ryan & Guerra, 2014), physical appearance and adornments by peacocks (Pavo cristatus; Dakin & Montgomerie, 2013), mate guarding by jumping spiders (Phidippus clarus; Elias et al., 2014), biting/mouthing or fighting by polar bears (Ursus maritimus; Stirling et al., 2016) and patterned movements or dances by Atlantic Cod (Gadus morhua L; Engen &
Folstad, 1999). One aspect of sexual selection that occurs in many species is same sex competition for access to the opposite sex (Alcock, 2009). Fighting amongst males of many species commonly leads to the victors gaining access to females and mating more often (Blanckenhorn, 2005). However, in cryptic species, such as the platypus, the behaviours involved in sexual selection are poorly understood. Male platypuses fight with each other, using their venomous spurs as weapons, during the breeding season rise in androgens, presumably to gain access to territories and females (Temple-Smith, 1973; New et al., 1998). Male platypuses also produce a strong, musky scent from cervical scent glands during the breeding season that may play a role in territoriality and individual identification (Temple-Smith, 1973). Many species that compete for territories and access to mates are sexually dimorphic, with the competing sex having a larger body size (Blanckenhorn, 2005). In accordance with observations supporting the male as the competing sex, platypuses show a strong sexual dimorphism with males up to 40% heavier than females in all populations that have been studied (Grant, 2007; Bino et al., 2015). Female platypuses appear to be more tolerant of each other than males, often occupying smaller home ranges that can overlap with one another (Serena, 1994).

Males use aggression and coercion (force copulation, intimidation, harassment, physical injury) as a strategy for improving their breeding success, and in response females will try to control encounters with males to reduce the risk of harassment and injury (Clutton-Brock & Parker, 1995). In some species, for example orangutans (*Pongo pygmaeus*), females will be coerced into breeding by the use of force by the much larger males (Mitani, 1985). However, in other species females will either resist encounters by manipulating situations or via physical avoidance (Orbach et al., 2015). Female cetaceans control breeding encounters by using evasive behaviour where they enter shallow water to exclude the larger males (Craig et al., 2014). Female southern hairy nosed wombats (*Lasiorhinus latifrons*) will turn away from a male and retreat when they are not receptive, and sometimes become aggressive towards the male if the encounter is unwanted (Hogan et al., 2011).

The platypus (*Ornithorhynchus anatinus*) is a semi-aquatic mammal inhabiting freshwater ecosystems along eastern Australia. Typically solitary by nature, platypuses are seasonal breeders that mate in late-winter to spring and
show a latitudinal cline in breeding season with northern populations breeding earlier than the southern populations (Grant & Temple-Smith, 1998). After mating, the female platypus constructs a nest of wet vegetation in a burrow where she lays between one and three eggs (Burrell, 1927). The period from the start of incubation to the hatching of eggs is estimated at 10 days (Griffiths, 1978). Hatching is followed by four months of lactation in which nestlings remain in the burrow while the mother leaves them unattended with increasing frequency to forage (Holland & Jackson, 2002).

Platypuses are very challenging to study in the wild because of the difficulties of observing them in their environment, the long distances they travel and their predominately nocturnal activities. In the wild it is very common to sight a platypus which then rapidly disappears under water, often not to be sighted again despite intensive scanning and monitoring of the water body. This makes wild studies very labour-intensive, data-poor, and creates enormous challenges in unravelling the complexities of courtship and mating behaviour that might assist in the conservation and management of this unique egg-laying mammal. Previous reports on mating and breeding behaviours have been from opportunistic wild encounters or captive-based observations (De La Warr & Serena, 1999; Holland & Jackson, 2002; Hawkins & Battaglia, 2009). Platypuses are notoriously difficult to maintain and breed in captivity, however there has been some success at Healesville Sanctuary and Taronga Zoo (Jackson et al., 2003). The captive breeding facility at Healesville Sanctuary provides a unique opportunity to view every aspect of the way in which these typically solitary animals interact with each other during the mating season. One of our authors (JT), along with other zoo keepers at Healesville Sanctuary and Taronga Zoo, has observed these behaviours for many years, yet very little from these observations has been recorded scientifically and reported. The most detailed published study was based on three successful breeding attempts and five unsuccessful attempts that showed similar behaviour interactions at Taronga Zoo (Hawkins & Battaglia, 2009). Despite the low sample sizes, our understanding of platypus behaviour is based on these wild and captive reports and other unpublished studies from which the observations have been aggregated to provide an understanding of many aspects of the biology of this cryptic mammal. Thus, our knowledge of
platypus reproductive biology remains rudimentary and many aspects of mate interactions and behaviour are unknown.

The aim of this study was to determine the time investment and types of interactions that occur between male and female platypuses during the mating season. We also aim to determine if females control behavioural encounters with males by using evasive strategies. We report the first observations of female-female competition for access to a male in the platypus and also describe a new preliminary courtship behaviour that suggests that females often control courtship and mating behaviour.

Material and methods

Animals

This study reports on four captive platypuses monitored between 2008 and 2015. Animals were housed in single male/single female breeding pairs from 2008–2013 and also in single male/two female breeding trios from 2014–2015. Individual platypus history and ages are listed in Table 4.1.

Housing and husbandry

Platypuses were housed in the breeding facility at Healesville Sanctuary, Zoos Victoria, VIC, Australia. The outdoor enclosure consisted of flowing freshwater and six ponds totalling 75,000 litres of water that were up to two metres deep and surrounded by areas of mulch along the water’s edge. The area was planted with native vegetation. The ‘deep’ and ‘shallow’ ponds included partially submerged logs, slow water flow and open space, while the ‘middle’ pond was smaller than the other two ponds and, although it also contained a partially submerged log, it had a higher water flow and high wall at the back. Three ‘feed tanks’ contained 5000 litres of water each and had few furnishings. Animals were fed an ad libitum diet of freshwater crayfish (*Cherax albidis* and *C. destructor*), earthworms (*Oligochaeta* sp.), mealworms (*Tenebrio molitor*) and fly pupae (*Musca domestica*). Two ‘dirt tanks’ consisted of 5000 litre water tanks filled with clay-rich soil and native plants were also available to the animals for burrowing and nesting.
Remote monitoring

Eight infrared closed-circuit television (CCTV) motion activated cameras (Vari-focal dome and bullet cameras, Jaycar electronics) were positioned to view all of the water areas and approximately 75% of the surface of the dirt tanks in the breeding facility all year round. All cameras were connected to a digital video recorder (DVR; Ganz DR16NRT Digimaster) and reviewed daily. Behaviours were described and classified as avoidance, non-contact courtship, contact courtship and mating (Table 4.2). From 2014–2015, animals were housed in a trio (one male, two females) and instances of female–female interactions were also recorded. The duration of courtship and mating behaviours (to the nearest minute) and the location within the enclosure they occupied was collected for the mating seasons of 2011–2015. Additional data, on which individual initiated the behaviours, were recorded in the 2015 mating season. The total activity time for each animal was recorded from 2008–2015 (to the nearest minute) as the time the animal emerges from their burrow to the time that they return. Data are presented as the premating season, consisting of the 90-day period prior to the first appearance of courtship behaviour; the mating season, consisting of the days from the first appearance of courtship to the last mating; and the post-mating season, consisting of the 90-day period after the last appearance of mating behaviour. This generated over 80,000 h of video footage, all of which was reviewed.

Definition of terms

The ‘mating season’ refers to the timeframe in which all courtship and mating behavioural interactions occurred between male and female platypuses. The ‘breeding season’ is defined as the whole season from the start of territorial behaviour in the male, through to the end of lactation by the female. ‘Breeding success’ refers to the years in which eggs were laid by females. In the case where eggs were laid but young not successfully reared to independence, we have indicated as such.

Data analyses

Two-way t-tests were used to compare means between successful and unsuccessful breeding years for pairs and trio groups and are expressed as $P$
values. All statistical calculations were conducted using SYSTAT (Systat Software, San Jose, CA, USA).

**Results**

Female platypuses were successful in laying eggs on five occasions during this study, and were successful in raising young on three occasions. The older female successfully raised 10 offspring between 2008–2015, while the younger female laid eggs on one occasion and was not successful in raising offspring (details in Table 4.1).

**Sequence of behaviours**

We constructed a flow chart (Figure 4.1) to show the sequence of the breeding season behaviours displayed by male and female platypuses, plus a description of each behaviour (Table 4.2). Briefly, the male platypus first increases his territorial and searching behaviour which leads to the females using avoidance strategies.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Sex</th>
<th>Year born</th>
<th>Approx. weight (g)</th>
<th>History</th>
<th>Age at first breeding (years)</th>
<th>Number of successful breeding years</th>
<th>Number of successful breeding years (eggs only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male A</td>
<td>M</td>
<td>1999</td>
<td>1600</td>
<td>Captive born</td>
<td>8</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Male B</td>
<td>M</td>
<td>2009</td>
<td>1100</td>
<td>Wild caught/rehabilitated</td>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Female A</td>
<td>F</td>
<td>2002</td>
<td>1200</td>
<td>Captive born</td>
<td>5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Female B</td>
<td>F</td>
<td>2007</td>
<td>1100</td>
<td>Captive born</td>
<td>8</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Successful breeding years are defined by the number of years in which young were successfully reared. Successful breeding years (eggs only) refers to the years in which eggs were laid but young were not reared.
The female then reduces the use of these strategies and starts non-contact courtship with the male, which involves swimming past each other in the same area. This progresses into contact courtship (tail biting) and then to mating. After mating, the female resumes avoidance strategies before constructing her nesting burrow. The male continues with courtship and mating until he has mated with all females, at which point he reduces his searching effort. The male is able to go through courtship and mating behaviours with more than one female at the same time. Because platypuses are seasonal breeders, the males are only fertile for about three months each year (Temple-Smith & Grant, 2001).
Table 4.2: Types of breeding behaviours and a description of their presentation that were observed. Territorial and searching behaviour was observed but not measured in this study.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Territorial and searching</td>
<td>Frequent swimming between the different pools, mostly on the surface of the water. The animal is on its own and appears to spend less time foraging for food.</td>
</tr>
<tr>
<td>Avoidance</td>
<td>One animal leaving the pool within 15 seconds of the arrival of the other or a change in activity pattern to avoid overlap.</td>
</tr>
<tr>
<td>Non-contact courtship (NCC)</td>
<td>The male and female swimming around in the same pool together without making an attempt to flee. There was no feeding or other functional behaviour during this time of close proximity and it was not observed outside of the mating season.</td>
</tr>
<tr>
<td>Contact courtship (CC)</td>
<td>The male biting onto the tail of the female and swimming in circles and twists on the waters’ surface.</td>
</tr>
<tr>
<td>Mating (M)</td>
<td>The male mounting the female and mating successfully.</td>
</tr>
</tbody>
</table>

**Timing of breeding behaviours**

Based on the onset of courtship and mating interactions between male and female captive platypuses, the mating season commenced as early as 5 August, and all interactions had ceased by 29 November. The mean date of the mating season onset was 20 August ± 6 days, and the mean completion was 24 October ± 15 days, data combined across all years.

**Other signs of breeding activity**

During the mating season, both females received superficial oval shaped wounds (< 10 mm diameter), on the ventral side of the tail at the tip where the majority of biting (by males) occurred (Figure 4.2a, b). Scratch-like grazes on both the hind feet and ventral side of the tail (Figure 4.2b) that appeared to be from claws or spurs were also recorded.
Figure 4.2: (a) A male and female platypus in contact courtship; (b) a puncture wound and scratches on the ventral side of the tail of a female platypus after courtship and mating behaviours.
Table 4.3: Time investment in courtship and mating behaviours in successful and unsuccessful breeding years including the number of days the behaviours occurred, the total time invested in each behaviour, the number of behavioural events and the average duration of each event.

<table>
<thead>
<tr>
<th></th>
<th>Non-contact courtship</th>
<th>Contact courtship</th>
<th>Mating</th>
<th>Total season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Number of days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Successful</td>
<td>33.6</td>
<td>6.4</td>
<td>29.6</td>
<td>5.1</td>
</tr>
<tr>
<td>Unsuccessful</td>
<td>38.5</td>
<td>4.5</td>
<td>38.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Time investment (min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Successful</td>
<td>3618.4</td>
<td>839.5</td>
<td>835.8</td>
<td>340.0</td>
</tr>
<tr>
<td>Unsuccessful</td>
<td>3937.0</td>
<td>308.0</td>
<td>859.0</td>
<td>500.0</td>
</tr>
<tr>
<td>Number of events</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Successful</td>
<td>556.4</td>
<td>166.2</td>
<td>340.6</td>
<td>112.1</td>
</tr>
<tr>
<td>Unsuccessful</td>
<td>666.5</td>
<td>62.5</td>
<td>340.0</td>
<td>115.0</td>
</tr>
<tr>
<td>Event duration (min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Successful</td>
<td>6.2</td>
<td>0.3</td>
<td>2.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Unsuccessful</td>
<td>5.6</td>
<td>0.9</td>
<td>2.5</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**Duration of courtship and mating behaviours**

The average duration of the mating season, from the first to the last appearance of courtship and mating behaviours, was 41.0 ± 6.6 days. Non-contact courtship (NCC) had the longest duration at 33.6 ± 6.4 days, followed by contact courtship (CC) for 29.6 ± 5.1 days and mating (M) for 13.8 ± 4.0 days (Table 4.3). These behaviours could occur simultaneously during a single day. In years where breeding was not successful, the average duration of the mating season was 43.5 ± 0.7 days. A similar number of days were spent in each behaviour in the successful and unsuccessful years and there was no significant difference between the number of days for each behaviour (Total duration: $P = 0.72$, NCC: $P = 0.56$, CC: $P = 0.37$, M: $P = 0.49$).
Time allocation to different behaviours

The time spent in each of the behaviours was different, with NCC occupying the largest amount of time and M the least amount of time for both a pair and trio (Figure 4.3). There was a significant difference between the average time spent in CC and M ($P = 0.01$), the average time spent in M and NCC ($P < 0.001$), and in CC and NCC ($P < 0.001$).

![Figure 4.3: Total time spent (mean ± SE) per platypus in each behaviour type in a mating season by animals housed in either a pair or trio. The pair contained one male and one female, while the trio contained one male and two females. There was no significant difference between pairs and trios for each behaviour ($P = 0.19$, $0.07$ and $0.61$, respectively). Dark grey, pair; light grey, trio; circles, data points for each year.](image)

Between the years where breeding successfully produced offspring ($N = 5$) and the years that were unsuccessful ($n = 2$), there was no significant difference in the total time spent on each behaviour (NCC: $P = 0.74$, CC: $P = 0.97$, M: $P = 0.43$), the number of times each behaviour occurred (NCC: $P = 0.56$, CC: $P = 0.99$, M: $P = 0.93$), the number of days spent on each behaviour (NCC: $P = 0.56$, CC: $P = 0.37$, M: $P = 0.48$), or the average duration spent on each behaviour (NCC: $P = 0.48$, CC: $P = 0.84$). There was, however, a significant difference observed between successful and unsuccessful years in the average duration of the mating sessions (M: $P < 0.001$). Every year, regardless of whether offspring
were produced, the females showed nesting behaviour and a short retirement underground for egg laying. This was determined by excavating a nest that was abandoned after less than 60 h of retirement, where two eggs were recovered. These behaviours were only observed in mated females and not in other females that were not paired with a male, housed at Healesville Sanctuary (n = 4).

![Figure 4.4](image)

Figure 4.4: Individual experience of the two females and the duration invested in breeding behaviours. (a) Total duration per year; (b) NCC; (c) CC; (d) mating behaviours per year. Black, female A, Grey, female B. Caret (`) denotes years that were unsuccessful.

No significant difference was found in the amount of time spent on NCC ($P = 0.10$), CC ($P = 0.19$) and M ($P = 0.49$) behaviours between the years for either pairs (one male and one female) or trios (one male and two females). This has likely resulted from the large standard errors in the data (Figure 4.3). There
was also no significant difference in the number of times each behaviour occurred during the mating season (NCC, $P = 0.06$; CC, $P = 0.18$; M, $P = 0.70$).

In Figure 4.4 (Continued.),

**Influence of individual experience**

The older female (female A) had bred successfully more times than the younger female (female B; Table 4.1) but the time each female invested in courtship and mating behaviours was not significantly different ($P = 0.32$). However, the older female showed a trend of spending less time in all behaviours than the younger female. The most obvious difference was the amount of time spent in mating by the younger female, which was much higher in 2015 than in other years (Figure 4.4). Two eggs were laid by this female, but were not successfully reared.
Evasive behaviour

Resistance

In 2015, 730 behavioural interactions (NCC, CC and M) were assessed to determine which animal left the encounter first. The female was considered “resistant” if she ended the encounter and “non-resistant” if the male ended the encounter (terms defined by Orbach et al., 2015). The females were resistant in 79% of all encounters observed. Of these encounters, 68% were behaviours involving one male and one female and 8% were between one male and two females. The younger female terminated the encounter first in most of the trio (74%) and female–female encounters (59%). Very few mating encounters were terminated overall and none were terminated by the male (Figure 4.5). The most frequently terminated encounter was NCC. The younger female terminated these encounters in 84% of the cases where NCC was ended by a female. However, the older female terminated 74% of the CC encounters that were ended by a female.

Flight

The mean number of flight events, where the female immediately left the pond when encountering the male, was 157 ± 21 times per mating season. Flight behaviour in response to male presence first appeared 17 ± 5 days prior to the first courtship (n = 5). The last appearance of this behaviour was variable; on three occasions it concluded prior to the last observed courtship or mating behaviour but it also occurred twice after the last courtship or mating behaviour was observed.

Activity patterns

Both males showed similar high levels of behavioural activity in the premating (three months prior to the first courtship and mating behaviour) and mating seasons (time frame where all courtship and mating behaviour occurred), with a decline in activity in the post-mating season (three months after the last courtship and mating behaviour; Figure 4.6). The females had significantly lower levels of activity than the males in the pre- and post-mating season (\(P < 0.001\) and \(P < 0.001\), respectively), but there was no significant difference in behavioural activity levels during the mating season (\(P = 0.123\)).
Figure 4.5: Percentage of encounters that were ended by either a male or a female. Light grey, non-contact courtship; dark grey, contact courtship; black, mating.

The average proportion of time the animals were awake and active during 24 h in each of the pre-mating, mating and post-mating seasons varied according to sex (Figure 4.7a–c). In the pre-mating season, the males were more active nocturnally and for longer than the females. During the mating season, males were more often active nocturnally, emerging at dusk and returning to their burrow at dawn. Females were more variable with their activity times, often emerging after midnight and remaining active diurnally until midday (Figure 4.7d). In the post-mating season, the males continued with the same nocturnal activity pattern that they exhibited during the mating season, while the females returned to a more nocturnal activity cycle and with less time active overall compared to the pre-mating season. When housed in a group, both females often emerged after midnight and the older female switched to a diurnal activity cycle.
Physical location of behaviours

Of the 4798 encounters analysed, 40% occurred in the deep pond, 32% in the shallow pond, 25% in a feed tank and 3% in the middle pond. The deep and shallow ponds were also the ones most frequently occupied when NCC (44 and 30%, respectively) and CC (37 and 35%, respectively) behaviours were recorded, while mating occurred most frequently in the smaller feed tank (45%).

Observations on mating ‘styles’

Each of the two males displayed different mating techniques. The larger male (average weight 1600 g), mounted the female for mating with his tail wrapped under her body and curled around her rump while holding onto her back and hind feet with his front feet. The smaller male (average weight 1100 g), mounted the females by laying on his side and partially on his back under her belly, using his bill to bite and hold onto the skin on her neck and using his hind feet to grip her lower body.
Figure 4.7: Mean daily spread of activity for males and females. (a) Pre-mating season activity (three months prior to the first courtship and mating behaviour); (b) mating season activity (duration of courtship and mating behaviours); (c) post-mating season activity (three months after the last courtship and mating behaviour); (d) pre-mating season activity when animals were in a trio in 2015. Black, male; dark grey, older female; light grey, younger female.
There were at least two records in each of the seven mating seasons of males attempting to mate with the females several weeks before the start of courtship. These events were brief (less than 30 s) and appeared to be an attempt at coercion by the male. Each time the female had been attempting flight behaviour when the male caught them. The response of both females was to scramble and flip around in the water to escape the situation to a different area away from the male.

**Observations of female–female competitive courtship behaviour**

The two females frequently engaged in competitive behaviour during the first year in which they were housed together with the male. The two females showed...
the following behaviours during October and November 2014 as each tried to

gain access to the male to mate:

• Approach: swimming in the vicinity of the other female and male

• Interruption: pushing herself into the interaction of the other pair

• Group courtship: all three animals biting each other’s tails and swimming in a
circle, a possible result from an interruption

• Female–female courtship: tail biting the other female and circle swimming and
twirling

• Fighting: forceful biting, climbing on top of the other female resulting in the
recipient female being submerged

In the following year (2015) the older and more experienced female switched
her activity pattern to a predominately diurnal cycle, which effectively reduced
interactions with the other female and the male. She switched her activity back to
a nocturnal cycle when she commenced courtship behaviours. By this stage the
younger female had ceased her courtship and mating behaviours with the male
and was starting to exhibit nesting behaviour.

Discussion

This is the first intensive study of courtship and mating behaviour in the
platypus across the mating season. We describe for the first time a preliminary
courtship behaviour — non contact courtship — in which the platypuses invested
the most time during the mating season, in comparison to other courtship and
mating behaviours. Female platypuses used a combination of three evasive
behavioural strategies to control breeding encounters with the male. These
strategies involved changing her activity pattern to avoid the male, a flight
response — fleeing from the male — and resistance by terminating the majority
of encounters. We also report the first observations of female–female competition
in the platypus for access to the male.

Courtship is a sexual behaviour under endocrine control that usually
signals when a female is receptive and ready to mate, or it can be used to select
a mate (Beach, 1976; Park & Rissman, 2011). A previous study estimated that
the period of receptivity in female platypuses was 4–6 days in spring and was defined by the number of days she had contact behaviours with the male (Hawkins & Battaglia, 2009). By this measure, our estimate of receptivity in the female would be considerably longer, 29.6 ± 5.1 days, which was the number of days in contact courtship and mating. Strahan and Thomas (1975) reported that ‘tail biting’ and ‘clasping’ behaviour occurred over 100 days while Fleay (1944), recorded contact courtship to mating occurred over a 28-day period, similar to our findings.

The female platypus controls the initiation of the courtship dance, the location in which it occurs and the duration. She leads the interaction by constructing the pattern of twists and twirls with the male securing his grasp on her tail and being pulled through the display. This display appears to have similarities to that of the other monotreme, the echidna (*Tachyglossus aculeatus*), which involves a male or males following a female in a nose to tail courtship ‘train’. Such displays may be associated more with high density populations and low breeding frequency in a solitary species (Rismiller & McKelvey, 2000) and it is possible that this may also be true for platypus which has a similar life history strategy to echidnas. However, further study in wild populations is required to confirm that the behaviour seen in captivity is comparable to wild animals.

Female platypuses used three evasive strategies to control breeding interactions with males. These included avoiding the male by switching female activity patterns to diurnal, exercising flight behaviour in his presence and being resistant in most of encounters to avoid him until she was receptive. Flight behaviour has previously been observed, but was generally described as avoidance behaviour in captive female platypuses prior to the mating season (Hawkins & Fanning, 1992). In mammals and other taxa, evasive females may reduce or change their behaviour and activity patterns in the pre-mating phase, as an avoidance strategy until they are receptive, to minimise contact with males and reduce the chance of injury from coercion (Clutton-Brock & Parker, 1995). In our study, the male platypus showed high activity levels each year for about 90 days prior to and during the mating season as he patrolled his territory in search of females. The absence of courtship and mating behaviours during this time show that males have little control in initiating courtship and that female avoidance is a driving factor in inhibiting or limiting these interactions. An
interesting example of female avoidance behaviour was that one female altered her activity pattern to diurnal prior to commencing courtship which we believe was to avoid the male. Diurnal behaviour in females has also been reported previously in captive (Fleay, 1944; Hawkins & Battaglia, 2009) and wild platypuses (Serena, 1994; Gust & Handasyde, 1995). It has also been observed in pinnipeds where females avoided male harassment by using high tide water levels and forming groups with other females (Chilvers et al., 2005). The wounds we found on the tails of female platypuses during the mating season indicate that the injury can occur during courtship and mating behaviour. However, in the wild these are rare compared with tail wounds in males (Temple-Smith, 1973). The need for females to control breeding behaviours is important in a species, like the platypus, where males are larger in size and are aggressive and venomous during the breeding season with the potential to seriously injure females.

Preliminary or non-contact courtship behaviours can provide females with an opportunity to evaluate attributes in a potential mate and help them to control interactions to protect themselves from injury and coercion (Orbach et al., 2015). During observations of courtship and mating behaviour in our study, the previously described tail biting courtship behaviour (Fleay, 1944; Strahan & Thomas, 1975; Hawkins & Fanning, 1992; Holland & Jackson, 2002; Hawkins & Battaglia, 2009), was preceded by a period of time where the female changed from her normal avoidance behaviour with the male, to staying in the same pond location with him for increasing amounts of time before contact courtship began. The non-contact courtship appeared to be an important preliminary behaviour before physical contact was allowed. A description similar to this was reported previously, but it was described as frontal, side or ventral passing of the female past the body of the male where the animals made contact with each other (Strahan & Thomas, 1975). It was possibly also reported as “proximity” behaviour of the animals using separation in time and/or space, although further details were not provided (Hawkins & Battaglia, 2009). This fits closely with our observations, but physical contact was not observed during this time in our study. This behaviour was more common than other courtship and mating behaviours and was usually terminated by the female. Non-contact courtship for the platypus is likely to be an evasive strategy for females to control breeding encounters, as it would allow her to identify a suitable mate without making physical contact.
Male aggression towards females can be elevated when females are receptive, for example in non-human primates (reviewed in Smuts & Smuts, 1993). Females may choose non-resistant strategies to coercion in favour of other qualities that increase the chance of survival for their offspring. Alternately, females in many species will show caution around males prior to them entering a phase of receptivity, where mating occurs, to protect themselves from coercion and injury. It is possible that this is the function of the non-contact courtship that we have described. In species where females are of similar size to males, more direct tactics can be used in the form of female-initiated aggression or chase (Randall et al., 2002). However, females will also minimise unwanted mating attempts by avoiding encounters with males by becoming less active or changing locations (Krupa et al., 1990).

In our study, the amount of time invested in courtship provided no indication of breeding success. There was also no pattern in breeding experience, familiarity or individual preference associated with duration of courtship behaviours between years. Although this lack of pattern held for the observations in this captive study, data from larger numbers of individuals of both sexes would be required to confirm whether this pattern reflects the breeding biology of wild platypuses. The only significant difference in behaviour between successful and unsuccessful years was the duration of mating, which was much higher in one year for one female. Despite this season resulting in eggs being laid, they did not successfully hatch and were likely abandoned less than 60 h after laying. This falls well short of the estimated 10-day incubation period (Griffiths, 1978). The stages in which breeding is likely to fail are those that occur after courtship and mating behaviours: for example, at conception, nest construction, egg laying/incubation or nursing of the nestlings. Whether monotremes lay infertile eggs in the same way as birds and reptiles has not yet been determined. The basal layer of the shell membranes starts forming after fertilisation in the distal third of oviduct and completes with the protective shell layer in the uterus (Flynn & Hill, 1939). When platypus eggs were abandoned by the female in our study it was not possible to determine if the eggs were infertile or if there was a problem with incubation and development. In captivity, female platypuses are often held in same sex pairings and in these situations, un-mated females have never laid eggs (J. Thomas, pers. obs.). The most likely explanation when eggs are laid, but
abandoned, is that they are either infertile from a failed mating/fertilisation, or they are fertile, but incubation or embryonic development failed which may occur due to inexperience in young females. The extreme challenge of successfully maintaining and breeding platypuses in captivity makes further investigation of this problematic.

The physical environment of our breeding facility appeared to influence the platypuses, with certain areas used more frequently for courtship and mating behaviours than others. Courtship behaviours occurred most often in the deep and shallow ponds which had a slow, but steady flow of water — these were also the two largest spaces available. One frequently used feature of the shallow pond was a partially submerged log which the animals would circle around. The female would occasionally hold the edge of this log during mating. However, the most frequently used space for mating behaviour was the feed tank which was the smallest area, had the lowest number of natural furnishings, but contained artificial ramps that females would also hold on to, still water and was where food was available. It is possible the animals were selecting these particular sites for features to assist them in their behaviours (i.e., females holding onto a log or ramp on the surface during mating) or to provide security (i.e., space for the female to be evasive). The use of a partially submerged log during mating has been observed in the wild on a single encounter of mating (De La Warr & Serena, 1999). Determining the best environmental conditions to encourage the most natural breeding activity for captive platypuses needs further study and may enhance captive breeding successes.

Female competition is often density and resource dependent (Stockley & Bro-Jørgensen, 2011). Significantly more time was spent per platypus in courtship and mating behaviours when two females were present with a male compared with only one female was present. We interpret this increase as female—female competition for a limited resource, i.e., the male. Consistent with this, females become competitive and aggressive with each other over food and nesting material during the mating season in captivity, (J. Thomas, pers. obs.) although this has never been measured. While the captive environment may not fully reflect behaviours of wild platypuses, as animals are not able to move freely in natural spaces, it does provide a representation of how animals may behave in high density populations and isolated waterbodies where population movement
is limited, such as on islands or in small natural lakes and tarns, and also in farm
dams. In each year during our study, when two females were present, only one
female was successful in breeding. High density populations and mate
competition can either improve or diminish reproductive success because of the
need for additional resources such as prey availability and nest sites (Sherley et
al., 2014), or to initiate reversed sex roles where competition for mates changes
depending on availability (Clark & Grant, 2010). Some of the major threats to
platypus populations have been identified to be stream fragmentation and altered
flow regimes from dams, agricultural development and activity, and urbanisation
which limit or prevent population movement (Grant & Temple-Smith, 2003;
Serena & Pettigrove, 2005), processes potentially creating high density
populations.

Recognising that females need to control breeding encounters highlights
important animal welfare considerations for breeding platypus in captivity.
Providing the animals with a large space, submerged logs and a variety of water
flows that allow females to be evasive is important for their breeding strategies
and safety, and probably influences breeding success. From the time since the
platypus was first bred in captivity in 1943, scientists have had the opportunity to
study the breeding behaviour and success of only six pairs of breeding
platypuses, some on multiple occasions. Although these studies and
observations remain limited, our work on captive breeding behaviour in the
platypus, along with data from previous studies and serendipitous records from
field and captive observations, have allowed us to describe, record, interpret and
understand many aspects of the cryptic breeding behaviour of the platypus.
These observations and their interpretation would be virtually impossible to
determine via studies entirely on platypuses in the wild.

Acknowledgements

This research was funded by Healesville Sanctuary, Zoos Victoria, and the
Violet Scott Foundation, and could not have been completed without the
dedicated assistance of the platypus keepers who took on additional work in order
to allow time for data to be collected. It was conducted under Zoos Victoria Animal
Ethics Committee approval ZV12014.
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Chapter 5 The platypus nest: burrow structure and nesting behaviour in captivity
The platypus nest: burrow structure and nesting behaviour in captivity

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Abstract

The platypus nesting burrow, where females lay eggs and rear their young, has not been well studied. We have little knowledge of its structure and the process of construction. This study aimed to investigate nesting behaviour of breeding females and to describe the structure and features of the burrow. We used infrared cameras to record behaviour of captive breeding female platypuses during the nest building period, over nine years. After the young had become independent, we excavated 11 nesting burrows and mapped their structure features. Nesting behaviour was observed between 7-15 days after mating and was an indicator of gravidity. Females invested an average of 8 hours 18 minutes over 3.5 nights, gathering and transporting wet nesting material to their burrows. The nests were composed mostly of native mat-rush leaves. Nesting burrows varied in length from 3.2 – 10.4 metres. They contained narrow tunnels, ‘pugs’ of backfilled earth, dead ends, multiple entrances and a chamber at the end that contained the nest. Appropriate nesting sites and nesting materials must be provided to female platypuses for captive breeding programs to succeed.
Introduction

Burrows are constructed and used in habitats from grasslands to rainforests by a wide range of mammals such as wombats (*Lasiorhinus* spp. and *Vombatus ursinus*) in Australia, armadillos in South America (*Chaetophractus* spp.) and naked mole rats (*Heterocephalus glaber*) in Africa (Davidson, Detling, & Brown, 2012; Fangyin et al., 2013; Machicote, Branch, & Villarreal, 2004; Sherman & Jarvis, 2002). Burrows are used for different reasons including as a secure retreat in which to enter torpor (Körtner, Pavey, & Geiser, 2008), shelter from environmental conditions and energy conservation (Finlayson, Shimmin, Temple-Smith, Handasyde, & Taggart, 2005), protection from predators (Masters & Dickman, 2012) and for raising or sheltering offspring (Beard & Grigg, 2000). Burrows that are used for rearing offspring may have additional structural requirements, such as a nest, that suit birthing or housing of young during lactation. Eutherian mammals, such as rodents, canids and felids (Nowak, Porter, Levy, Orgeur, & Schaal, 2000), give birth to relatively well developed offspring compared with those of marsupials (Tyndale-Biscoe & Janssens, 1988) and newly hatched monotremes (Griffiths, 1978). The latter require different environmental conditions and security structures to cater for their less developed offspring (Tyndale-Biscoe & Janssens, 1988). While marsupials and echidnas carry their dependant young in a pouch or pouch-like structure, some will leave the older furred young in a nest or burrow for protection (Russell, 1982). However, unlike marsupials and echidnas (*Tachyglossus aculeatus*), the platypus (*Ornithorhynchus anatinus*), cannot carry its offspring because it lacks a pouch, thus females are entirely dependent upon a burrow to successfully rear their young.

The platypus is a semi-aquatic, semi-fossorial, egg-laying mammal. They dig burrows into a river bank to provide shelter and protection from predators, floods and environmental temperature extremes (Burrell, 1927; Grant, 1983). Platypuses build and use two types of burrows: a resting burrow and a nesting burrow (Burrell, 1927). The resting burrow is used by males and females at all times of the year and is generally simple in structure, with no nesting material or specialized features (Burrell, 1927). Burrows may be used by different individuals, and each individual uses multiple burrows (Serena, 1994). In
contrast, a nesting burrow is complex in design and used by a single breeding female and her offspring (Burrell, 1927). The nesting burrow contains a series of narrow tunnels leading to a larger nesting chamber that is filled with wet vegetation. Females will plug up sections of tunnels by blocking it with backfilled soil, creating what has been termed a ‘pug’ (from the verb used in mining meaning ‘to fill with clay’) by Burrell (1927). This gives the illusion to any intruders that the end of the burrow has been reached and likely protects the dependant young inside the nesting chamber from predation and to some extent rising flood waters. The burrow has a critical role in breeding, because it provides a place for incubating their soft-shelled eggs and housing dependant offspring during lactation when the female leaves the burrow to forage in adjacent water bodies. Thus, the burrow must provide the appropriate environmental temperatures, humidity and protection required for the survival of young in the same way as a pouch does in a marsupial.

There has been little research on the construction and use of nesting burrows due to the difficulty in observing animals in the wild and capturing females during the narrow time period of nest-building. Platypus breed seasonally in spring (Temple-Smith & Grant, 2001). After mating, a gravid female will build a nesting burrow and a nest from wet vegetation which she carries into the burrow using her tail. The female will then lay one to three eggs which she incubates for approximately 10 days (Burrell, 1927; Griffiths, 1978). During the initial stages of incubation and lactation, the female spends most of her time underground in the nest (Fleay, 1944; Hawkins & Battaglia, 2009; Holland & Jackson, 2002), initially leaving the nesting burrow and entering the water for only 30 – 40 minutes at a time (Fleay, 1944; Hawkins & Battaglia, 2009; Holland & Jackson, 2002). The offspring remain in the burrow for around four months, feeding on their mother’s milk as they grow from small neonates to independent juveniles (Holland & Jackson, 2002). At the time of emergence from the burrow, young are roughly 80% of their adult length and 67% of their adult weight (Grant & Temple-Smith, 1983).

Platypus nesting behaviour and burrow structure was first described and reviewed by Burrell (1927) and described for captive animals (Fleay, 1944; Hawkins & Battaglia, 2009; Holland & Jackson, 2002). The aim of our study was
to describe the behaviour associated with construction of the nest, and describe the structure of the platypus nesting burrow where offspring are reared.

**Methods**

**Animals and housing**

Four platypuses (two females, two males) were housed and studied in the platypus breeding facility at Healesville Sanctuary, Victoria, Australia, between 2007 and 2017. The animals successfully bred on seven occasions, producing a total of 10 offspring that survived to adulthood. On one additional occasion, breeding was successful with young produced, however the offspring did not survive to lactation independence and died at 90 days of age in the nesting burrow. On four occasions eggs were laid and recovered, but the young did not survive through incubation (Table 5.1). The facility consists of three large flowing ponds (50,000 L) containing rain and mains water filtered using four sand filters and multi-cyclone units flowing at 353 L minute\(^{-1}\), plus three 5000 L aquaculture feed-tanks which were cleaned twice weekly. The facility also contained two 5000 L water tanks (diameter 2.8 m, depth 1.2 m), filled with a clay-rich soil and planted with native grasses, ferns and a tree stump to allow platypus to build their own burrows in a stabilised soil matrix. In addition, two temperature controlled rooms containing nest boxes were provided. These contained six wooden nest boxes (500 x 400 x 400 mm) connected to each other via 200mm PVC pipe lined with non-slip rubber matting. The boxes contained leaf litter and other organic plant matter as nesting material.

Platypuses were fed *ad libitum* daily. The diet consisted of live freshwater crayfish (*Cherax destructor* and *C. albidis*), mealworms (*Tenebrio molitor*), earthworms (*Oligochaeta sp.*) and fly pupae (*Musca domestica*). House crickets (*Acheta domesticus*), blackworms (*Lumbriculus variegates*) and freshwater shrimp (*Paratya australiensis*) were offered twice per month at ~50 grams each as part of their enrichment program.
Table 5.1: Breeding history of platypus at Healesville Sanctuary from 2007-2017. The number of offspring is denoted as: male offspring, female offspring, unsexed offspring. All offspring listed as unsexed did not survive through lactation to independence.

<table>
<thead>
<tr>
<th>Year</th>
<th>Sire</th>
<th>Dam</th>
<th>Number of offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007/08</td>
<td>A</td>
<td>C</td>
<td>1.1.0</td>
</tr>
<tr>
<td>2008/09</td>
<td>A</td>
<td>C</td>
<td>0.1.0</td>
</tr>
<tr>
<td>2009/10</td>
<td>A</td>
<td>C</td>
<td>1.0.0</td>
</tr>
<tr>
<td>2011/12</td>
<td>B</td>
<td>C</td>
<td>1.1.0</td>
</tr>
<tr>
<td>2012/13</td>
<td>B</td>
<td>C</td>
<td>2.0.0</td>
</tr>
<tr>
<td>2013/14</td>
<td>B</td>
<td>C</td>
<td>0.0.0</td>
</tr>
<tr>
<td>2014/15</td>
<td>B</td>
<td>C</td>
<td>0.0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>0.0.0</td>
</tr>
<tr>
<td>2015/16</td>
<td>B</td>
<td>C</td>
<td>2.0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>0.0.2</td>
</tr>
<tr>
<td>2016/17</td>
<td>B</td>
<td>D</td>
<td>0.0.1</td>
</tr>
<tr>
<td>2017/18</td>
<td>B</td>
<td>C</td>
<td>0.0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>0.0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total: 7.3.8</td>
</tr>
</tbody>
</table>

Nesting behaviour

All parts of the facility were monitored year round using CCTV motion activated infrared cameras (Techview, 800TVL CMOS bullet camera). Once a female had mated, we provided large amounts of nesting material in the form of local native mat-rush leaves and tussock grasses (*Lomandra logifolia* and *Poa ensiformis*) and varieties of eucalypt bark and leaves (*Eucalyptus viminalis* and *Eucalyptus ovata*) that would float on the water surface. The leaves from the mat-rush often sank to the bottom of the tank, so were also secured to the edge. Mat-rush was first observed being pulled into the water by the breeding female from a pot plant in the exhibit. It was subsequently provided each year based on this observation. We watched the full night’s footage daily until nesting behaviour was shown and then analysed footage for the duration spent collecting nesting material and the number of visits to the nesting burrow. Data was analysed from over 80,000 hours of video footage for the females from 2011 – 2016 (n = 9 platypus monitoring years, as for 3 years we housed two females). The data from 2007 – 2010 was not included as it was incomplete for these behaviours.

We were unable to determine the duration it took the female platypus to excavate the nesting burrow or when it began as the animals were already using
earth burrows in some years, prior to mating. At this time we were only able to view their behaviour on the surface, not underground, and therefore unable to differentiate sleeping in the burrow with excavation of the burrow.

**Mapping the burrow**

At the conclusion of the breeding season, once their offspring were no longer using the nesting burrow, or the female had abandoned the nest, we excavated the contents and mapped the structure of 11 burrows. These included both burrows that contained young that were successfully reared and those that did not. We recorded the number of openings to the surface, dead ends and pugs; a pug is an earth plug formed where the tunnel had been back filled or closed, originally a mining term proposed by Burrell (1927). Measurements were taken of the overall length and the maximum depth of the burrow (from the base of the tunnel to the soil surface). The height and width were also measured along the tunnels at three to six points, at random intervals. The height, width and depth of all nesting chambers were measured to the nearest centimetre (cm). We excavated burrows by starting at the entrance and removing sections of the earth in roughly 3 cm increments with a small spade. The burrow map was considered complete when no other tunnels were found within approximately 30 cm of the last point.

**Observations of the nesting chambers**

In 2015, one female abandoned the nest she had constructed two days after retirement into the nesting burrow. In 2016, the same female abandoned the nest after 18 days and in 2017, both females abandoned their nesting burrows after 10 – 11 days of retirement. We excavated these burrows within seven days of abandonment and the fresh nesting material, in the form of grasses, rushes, bark and leaves were sorted and weighed, to determine the overall quantity of different materials. We also made observations on the structure of the nest and recorded the position of any abandoned eggs or egg shells.

**Statistical analysis**

The shape of the nesting chambers resembled an ellipsoid: a three-dimensional shape similar in appearance to a flattened sphere. Thus the volume
of the nesting chamber \((C_v)\) was determined using the radius of height \((a)\), width \((b)\) and depth \((c)\) in the formula:

\[
C_v = \frac{4}{3} \pi abc
\]

The total burrow volume \((BV)\) was determined by the formula used by Smallwood and Morrison (1999) to estimate burrow volumes of pocket gophers (Geomyidae)

\[
BV = A \cdot L + n_c (C_v)
\]

where \(A\) is the average cross section of tunnel \((m^2)\) and \(L\) is the length of the tunnel and backfilled pugs \((m)\), \(n_c\) is the number of nesting chambers and \(C_v\) is the volume of the chamber \((m^3)\).

All means are presented ± standard error of the mean (SE). Nesting behaviour for the total duration spent, number of nights and number of trips with material was compared for years with either zero, one or two offspring produced using analysis of variance (ANOVA). Burrow morphology data were compared using an ANOVA or a two-way t-test in SYSTAT software (Systat software, San Jose, CA). P values equal to or less than 0.05 were considered significant. Where significance was found, Bonferroni post hoc tests were used to determine significance between the morphologies of burrows that produced either zero, one or two offspring. We acknowledge the small sample size and perhaps contrived aspects of the earthen tanks used for burrow construction in this study. However, females chose to construct their nesting burrows in the environment provided and as there is little published information on platypus nesting behaviour and the structure of their nesting burrows, we consider that the data from this study contributes significantly to our understanding of captive nesting behaviour in this species. Data from the excavation of three wild nesting burrows (Temple-Smith, 1973) and two wild resting burrows (Serena, 1994), have been included with this data for comparison.

Results

Nest construction behaviour

Nesting behaviour was first observed between seven and 15 days after mating, and three to eight days before egg laying. Two eggs were found in the
nest of a female who abandoned her nest after retiring underground for 59 hours 22 minutes. Based on this, the day on which this female retired to her burrow for longer than 24 hours was assumed to be the day of egg laying.

Female platypuses only collected nesting material from the surface of the water, rather than anything that had become submerged. Nesting behaviour involved females gathering wet plant material from the surface of the water with their bill, then passing it under their body on to the underside of their tails, which they curled downwards and forwards into a hook. This material was then carried back to their burrow. In every year of the study the most commonly selected materials for nest construction were cut strands of *Lomandra longifolia*, followed by strands of *Poa ensiformis*. Very little of the bark and leaves were collected. Nesting material was collected over 3.6 ± 0.3 nights (range: 2 - 5 nights), and the mean total time invested in this activity was 8 hours 18 minutes ± 1 hour 36 minutes (range: 2 hours 30 minutes – 16 hours 48 minutes), and included 24 ± 4 trips (range: 9 – 39) from the water back to the burrow with material. The mean duration of a single nesting event was 18 minutes 24 seconds ± 48 seconds (range: 3 minutes – 2 hours 24 minutes). The highest number of trips to burrows with nesting material occurred on nights 2 and 3 (Figure 5.1). On one occasion, nesting material was collected, but the animal was not successful taking the material back to the burrow. This was recorded as zero trips (Figure 5.1). However, there was no significant difference between the number of trips made per night ($F_{4, 26} = 2.57, P = 0.06$). There was also no significant difference between the number of trips to the burrow with nesting material, the total duration spent nesting or the number of nights spent nesting between years where there were zero, a single or twin offspring produced ($F_{2, 6} = 0.61, P = 0.57; F_{2, 6} = 0.68, P = 0.54; F_{2, 6} = 0.12, P = 0.89$ respectively). There was also no significant difference between individual females ($t = 0.45, \text{d.f.} = 2.96, P = 0.68; t = -1.65, \text{d.f.} = 3.58, P = 0.18$ and $t = 0.56, \text{d.f.} = 6.98, P = 0.59$ respectively).
Figure 5.1: Mean number of trips carrying nesting material by female platypuses per night from nine breeding seasons (n=2 individual females). Grey bars = mean; black points = individual values for each night of nesting behaviour. There was no significant difference between the number of trips made per night (P = 0.06).

Burrow structure and morphology

Each burrow exhibited common characteristics. The shape, and layout of the burrow tunnels varied between years and between females, with a new burrow constructed each season, except in 2008 as it was the second year of the program and we aimed for minimal disturbance to the area (Figure 5.2A - K). The shape of the tunnels followed a curved pattern and were comparable in layout to a burrow excavated in the wild (Burrell, 1927; Figure 2L). The number of openings to the surface (Figure 5.3A and B), pugs and length of each burrow, were similar for burrows in which twins or a single offspring were reared, or burrows that were unsuccessful and produced no offspring (Table 5.2). In every burrow, numerous small compact balls of soil, ranging in size from 2 mm to 20 mm, were found around the openings to the surface and in parts of the tunnels (Figure 5.3C).
Figure 5.2: Drawings of captive platypuses nesting burrows from above. A: 2007 and 2008 burrow (same burrow used over two years) containing twins and a single young; B: 2009 single young; C: 2011 twins; D: 2012 twins; E: 2014 single young; F: 2015 twins; G: 2013 no young; H: 2015 no young survived, two eggs laid; I: 2016 one egg shell but no young survived; J: 2017 no young survived, one intact egg shell, one torn egg shell; K: 2017 no young survived, two torn egg shells; L: Sketch of a wild nesting burrow by Burrell (1927). Arrows indicate openings to the surface, N indicates the nesting chamber. Figures are not drawn to scale.
Figure 2 (continued)
Figure 5.3: Features of the platypus nesting burrow. A and B: burrow entrances, black arrows denote openings; C: compacted balls of soil; D: excavated nesting chamber with nest material; E: a nest removed from the nesting chamber; F: two platypus egg recovered from an abandoned nest.
Data on the morphology of various components of burrows are presented in Table 5.2. The mean length of captive nesting burrows was 5.0 ± 0.8 m and the mean burrow volume was 6.2e^{-2} ± 1.1e^{-2} m^3. The mean nesting chamber volume was 0.7e^{-2} ± 0.2e^{-2} m^3 and mean tunnel volume 5.6e^{-2} ± 1.1e^{-2} m^3. There was no significant difference in chamber, tunnel or total burrow volume between burrows where single, twin or no offspring were reared (F_{2, 5}=1.10, P = 0.40; F_{2, 5}=0.59, P = 0.59 and F_{2, 5}=0.49, P = 0.64 respectively). There was a significant difference in length between burrows where single, twin and no offspring were reared (F_{2, 8} = 4.33, P = 0.05); those burrows in which young were not successfully reared had the shortest length. Therefore, the mean length of burrows containing young was 6.3 ± 0.9 m. However, a post hoc comparison of these data found no difference (0 and 1 offspring: P = 0.08; 0 and 2 offspring: P = 0.08; 1 and 2 offspring: P = 0.42).

Table 5.2: Comparison of mean morphology parameters of burrows in which a single offspring, twins or no offspring were produced, and of all the burrows combined regardless of offspring. * denotes significance

<table>
<thead>
<tr>
<th></th>
<th>Single</th>
<th>Twins</th>
<th>None</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (m)</td>
<td>7.3 ± 3.4</td>
<td>5.6 ± 2.4</td>
<td>2.6 ± 0.7</td>
<td>4.33</td>
<td>0.05*</td>
</tr>
<tr>
<td>Number of entrances</td>
<td>2.3 ± 0.0</td>
<td>2.2 ± 0.5</td>
<td>1.0 ± 0.0</td>
<td>4.94</td>
<td>0.04*</td>
</tr>
<tr>
<td>Number of pugs</td>
<td>2.3 ± 1.0</td>
<td>3.0 ± 0.5</td>
<td>0.5 ± 0.5</td>
<td>5.48</td>
<td>0.03*</td>
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<tr>
<td>Depth of chamber (m)</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.3</td>
<td>0.4 ± 0.1</td>
<td>0.35</td>
<td>0.72</td>
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<tr>
<td>Nesting chamber volume (m^3)</td>
<td>0.8e^{-2} ± 0.7e^{-2}</td>
<td>0.2e^{-2} ± 0.3e^{-2}</td>
<td>0.9e^{-2} ± 0.4e^{-2}</td>
<td>1.10</td>
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<td>Tunnel volume (m^3)</td>
<td>7.7e^{-2} ± 2.0e^{-2}</td>
<td>5.8e^{-2} ± 1.8e^{-2}</td>
<td>4.3e^{-2} ± 1.8e^{-2}</td>
<td>0.59</td>
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<tr>
<td>Burrow volume (m^3)</td>
<td>8.5e^{-2} ± 1.9e^{-2}</td>
<td>6.1e^{-2} ± 3.1e^{-2}</td>
<td>5.3e^{-2} ± 1.9e^{-2}</td>
<td>0.49</td>
<td>0.64</td>
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</table>
Comparison of burrow morphology from our study and two others (Serena, 1994; Temple-Smith, 1973) showed no significant difference between the length, depth or chamber volumes of captive nesting burrows and wild nesting burrows (Table 5.3). There was a significant difference in the tunnel volume and total burrow volume between captive nesting and wild nesting \( (t = -3.13, \text{ d.f.} = 4.01, P = 0.03 \) and \( t = -3.14, \text{ d.f.} = 4.01, P = 0.03 \) respectively), with the largest volumes recorded in the wild nesting burrows. There was also a difference in tunnel and total burrow volume between wild nesting and wild resting burrows \( (t = -6.28, \text{ d.f.} = 2.01, P = 0.02 \) for tunnel volume and \( t = -6.36, \text{ d.f.} = 2.01, P = 0.02 \) for burrow volume).

**Nest structure**

The nests were removed from four failed burrows that were abandoned and the material weighed. These nests were composed of 31%, 43%, 52% and 52% of the material that was carried by the female into the burrow, with the remainder found lying in the tunnels. In each of the nests, the grasses and rush leaves were folded and wrapped around each other in a spherical pattern (Figure 5.3D and E) and mostly composed of long strands of *Lomandra longifolia* (Figure 5.4). These large rush leaves were also held together by the thinner tussock grass *Poa enciformis*. In one nest, the inside of the nest was lined with *Eucalyptus* leaves.

**Observations inside the nest**

After one week, the *Poa* had browned and was quite fragile, breaking apart easily when touched. The thicker blades of *Lomandra* remained strong and fresh in appearance. From an abandoned nest in 2015, two eggs, with their shells intact were recovered. Although the shells appeared intact, they had started to form pin-sized holes and were empty inside. In 2016, the remains of an egg shell was recovered. The shell was split and torn through the top at the centre and appeared as if a young platypus had successfully hatched, but had not survived through early lactation. In 2017, one intact and one broken egg were recovered (Figure 5.2F). A second female abandoned a nest in 2017 and two broken egg shells were recovered.
Table 5.3: Comparative female platypus nesting burrow morphology from this study and two other studies. ¹ data from this study, ² data from Serena (1994), ³ data from Temple-Smith (1973); * denotes significance

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<tr>
<th>Type</th>
<th>Length (m)</th>
<th>Depth of chamber (m)</th>
<th>Chamber (m)</th>
<th>Chamber volume (dm³)</th>
<th>Tunnel volume (dm³)</th>
<th>Whole Burrow volume (dm³)</th>
<th>Entrances</th>
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<td>d.f.</td>
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</table>

Where females successfully raised young to independence and young had emerged from the burrow at 124-134 days since the eggs were laid (n = 11 nests), the vegetation had completely decomposed with only a few fine pieces less than 3 mm long remaining.
Figure 5.4: Nesting material collected by females over two years. Black = female D 2015, dark grey = female D 2016, light grey = female D 2017, white = female C 2017. Vegetation types found included *Lomandra longifolia*, *Poa enceformis*, leaves and bark from Eucalyptus species.

**Discussion**

Breeding female platypuses invest a significant amount of time and effort in the construction of their burrow and nest to provide the conditions required to successfully rear their young. While nesting burrows of captive females varied in shape from year to year, each contained similar features – narrow tunnels that led to a larger nesting chamber, pugs, dead ends and multiple entrances that led to the surface. All of these features created a concealed and secure space that would function to protect the young from predators, other platypuses and potentially flood waters. Pugs in burrows also effectively seal them, which probably assists in maintaining a suitable microclimate and preventing offspring from emerging prematurely.

We found platypus nesting behaviour first appeared seven to 15 days after mating, which suggests that maternal recognition of pregnancy occurs at least as
early as one week after conception. We were unable to determine the time of the
burrow construction as the female was already using an earth burrow in some
years prior to mating, so we do not know whether this behaviour is also involved.
A study from Taronga Zoo saw the female excavate a burrow in the week
between mating and nest building which would indicate that it was in this case
(Hawkins & Battaglia, 2009). However, the observations we have made would
suggest that further study is required to determine when the timing of the
construction of the nesting burrow. Changes in behaviour can indicate maternal
recognition of pregnancy (Renfree, 2000). In macropodid marsupials, maternal
recognition of pregnancy presents itself behaviourally just prior to parturition, with
the female adopting a birthing posture, under the hormonal control of
prostaglandins (reviewed in Renfree, 2000). In other mammals, nesting
behaviour is initiated by the changing hormone levels associated with gestation
(González-Mariscal, Caba, Martínez-Gómez, Bautista, & Hudson, 2016). In our
study, nesting behaviour was only observed in females that successfully mated.
While young were not always successfully produced, egg remnants were
sometimes found in abandoned nests. We did not see nesting behaviour and did
not find eggs in the enclosures of un-mated female platypuses, suggesting that
nesting behaviour occurs as a result of maternal recognition of pregnancy in this
species or that copulation might induce ovulation. In this case, any successful
mating where conception was not achieved, may still lead to nesting behaviour.

Female platypuses spent an average of 8 hours distributed over two to five
nights collecting nesting material. This time investment in nest construction
finished only one day prior to the start of incubation, during which time the female
initially does not feed for several days and subsequently for less than two hours
compared to normal nightly foraging bouts of eight to 12 hours for females in
captivity (J. Thomas, pers. obs). This is consistent with observations by Hawkins
and Battaglia (2009) of three to five days collecting nesting material and Holland
and Jackson (2002) of five days. Fleay (1944), however observed only one night
of nest building. These breeding activities, and possibly excavation of the burrow,
impose large energetic costs on female platypuses likely requiring them to rely
on their body fat stores for energy during incubation and early lactation.
Platypuses require a large food intake and consume up to 30% of their body
weight in food matter daily (Holland & Jackson, 2002). This is consistent with data from wild female platypuses, in which tail fat stores were found to decline during this time (Handasyde, McDonald, & Evans, 2003). We did not determine this in our study to minimise human contact with the animals during breeding.

Where nests could be analysed soon after construction (four nests abandoned by females within the first week), more than half of the nesting material retrieved was dropped in the tunnels, suggesting that gathering and delivering the material was difficult. It is also possible that females gather excess material to what they require for this reason. In captivity, when a platypus carrying nest material was disturbed by another animal, material was usually dropped (J. Thomas pers. obs.). If dropped on land, it was not re-collected, which has also been reported previously (Fleay, 1944). Our observations suggest that suitable vegetation in close proximity to the nesting burrow, as well as undisturbed space and time for collection by a breeding female platypus, is critical for the successful construction of a nest.

Nesting material is an important component of the nesting burrow, as different materials have different insulation and decaying properties. The type of nesting material selected can reduce the rate of heat and humidity loss in nests during incubation, which may be particularly important when a female needs to temporarily leave young to feed herself (Hilton, Hansell, Ruxton, Reid, & Monaghan, 2004). A study comparing insulation properties of different bird nesting material found that grass, the material most commonly selected by platypuses, was the worst insulator of eggs (Hilton et al., 2004). However, the underground burrows of platypuses are not exposed to the same environmental stressors, such as temperature fluctuations, rain and wind, that the nests of many birds experience. Platypus burrows are relatively stable in temperature with mean burrow temperatures ranging from 14.2°C - 17.5°C throughout the year (Bethge, Munks, Otley, & Nicol, 2004). Platypus do not have a pouch to provide the required moisture and warmth to neonates as in marsupials or echidnas, instead the stable temperature and high humidity within the burrow during incubation and early lactation prevents eggs and neonates from desiccation (Temple-Smith & Grant, 2001). Female platypuses always collected wet vegetation for their nest
from adjacent water bodies and this additional moisture is probably important for maintaining the humidity in the nest.

Of the small number of captive platypus nests examined previously, the nesting material consisted mostly of eucalyptus leaves and secondarily grasses with fern fronds also offered but not taken (Fleay, 1944; Holland & Jackson, 2002) or a combination of eucalyptus leaves, grasses, tree fern fibre and stringy bark stripped from logs, although it was not specified the other materials that were offered in this study (Hawkins & Battaglia, 2009). Early reports also found variation in wild platypus nests with one containing grasses and eucalyptus leaves, another willow roots, leaves and reeds, a third nest contained only reed roots, a fourth grass and eucalyptus leaves and a fifth contained reed stalks and flags (Burrell, 1927). This strongly suggests that the type of nesting material used is likely to be influenced by what is available in the immediate environment of the female when she is collecting it. Although in our study, the nests we examined were still fresh at approximately two week old, the older dried out nests from Burrell (1927) were still similar in shape with a circular structure, with the material interwoven. The primary material used for nest construction by our captive females was *Lomandra* rush leaves, followed by *Poa* grass, eucalyptus leaves and bark. The *Lomandra* strands are a thick and fibrous material, which may hold more moisture and breakdown at a slower rate than finer materials. As nestlings grow, become furred and develop homeothermy, the humidity and thermal requirements within the nesting burrow to prevent desiccation and cold stress gradually become less important; by the end of lactation after four months the vegetation in the nest was completely broken down. The large and fully furred offspring were also more mobile by this time which likely assisted in the degradation of nesting material as it was trampled by the growing young. This suggests that the selection of appropriate vegetation for nest construction is important for maintaining nest integrity and supporting nestlings, especially during the early stages of their development. Provision of a variety of appropriate nesting materials is likely to contribute to the success of captive breeding programs for platypuses.

The physical features of platypus nesting burrows undoubtedly provide young with security from external threats. Pugs, looping tunnels, dead ends and
multiple openings to the surface all provide complexity to the structure. Burrell (1927) suggested that the loops and shapes of the burrows were too variable to be classed into types and were a consequence of the ‘vagaries of the builder’, although they were possibly also shaped by the type of soil and surrounding obstacles such as plant roots and rocks (Burrell, 1927). The former explanation appears to be plausible, at least to some degree, as we found that individual females constructed different shaped burrows across years. However, although the shape changed, the features did not. At the time of construction, it is likely that the female does not know how many offspring she will have thus the nest is built to accommodate all possibilities. There were significantly fewer entrances and pugs, and a shorter overall length in burrows that did not produce offspring, compared with those that either had a single offspring or twins. The two most likely possibilities explaining this are that either this simple burrow structure did not contain the appropriate microclimate for successful incubation and development of young, or that the full final complexity of the burrow is created over the course of the four month lactation period. Anecdotally, we observed a female creating additional burrow entrances part way through lactation when offspring were successfully produced. Females were also observed spending time in the burrow, but not in the nest with the young. While this was not measured in our study, it would be interesting to assess this behaviour in the future.

Nesting burrows previously measured were on average 7.3 m long, but some up to 30.5 m long were recorded (Burrell, 1927). Due to logistical constraints, we were only able to provide our captive animals with limited space in the dirt tanks for building their burrows, which may have influenced burrow length and shape. Despite this, the average length was 5.0 metres and tunnels rarely ended at the wall of the tank, suggesting that limited space was not a constraint in nesting burrow construction. During the construction of the burrow, no soil mound is produced around the burrow entrance, as is found, for example, around wombat burrows, suggesting that no soil is removed. Instead, soil was firmly compacted into the structure using the body and tail, previously described as ‘tamping’ as the platypus moves backwards and forwards through the tunnels (Burrell, 1927). The smooth and highly compacted nesting chambers we excavated appeared structurally robust and highly unlikely to collapse. The balls
of compacted clay soil found intermittently throughout the burrow are a feature of platypus burrow construction in heavier clay-based soils where compaction by ‘tamping’ is difficult. These balls allow excess soil to be rolled out of the burrow and are particularly effective where the river banks have a natural camber that is followed during the burrow construction (Burrell, 1927). Soil balls have not been observed in burrows constructed in sandy soils where soil compaction during burrow construction is likely to be easier (P. Temple-Smith pers. obs.). Burrell (1927) speculated that these soil balls were also used to create and remove pugs in the tunnels. Many of the features that are incorporated into the complex nesting burrow appear to have important functions for safety and security. The ability of platypuses to build burrows with such features would be dependent upon soil type, moisture content and ground stability. While in captivity these can be controlled - careful selection of soil with high clay contents, native plants and watering systems should be provided to assist in successful breeding and management of platypuses.

Pugging the burrow may function to maintain heat and humidity and prevent desiccation of the eggs, which have been observed to become dented within a few minutes of exposure to external air (Burrell, 1927), and the dehydration of the early neonates, which are very small and underdeveloped (Grant, 2007). Plugged burrows of kangaroo rats (*Dipodomys spectabilis*) maintain 100% relative humidity even in dry environments, whereas open burrows had only 30% relative humidity (Schmidt-Nielsen & Schmidt-Nielsen, 1950). Blocking the burrow with pugs is also likely to reduce egg damage/loss or drowning of young from floodwater (Burrell, 1927). Pugging likely provides additional protection from external threats such as predators or other platypus trying to use the burrow. Other burrowing species such as spinifex hoping mice (*Notomys alexis*; Thompson, 2007), short-beaked echidnas (*Beard & Grigg*, 2000) and prairie dogs (*Cynomys ludovicianus*; Hoogland, 1995) also backfill their burrows for these reasons. Pugs may also provide internal security to keep mobile offspring from prematurely leaving the safety of the burrow (J. Thomas, pers. obs).

Nesting burrows in the wild may have a single entrance (Temple-Smith 1973) or two or more entrances (Serena, 1994). We also observed this in
captivity, where some burrows had a single entrance whereas others had as many as three. The function of multiple entrances for the platypus is unclear. The gerbillid rodent, *Meriones crassus* use multiple entrances for additional ventilation (Brickner-Braun et al., 2014), however this is unlikely to be the explanation for the platypus as the presence of pugs seals the tunnels from air flow. Multiple entrances may provide additional security for escape from predators as they do in prairie dogs (Hoogland, 1995).

We found a significant difference in tunnel volume between wild and captive nesting burrows, yet no significance for their overall length. We suggest that tunnel size is created for the width of the animal that constructed it. A north-south cline in body size of platypus (Grant, 2007), and different origin locations of the females in this study, likely explains the difference seen in tunnel volumes. The origin source of our captive platypuses was mixed with some central NSW coast and central Victoria, and the wild platypuses in Temple-Smith (1973) were from south-east NSW. The construction of narrow tunnels by females may have evolved to exclude larger males as platypuses are sexual dimorphic (Burrell, 1927; Furlan et al., 2012; Gust & Griffiths, 2011). During the 2008 platypus lactation period at Healesville Sanctuary, a male platypus made multiple attempts to enter a nesting burrow, before becoming partially stuck at the entrance. When the female returned to the nest from feeding, her behaviour became agitated and she started re-shaping the entrance immediately after the male left (J. Thomas, pers. obs.). In this case, it was likely the male’s larger body size and the female’s construction of a small tunnel that prevented the male from gaining access. While burrow sharing has been observed in resting burrows in some wild adult platypuses (Serena, 1994), it does not appear to be common. There was no significant difference between the captive nesting burrow volumes and wild resting burrow volumes, however there were differences between wild nesting and wild resting burrow volumes. Without knowing the size of the animals that constructed each type, we are unable to determine if body size influences tunnel volume. However, to properly compare the two tunnel sizes, resting and nesting tunnels constructed by the same female should be studied to determine if tunnel size is influenced by the burrows purpose (i.e. for ‘resting’ or ‘nesting’ to rear their young).
This is the first detailed study of the construction, structure and morphology of platypus nesting burrows. Female platypuses invest considerable time in the construction of their nests, which likely imposes high energy costs. The consistent structural features of the burrow suggest they are constructed to provide a stable microclimate and to minimise disturbance from other platypus, predation and flooding. This study provides important new information to guide animal management to improve breeding outcomes in captive platypus colonies. In particular, suitable nesting material should be provided for breeding females in captivity and the provision of large external tanks, filled with appropriately compacted and stabilized clay-based earth, is also an essential requirement for breeding success.

Acknowledgements
This work was funded by Healesville Sanctuary, Zoos Victoria, the Holsworth Wildlife Trust and Winifred Violet Scott Trust. It could not have been completed without the assistance of keepers Sarah Boyd and Mason Hill who we thank for their help with the excavations, which took many hours of their personal time. Special thanks to Amie Hindson for her photos of the platypus eggs and to Gavin McCall for identifying the species of leaves in the nest. This research was conducted under Zoos Victoria Animal Ethics Committee approval ZV12014.
References


Chapter 6 *Maternal care of platypus nestlings*
Chapter 6

Maternal care of platypus nestlings

Abstract

Maternal care in mammals is critical for the survival of their offspring. However, maternal care in platypuses has never been rigorously studied due to the difficulty in locating the nesting burrows. Our aims were to describe the maternal behaviour of a captive female platypus and the growth and development of her offspring. We were able to locate an underground nesting burrow and insert a camera inside the chamber to record the activity and behaviour of a breeding female platypus and her two dependent nestlings. We also gathered data on maternal activity outside the burrow via infrared cameras and measured her food intake during lactation. The ages of the offspring were assigned to developmental milestones including the opening of the eyes and development of pelage. Twins were left alone for periods of more than 24 hours by the mother at an earlier stage of 44 – 52 days of age, than the single nestlings who were left alone at 71-72 days of age. The dietary energy intake of the breeding female was more than double that of the non-lactating females in the last month of lactation indicating the large energy investment required for milk production. The mean age of young at emergence from the burrow was 128 ± 1 days and occurred after weaning in 60% of nestlings. This suggests they instantaneously transition from a completely milk-based diet to a completely aquatic macroinvertebrate diet. Developing a technique to study maternal care in platypuses has allowed us to understand how nestlings develop and are reared, an aspect of their biology that was previously very poorly understood.

Introduction

Maternal care is an essential component of the breeding biology of mammals required for the survival of offspring. While maternal care strategies vary across species, a common feature across mammals is providing nutrition for the offspring, with nutritional composition of the milk closely regulating the development of young (Rosenblatt, Mayer, & Siegel, 1985). Maternal care is also important for protecting dependant young from predators and environmental
extremes, and to impart survival behaviours such as seeking shelter and finding food (Rosenblatt et al., 1985). Lactation in monotremes has been considered a more primitively developed system compared to that of therian mammals (Lefèvre, Sharp, & Nicholas, 2010). Similar to marsupials, monotreme milk changes its composition from carbohydrate-enriched in early lactation to lipid-enriched later in lactation as the young grow (Griffiths, Green, Leckie, Messer, & Newgrain, 1984; Messer, Gadiel, Ralston, & Griffiths, 1983). Maternal care starts with incubation of the eggs and extends through lactation in monotremes. One species of monotreme, the platypus (*Ornithorhynchus anatinus*), requires a specialised strategy of maternal care as it lacks the ability to carry its altricial dependant air-breathing offspring in its aquatic environment. However, there have been few studies of maternal care in platypuses as their semi-fossorial, semi-aquatic and nocturnal lifestyle has made maternal care difficult to observe in the wild and also challenging to study in captivity.

The most significant challenge in studying the growth and development of platypus nestlings is accessing them during this phase of their life. Breeding females excavate a nesting burrow where they house their eggs and offspring during incubation and lactation (Burrell, 1927). These burrows are complex in design and are only used for approximately four months of the year (Burrell, 1927). They also contain sections of backfilled soil or ‘pugs’ which are thought to provide additional security from rising flood waters, predators and con-specifics (Burrell, 1927; Thomas, Handasyde, Parrott, & Temple-Smith, in press-a). There have only been a few observations on platypus nestlings and no detailed studies on animals throughout this period. While the physical features of platypus nestlings from the Burrell Collection series in the National Museum in Canberra have been described in detail by Manger, Hall, and Pettigrew (1998), the age of these specimens were estimated. The behavioural milestones associated with the development of the external features of living platypus nestlings have not been determined. For example, the age at which the eyes open is unknown. There is considerable value in understanding development milestones in platypuses, not only to improve our knowledge on the breeding biology of this cryptic species, but also to improve decisions on the use of rehabilitation
techniques for wild orphaned nestlings. This is especially important since information on natural growth and development is not yet available.

In one study, three wild female platypuses that were rearing young, were located to their nesting chambers using specialised glue-on ‘Ferret finder’ transmitters (Temple-Smith et al., 2003). The transmitters are able to signal depth underground which allowed accurate location of the chamber. The nesting burrows were monitored with the ABC Natural History Unit, using endoscopy equipment for the documentary ‘Platypus – world’s strangest animal’ (Koch, Utesch, Munks, & Moore, 2002). However, this study was not able to ascertain the age of the nestlings, and has not been published. My study adapted this technique to study the nesting burrow of a lactating female in a captive breeding program at Healesville Sanctuary. The advantage of studying captive animals is that the exact ages of nestlings can be determined from close daily observations of breeding events. The aims of this study were to 1) develop a method for monitoring platypus nestlings in a burrow, 2) study the growth and development of platypus nestlings and 3) study maternal care, in terms of both diet and behaviour, invested by female platypuses when rearing offspring.

Methods

Animals

The platypuses in this study were held at Healesville Sanctuary, Victoria, Australia between 2007 and 2018. The breeding facility housed two captive bred females and either one captive bred male (2007 – 2009) or one wild-born rehabilitated male (2010 – 2018). The breeding facility consisted of three large flowing pools of approximately 50 000 litres of water, three aquaculture feed tanks holding 5000 L of water and two ‘dirt tanks’ with 5000 L of clay-rich soil. The enclosure was furnished with native vegetation and partially submerged logs.

Diet and food intake

The diet of one individual female platypus was measured during three periods of lactation (i.e. four months of lactation over two years, plus three months of lactation in one year when ended early when the nestling died) and compared with the diet of five non-lactating females from the same period (Thomas,
Briefly, all platypuses were fed *ad libitum* a diet of live mealworms (*Tenebrio molitor*), earthworms (*Oligochaeta* sp.), freshwater crayfish (*Cherax albidis* and *C. destructor*), and frozen fly pupae (*Musca domestica*). All food items were weighed prior to entering the tank. The feed tanks were cleaned twice per week, and all remaining food was collected, sorted and weighed to determine the amount of each food type that was consumed as per Thomas et al. (in press-b).

All food items were analysed by the National Measurement Institute, Melbourne, Australia for a nutritional panel of macronutrients, including total energy, fat, protein, and sugars (Thomas et al., in press-b).

**Recording activity outside the burrow**

All animals were monitored continuously via infrared closed circuit television (CCTV) cameras (Vari-focal dome and bullet cameras, Jaycar electronics) which were located above each pond and dirt tank. Cameras were connected to a digital video recorder (DVR; Ganz DR16NRT digimaster) and the footage reviewed daily. Data was collected on activity levels, movement patterns and was also used to identify key breeding events such as mating and nest building (Thomas, Parrott, Handasyde, & Temple-Smith, 2018).

**Locating the nesting chamber**

In 2015/16, one 13 year old female successfully reared two male offspring in a nest monitored via infrared camera. The breeding female was radio-tracked to her burrow location using a 14 g Ferret finder MK 3M transmitter and receiver (Deben, UK). A patch of fur the size of the transmitter was removed and shaved down to the skin. The transmitter was attached with a fast setting adhesive (Selley’s Quick fix Supa glue) and the surrounding fur smoothed around the transmitter so it sat flush with the skin/fur. The animal was released back into the water and radio-tracked twice daily for the duration of the battery life, which was approximately 10 days. The daily location of the female was marked on the ground as the transmitter gives approximately 15 centimetre variation on signal, depending on how the animal is lying in the burrow. The depth of the transmitter below the soil surface was also recorded daily. The middle position of these
points was taken as the centre of the burrow. The transmitter was allowed to fall off the platypus naturally.

**Burrow scope set up and recording**

When inserting the camera into the nesting chamber, firstly water was misted over the surface to wet the soil to minimise the instability of the top soil. A 13 mm auger drill bit was placed on a 45° angle and turned manually by hand with little pressure through the soil, taking care to remove the soil during the process. We found this angle reduced the pressure the camera put on the roof of the chamber and provided the camera with more stability. Data from the ferret finder indicated the chamber depth was 0.45 m below the surface. In previous studies we found the average chamber depth dug by this female was 0.40 m from the surface to the roof of the chamber (Thomas et al., in press-a), thus for safety, a distance of 0.40 m was marked on the drill bit, as the transmitter was also deeper than the roof of the chamber. The point at which the burrow roof was punctured could be detected by the operator. A piece of 20 mm PVC rigid conduit was cut to length so that it would sit from the roof of the nesting chamber to 50 mm above the surface. By using a drill bit that was smaller in diameter than the conduit, allowed us to make the hole a tight fit which held the conduit in place. The camera was then slid through the conduit (Figure 6.1) and secured in place with duct tape (Perflex, China). The tape also sealed any gaps around the camera to make the burrow climate secure again. The infrared camera (Faunatech, Australia) was 14 mm in diameter and mounted on a 1 m flexible gooseneck that was connected to a mains power supply. The instillation of the burrow camera was conducted only after the female had left the burrow to forage in the water, to avoid disturbing the animal.

Footage was recorded continuously on a digital video recorder (DVR Ganz DR16NRT digimaster) from 3/12/2015 to 26/2/2016. During the course of the study, the hard drive became corrupted and the majority of the footage was destroyed. Some footage was recovered from each day between 3/12/2015 and 5/2/2016. Thus the analyses only includes the behavioural development and maternal care in the files we recovered. In total, 460 hours of footage over 85 days, was reviewed.
Definition of terms

All ages of the nestlings are described in days post egg laying (PEL) as the exact length of incubation is unknown and this was the most accurate time frame that could be obtained. Rose (1989) suggested the greater part of development occurs during incubation so the ‘t₀’ for development occurs at the time of egg laying.

Emergence is defined as the time at which the nestlings leave the underground burrow and enter the water for the first time. Weaning is defined as the last time a nestling rested or slept in the same burrow as their mother and therefore would not have had access to milk past this point.

The baby platypuses are referred to as ‘nestlings’ while they are dependant and permanently housed in the nesting burrow and referred to as ‘juveniles’ after emergence.
Analyses

The length of gestation was determined by calculating the number of days between mating and the first day of retirement underground into the burrow. This was assumed to be the day of egg laying which was determined through the excavation of an abandoned nest in 2015 (Thomas et al., in press-a).

The mean kilojoules consumed by the lactating female over four months were compared using an Analysis of Variance (ANOVA). Significant results ($P < 0.05$) were analysed with a Bonferroni post hoc comparison. Two-way t-tests were performed to compare the mean energy intake of the lactating female with that of non-lactating females. We acknowledge that the lactation data comes from a single animal over three years. The data is presented as the mean amount of food eaten through the four months of lactation. Analyses were performed using SYSTAT (Systat software, San Jose, CA).

The means from successful and unsuccessful breeding years were compared by two-way t-tests.

All available video footage was reviewed daily and the physical and behavioural development of the nestlings described. In addition, for each day from day 73 to day 113 of age ($n = 41$), 10 minutes of footage were randomly selected and analysed using a behavioural ethogram. Footage was selected based on available footage, the mother’s absence from the nest and spread across a 24 hour period to include different times of day/night. Behaviour was scored every 15 seconds as either walking, rolling around on the spot, sleeping, interacting with their sibling or not visible. Yawning and grooming were considered instantaneous behaviours and were recorded per event during the 10 minutes.

Results

Transmitter

The transmitter fell off the female platypus after 63 days. This left a bald patch on the skin of the platypus until the fur started to grow back after
approximately 40 days. By 55 days after the transmitter fell off, the undercoat had regrown to approximately half its height and over 80% of the bald section.

**Gestation**

The mean length of gestation was $16 \pm 1$ days (range: 13 - 23 days), from the time of mating to the day female retired underground in the burrow. However, a 23 day gestation occurred only once in this study; all other successful pregnancies ($n = 6$) were between 13 and 17 days in length.

**Incubation**

In the years where young were successfully bred ($n = 7$), the mean duration spent away from nesting burrow, feeding in the water, over the first 15 days to include the assumed incubation period of 10 days (Griffiths, 1978), was 7 hours 18 minutes $\pm$ 1 hour 15 minutes (2% of the time). On two occasions, no nestlings were produced, but eggs were found in the burrow and ‘incubated’ by the female for 10 and 12 days (the estimated length of incubation). In one of these nests, one whole egg shell and one torn egg shell was found and in the other two torn egg shells were found. The tear in the egg shell resembled that of a hatched echidna shell (Figure 6.2A and B). The mean duration females spent away from the failed nests was 6 hours 58 minutes $\pm$ 2 hours 55 minutes (2.8% of the time, $n = 2$). There was no significant difference in the total duration of time spent with the eggs between successful and unsuccessful years ($t = -3.21$, d.f. = 2.57, $P = 0.06$). The mean duration of each feeding trip away from the eggs was 1 hour 22 minutes $\pm$ 11 minutes in successful years and 1 hour 30 minutes $\pm$ 8 minutes in the unsuccessful years. There was no significant difference in the mean durations during feeding trips during incubation between successful and unsuccessful years ($t = 0.54$, d.f. = 53.01, $P = 0.59$).
Figure 6.2: A) an echidna hatching from an egg (Rismiller & Seymore, 1991); B) a whole and torn egg shell that were recovered from an abandoned platypus nest. The similar appearance of the torn egg shell resembles the opening created by an echidna in a) and is likely from a hatched platypus; C) platypus nestling aged 74 days PEL; D) the same platypus nestling aged 80 days PEL.
Mother’s activity during lactation

The proportion of time the mother spent in the nest over the entire lactation period for each breeding season decreased over time (Figure 6.3). The female first resumed a full night of activity (> 8 hours; Thomas et al., 2018) the nestlings aged 23 – 36 days PEL. The amount of time spent in the nest declined significantly over time.

The female reduced her time in the nesting burrow to every second day, when nestlings were at an earlier age when there were two offspring (n = 4 years) compared with a single offspring (n = 2 years; Figure 6.3). The female first left a single nestling alone for a night (> 24 hours) at 71 – 72 days of age PEL and at 44 – 52 days of age PEL for twins. The mother spent significantly more time in total across the whole lactation period with a single offspring (mean: 2104 ± 34 hours), compared to twins (mean: 1455 ± 127 hours; t = -9.57, d.f. = 3.69, P < 0.001). Normal rest cycles were maintained by the female in an alternate nest during the periods of reduced attendance in the maternal nest.
Figure 6.3: Proportion of time spent in the nesting burrow by the female with the nestlings from egg laying to emergence. In years 2008 - 2009 and 2009 – 2010, a single offspring was produced. In all other years, twins were produced.
The mean number of kilojoules consumed over the lactation period by the lactating female compared with non-lactating females was similar in the first two months and increased dramatically in the lactating female in the last two months (Figure 6.4). This equates to 154% of a non-lactating female’s energy requirements in the third month and 210% in the final month of lactation. The volume of food consumed increased from 14.4% of this female’s body mass per day in the first month, to 36.4% per day in the fourth month. This is 18.4% higher than in the non-lactating females at the same time.
There was a significant difference in the number of kilojoules consumed by the lactating female between each month ($F_{3,7} = 11.46, P = 0.004$). The post hoc results show that the first month was significantly lower than the second, third and fourth months ($P = 0.03; P = 0.02$ and $P = 0.01$). There was also a significant difference in the number of kilojoules consumed by lactating vs non-lactating females in the second and fourth month ($t = 4.59, d.f. = 2.25, P = 0.03; t = 5.62, d.f. = 1.76 P = 0.04$, respectively). The lactating female changed the amount of different prey types consumed over time (Figure 6.5). The amount of crayfish meat she consumed increased in the third month of lactation, and mealworms and fly pupae in the third and fourth months. Earthworm consumption also increased in the fourth month. Mealworms and crayfish had the highest energy and fat content of all prey items consumed (Thomas et al., in press-b).

Figure 6.4: Mean kilojoules consumed per day over the four months of lactation of a lactating female, compared to 5 non-lactating females. Light grey = the lactating female, dark grey = non-lactating females, black dots = data points (1 - November, 2 - December, 3 - January, 4 - February). ^ denotes significance ($P > 0.05$) between lactating and non-lactating females.
Nestling behaviour and development

The structure of the nesting material within the nesting chamber changed over time. Little could be seen of the nestlings for the first 20 days of footage (day 49 – 69 of age), due to the large amount of nesting material in the nest. Further, across the lactation period, the female consistently moved nesting material prior to leaving which obscured the camera view of the nestlings. Initially the grasses
were woven to form a spherical nest structure that was closed from above (Thomas et al., in press-a). On day 69, the nest started to open from the top and the chamber was half filled with material allowing a full view of the nestlings. At day 73, the nest was composed of a mix of short and long grass strands. At day 89, the chamber was only a quarter filled with material that still formed an open nest structure. At day 106, the grasses were all in short and straw-like strands and at day 108 the nest had no obvious structure. From day 111, there was only a floor covering of material left in the nesting chamber until emergence.

Observations of the nestlings during the period of lactation provided a comparative timeline for their physical and behavioural development including fur appearance, opening of the eyes, body shape, grooming behaviour and general movement (Figure 6.6). The fur on the back was the first to develop, followed by the fur on the ventral side and finally the tail. The eyes went through a transitional phase of opening involving a blinking type movement around the eye socket from day 87 to day 93 when the eyes were fully open. Spurs were first sighted on day 89 of age, however the quality of the footage did not provide enough definition to determine their size. Both the grooming response and general movement developed at a similar time, with slow movements progressing to full, more vigorous and purposeful movement at day 84 and 89 respectively. Slight movement of the grasses in the nest was observed from day 49 when only nestlings were present and the mother was out feeding. In one year, a nestling was found wandering around outside of the nesting chamber at day 74 of age and again after being returned at day 80 of age. While this nestling did not survive, its physical development was clearly seen (Figure 6.2c and d). The eyes were still closed and the fur had grown from velvet to a short covering on the back.
Figure 6.6: Physical and behavioural development of nestlings as recorded from first visibility at day 68 – 70 of age until emergence at day 133 of age. Fur appearance: white = no fur present, light grey = velvet fur, dark grey = short fur, black = long or glossy fur; eyes: white = eyes closed, patterned = partially open, black = eyes open; body shape: black = straight; grooming: dark grey = slow foot motion, black = fast and full foot motion; movement: dark grey = slow crawl, black = full walk.

Common behaviours of the nestlings included walking, rolling around on the spot, sleeping (in both a curled position and upside down with legs spread out), yawning and grooming. Other behaviours included slow digging around in the nesting material, covering themselves with the grasses and a chewing motion of the bill. Occasionally a nestling was seen chewing on a piece of grass, and investigating the boundary of the nesting chamber by touching the walls with their bills. On a single occasion we observed urination by a nestling in the nest when the mother was not present, where the nestling walked a few steps out of the central nest area and lifted their tail to urinate in front of the camera lens. The twin nestlings commonly slept curled up together with their bills and feet tucked under their bodies. They occasionally climbed over each other, but otherwise rarely interacted with each other when awake. On two occasions, we observed
one nestling chewing on the hind foot and the front foot of the other and, on another occasion, chewing on the front shield of the other’s bill. On one other occasion the nestlings were seen touching each other’s bills.

Suckling behaviour was also observed in the nest. When the mother was in the nest, she lay either on her side or flat with her ventral side to the base of the burrow, with the nestlings laying upside down under her abdomen. The whole body of the nestlings moved in a pulsing motion when sucking. The nestlings were also observed pushing on her abdomen with their front feet. Prior to the mother leaving the nest, she moved around the sleeping nestlings in a circular pattern, pushing the nesting material around and over them. Occasionally, the mother would rub her bill over the bills of the nestlings after they had suckled.

Using a behaviour ethogram to assess behaviour from 73 to 113 days of age, the most common behaviour was sleeping (83 – 100% of assessed time), followed by rolling around on the spot and walking (3.4% and 0.4% respectively; Figure 6.7). After 85 days of age, the nestlings were visible in all observation sessions. Yawning was observed in five out of 38 sessions (13%) and grooming behaviour in 10 out of 38 sessions (26%).
Figure 6.7: Behaviour of platypus nestlings in the burrow. Black = nestlings were not visible; dark grey = rolling around on the spot; moderate grey = sleeping; light grey = walking.

**Emergence and independence**

The mean number of days of maternal care for dependant young, from egg laying to emergence, was $128 \pm 1$ days ($n = 10$; range $124 – 134$ days) and the average length of time to weaning (the last time the female and young were together in a burrow) was $132 \pm 5$ days (range: $122 – 172$; Table 6.1).

Sixty percent of the offspring were weaned prior to emergence. Seven nestlings commenced emergence on their own, while the mother was away from the nest. Three emerged with the mother inside the nest at the time. One of these nestlings was viewed on camera being pushed out of the nest by the mother, followed by the mother collapsing the nesting burrow behind them, including the entrance at the surface.

There was no relationship between body weight at emergence and the duration of lactation (Figure 6.8). The mean body weight of the mother was $1215 \pm 14$ g and the mean body weight of each father was $1697 \pm 17$ g and $1120 \pm 17$ g.
g. The nestlings weight at emergence ranged between 47 - 97% (mean = 72%) of their mother’s weight and 51 - 105% (mean = 66%) of their father’s weight. The female nestlings were 70 – 75% of their mother’s weight and the male nestlings were 51 – 105% of their father’s weight at emergence.

Figure 6.8: Body weight of each nestling at emergence. Bars that are paired together indicate years with twins and standalone bars indicate years with single offspring. Black bars = males, grey bars = females. Hollow circles on the secondary axis indicate the individual’s age PEL at emergence.
Table 6.1: A comparison of emergence details of juvenile platypuses including their age at emergence, their age at weaning, first body weight that was measured, if the mother was in the burrow at emergence and if weaning occurred prior to emergence. Ages are presented in days post egg-laying. Twins are denoted by 2 columns within the same year.

<table>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<td>132</td>
<td>132</td>
<td>132 ± 5</td>
<td>-</td>
</tr>
<tr>
<td>Weaning prior to emergence</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Y: 60%</td>
<td></td>
</tr>
<tr>
<td>First weight (g)</td>
<td>1050</td>
<td>850</td>
<td>875</td>
<td>998</td>
<td>1175</td>
<td>914</td>
<td>788</td>
<td>870</td>
<td>634</td>
<td>570</td>
<td>M: 869 ± 84 g</td>
<td>F: 880 ± 19 g</td>
</tr>
</tbody>
</table>

**Development after emergence**

Weight gain of juveniles continued after emergence from the nesting burrow but slowed and plateaued between 200 – 240 days of age (Figure 6.9). All juveniles had reached their adult weight at approximately eight to nine months of age.
Discussion

This study is the first to describe the growth and development of live, accurately aged, nestling platypuses and the corresponding level of care provided by the mother. The mother’s behaviour changed during lactation, with time spent in the nest reducing as the nestlings grew. The technique used to place the burrowscope in the nesting chamber, while challenging, was also successful and our observations showed that the major developmental milestones, such as fur growth, eyes opening and walking movement, occurred in individual nestlings at similar ages in development and were aligned with the degradation of the nesting material.

Gestation

Gestation was estimated at 16 days, based on the time between mating and egg laying with offspring successfully reared on each of these occasions. Our estimates of gestation were similar to those from previous years at
Healesville Sanctuary and Taronga Zoo of 15 – 21 days (Hawkins & Battaglia, 2009; Holland & Jackson, 2002). However, the estimate calculated at Taronga Zoo included the period of courtship behaviour prior to mating, thus gestation is likely to be shorter than they stated. Our study found a large variation in the duration between different years (10 days) over a short time frame of 2 – 3 weeks, however data from future years will produce a more accurate mean. In the closest relative to the platypus, the short-beaked echidna (*Tachyglossus aculeatus*), gestation was estimated from field studies to be 20-23 days duration (reviewed in Temple-Smith & Grant, 2001) which is longer than the average we observed.

**Incubation**

We were unable to determine the exact length of incubation using the activity of the mother, thus this remains unknown. Griffiths (1978) suggested that the length of incubation in the platypus is likely to be similar to that of the echidna at 10 -11 days due to the similarities in stage of development of hatchlings and the similar body temperatures of both species. While both create an ‘incubatorium’ - a place of stable temperature and humidity for the eggs, the echidna uses pouch-like skin folds, while a platypus adopts a curled body position with the eggs on her abdomen, as she does with her newly hatched young (Burrell, 1927). Echidnas are mobile with their eggs, whereas platypuses lack a pouch and appear to leave their eggs in the nesting burrow while they feed. We found that female platypuses will leave their eggs in the nest for an average of 80 minutes every couple of days while they feed in the water. Humidity in the nest, maintained by the wet vegetation and plugged entrances, likely prevent the parchment-like egg shells from desiccating (Temple-Smith & Grant, 2001). However, removal of the female’s body heat for this short duration may slow the development of the embryo as they are left in an environment of 19°C, compared with 32°C platypus body temperature (Burrell, 1927). For this reason, it is possible that the duration of incubation may be slightly longer in the platypus compared to echidnas. Small parchment shelled eggs (such as those produced by the platypus) can lose more than a third of their water content in 12 hours if there is a differential between the temperature of the egg and environment (Oftedal, 2002). A gradient as small as 4°C, can cause embryonic death (Oftedal, 2002), which could occur when eggs are left unattended in a nest for too long. We found
no difference in the time spent away from the nest during incubation in years that were successful verses years that failed. In 3 out of 4 of the failed nests, torn egg shells were found after 10 and 12 days indicating young had hatched, suggesting failure occurred at lactation. We suggest that the short feeding durations by the female are important for maintaining egg hydration and survival.

**Female diet during lactation**

The increase in energy intake by lactating platypuses is comparable to changes observed in other mammals such as tammar wallabies (*Macropus eugenii*) that consumed 174% (Cork, 1991) and red-tailed phascogales (*Phascogale calura*) that consumed up to 219% (Stannard & Old, 2015) of their normal (non-lactating) energy intake during lactation. Similarly, the lactating female in our study consumed more than twice that of non-lactating females suggesting that she had similar energy requirements during lactation as those species. Platypuses typically consume 921 kJ/kg/day or up to 21% of their body weight in food items daily (Thomas et al., in press-b). Food consumption by lactating females in our study reached a maximum of 2093 kJ/kg/day (36.4% of her body weight) during the final month. Holland & Jackson (2002), by contrast, estimated that one lactating captive female consumed 90 – 100% of her body weight per day in food. However, Holland and Jackson (2002) estimated food consumption whereas we used precise measurements to quantify food remains (Thomas et al., in press-b). It is interesting that there was no significant difference between the amount of kilojoules consumed in the first month of lactation compared with the non-lactating platypuses, which is likely due to the reduced activity of the lactating platypus during this time. Wild platypuses are typically active for 8.5 – 12 hours a night (Otley, Munks, & Hindell, 2000), however we found that our captive lactating females were able to consume the same number of kilojoules in a fraction of that time. This suggests the females used efficient foraging technique to obtain adequate nutrition. The prey consumed in the highest quantity across all months were the mealworms which were also the highest in kilojoules of all the prey items offered and they were relatively immobile in the tanks, making their acquisition easy (Thomas et al., in press-b). The female was likely able to meet her increasing energy demands by consuming the most nutritious food available.
Lactation in mammals is energetically costly (Rogowitz, 1996). To accommodate the increased food intake during lactation, the intestines of the mother can expand to increase the surface area of the gut and therefore nutrient absorption (Hammond, 1997). If energy demands are not met, a trade-off is made between self-preservation or diminished nutrient exchange to the offspring. In some species (baleen whales, sea lions and true seals) the energy required for lactation is sourced from fat reserves, rather than directly from their diet, as their lactation occurs during a period of fasting (Oftedal, 2000). In other mammals (red-tailed phascogales), energy for lactation is acquired directly from diet as well as fat reserves (Stannard & Old, 2015). Platypus store fat in their tail (Grant & Carrick 1978; Temple-Smith, 1973) and tail fat stores are known to decline during late lactation (Grant, Griffiths, & Temple-Smith, 2004; Handasyde, McDonald, & Evans, 2003). However, glucocorticoid levels in lactating female platypuses are not elevated during this period indicating they may not be mobilising all of their energy from fat reserves and thus must be gaining it from the diet (Handasyde et al., 2003). Females are likely to draw energy for lactation primarily from fat stores in the first two months, when most of their time is spent feeding the nestlings. In the last two months of lactation, the young are significantly larger and require larger volumes of higher quality milk (Griffiths et al., 1984). At this time, fat stores of the lactating female are likely to be diminishing and more energy is required from the diet. As the nestlings are furred and likely to be endothermic at this stage, the female would be able to leave them for longer periods to forage. Presumably the trade-off between the needs of growing nestlings in the last month obtaining large amounts of energy to continue their growth, and the need for the mother to provide that energy, eventually provides the signal for the nestlings to wean and leave the nesting burrow to find their own source of food.

In many species that rear young from a very undeveloped stage, such as marsupials and monotremes, the nutritional composition of milk changes during lactation (Green & Merchant, 1988). In the platypus, milk from the first half of lactation is more dilute (containing only 19.8 - 29.0% solids), compared with milk produced later in lactation (containing 41.4 – 45.0% solids) which indicates that there is a change in milk composition as the young grow (Griffiths et al., 1984). The increase in food consumption and energy requirements of the female
towards the end of lactation may also be due to these changes in milk composition. The high fat content of platypus milk is likely acquired from both fat reserves and from the diet. Polyunsaturated fats found in platypus milk have been found to originate in their diet of macroinvertebrate prey in the wild (Gibson, Neumann, Grant, & Griffiths, 1988). In the food items provided to our captive animals, the major polyunsaturate, linolenate, were in the highest levels in fly pupae and mealworms (20 – 27%; Thomas et al., in press-b) which is higher than in foods recorded in wild diets (10 – 14%). Eicosapentaenoate were highest in earthworms, although only at 0.6% (Thomas et al., in press-b), yet in the wild diet, the levels are 6 – 12% in their prey (Gibson et al., 1988). This suggests that while breeding was successful, the captive diet did not contain a comparable source of eicosapentaenoate as the wild diet, thus this polyunsaturate may be derived from a females body reserves as well as the diet. Interestingly, when energy intake was at its highest, attendance in the nest by the female was at its lowest. We found the female will only feed her young every two days in late lactation. This suggests that an increase in foraging time is required to obtain enough energy from the diet to increase the energy content of the milk and possibly the increasing quantity required to feed the growing young.

Female activity

We found the amount of time the mother spent with her young differed when she had one compared to two nestlings in the burrow. The nestlings were left alone for more than 24 hours earlier with twins compared to a single young. At 44 – 52 days of age, when twins were first left alone, the nest was still full of nesting material and it was difficult to see the nestlings, apart from the small movements they created. Holland and Jackson (2002) similarly found the mother first left the nest for more than 24 hours with twins at 39 days of age and Hawkins and Battaglia (2009) found twins left alone at 38 and 48 days. Nestlings at this age have a C-shaped body position and no fur (Manger et al., 1998). Marsupials are born ectothermic and develop endothermy around the time the pelage grows (Russell, 1982); monotremes likely follow the same pattern of development. It is possible the platypus twins were able to be left earlier as they have already developed endothermy and have each other for warmth. At 71 – 72 days of age, when single nestlings were first left alone for more than a night, the footage
showed nestlings had no fur present on the underside of the body but their backs were covered in a velvety layer of fur. These nestling were likely to be large enough and adequately protected with fur growth to be left alone. In contrast, Hawkins and Battaglia (2009) found when a single offspring was produced, the mother behaved the same as with twins and first left the nestling for more than 24 hours at 40 days of age. These differences are difficult to interpret without further information but the loss of one nestling (Hawkins & Battaglia, 2009), or the individual behaviour of females may explain the difference.

**Nestling development**

We experienced several challenges using the burrowscope. For the first 20 days when the camera was in the burrow, there was an abundance of nesting material which obscured the view, except when the mother was in the nest. As the mother and nestlings moved around the nest, they also brushed up against the camera or touched it on a daily basis. This often meant the camera needed to be refocused or have the lens cleaned of dirt. This involved removing it from the conduit and exposing the burrow for a few minutes while this was done. The location of the burrowscope was right on the edge of the chamber which provided a view of about half to ¾ of the total space. At times, the nestlings were not in view. We left the camera in this position as we were cautious about weakening the integrity of the burrow with an additional hole or disturbance without knowing at the time if this would affect successful rearing of the nestlings. At the time the camera was inserted, the loose top soil did not provide enough stability for drilling until approximately one inch below the surface where it was compacted. The technique was improved by spraying the earthen surface with water prior to drilling. This reduced the amount of soil fell in the chamber as the conduit was inserted, to less than one teaspoon. When the female first returned to the nest after the camera had been placed, she touched the edges of the chamber, including the camera, with her bill, and then re-covered the camera with nesting material before leaving. Rearranging nesting material by the mother prior to leaving was a consistent behaviour and did not change from the first day the scope was placed into the burrow through the rest of lactation.

An accurate age-related growth and development sequence of nestling platypuses has not been reported previously. The only study to describe the
physical development of platypus nestlings was from a series of museum specimens (Manger et al., 1998) collected from a variety of wild populations by Burrell (1927), who provided an estimate of their ages. While many of the specimens and ages described by Manger et al. (1998) appear to fit with our observations, the final specimen does not. We found the majority of developmental milestones such as the eyes opening and a full body of fur, occurred at day 91-92 of age, 100 days earlier than the described specimen. A nestling bred at Healesville Sanctuary in 1944 was also observed with eyes open at 80 days of age and with full fur by 102 days of age (Fleay, 1944). However, most of the age classes that have been described, were for ages younger than we were able to observe in the nest (Manger et al., 1998). The rate of nest decomposition coincided with the development of the nestlings. The nest remained as a closed structure until approximately halfway through nest-life. At this point the nest opened up and the nestlings had developed to the point of having velvety fur over their body and were able to move around in a slow crawl. The timing of the nest opening up, coincided with many developmental milestones such as fur development, eyes opening and full movement being achieved. This timing is likely assisted by the increased movement of the young in the nest, aiding the breakdown of the vegetation.

Platypus nestlings spent most of their time sleeping or in a sleep-like behaviour when unattended in the nest. This state exists in newborn mammals until they develop cognitive maturity in terms of sensory processing and the behavioural responses associated with it (Mellor & Lentle, 2015). REM sleep in young mammals is high in early life as the central nervous system develops (Marks, Shaffery, Oksenberg, Speciale, & Roffwarg, 1995). It has been suggested that marsupials are cognitively developed to the point where they show true REM sleep just prior to leaving the pouch which is roughly 60% of their pouch life for tammar wallabies (*Macropus eugenii*) and almost 100% of the pouch life of the Virginian opossum (*Didelphis virginiana*; Mellor & Lentle, 2015). At this stage, most of the physical development required for sensory input has occurred such as eyes and ears opening (Mellor & Lentle, 2015). Sleep position varied with nestlings using both a curled up position and laying on their back with their ventral side exposed. The curled position was previously suggested to be
used by adult platypuses for heat conservation, protecting the extremities (Grant & Dawson, 1978), while the exposed position has been described in adults when their core body temperature reached 35°C and they were overheated (Robinson, 1954). Burrow temperatures are typically a stable environment (Bethge, Munks, Otley, & Nicol, 2004) so it seems unlikely this behaviour in the nestlings, resulted from exposure to high temperatures. However, to assess this with certainty, temperatures should be recorded within nesting burrows. Platypus nestlings opened their eyes at 68% of their equivalent of pouch life in a marsupial, suggesting that are cognitively aware and capable of behavioural responses to stimuli at this age. However, as the platypus nestlings were not observed leaving the nest until weaning, they would receive much less stimulation inside the nest than the young of other species, like marsupials, that are carried externally by the mother.

Adult platypuses use a chewing motion when cleaning out their cheek pouches (pers. obs). This chewing motion was another behaviour observed in nestlings in the later stages of dependence but was not seen regularly. Platypus nestlings have enamel teeth in their bill, which are shed around the time of emergence and replaced with keratin grindings pads (Grant, 2007; Green, 1937). Although nestlings were observed chewing on nesting material, they did not take solid food until after they left the nesting burrow. Possible explanations for this behaviour include clearing out old milk from the mouth and cheek pouches prior to the next feed and/or the more deterministic preparation of nestlings for eating solid food, through changes in dentition at weaning, when they leave the nesting burrow and start to forage for themselves. Yawning was also observed, but the purpose of yawning across all taxa is poorly understood (Guggisberg, Mathis, Schnider, & Hess, 2010). Grooming was frequently observed. This behaviour is common in adult platypuses to maintain the two dense layers of fur which function to trap air close to the skin and keep them warm and dry in water (Grant, 2007). Grooming in the nestling first appeared as a slow and awkward behaviour until the hind feet became oriented in the adult position and they had a full range of movement. This occurred as the fur developed from short velvet to a dense and glossy coat which would likely need greater maintenance.
Emergence

Weaning occurred abruptly in all but one of the breeding seasons we studied, sometimes occurring prior to emergence. Weaning occurs when the offspring has sufficient immune defences and is physically capable of obtaining, processing and digesting an adult diet (Humphrey, 2010). At Taronga Zoo, the female platypus continued to return to the nesting burrow with the juveniles post-emergence for 7 – 14 days (Hawkins & Battaglia, 2009), and at Healesville Sanctuary for 24 and 29 days post-emergence (Fleay, 1944; Holland & Jackson, 2002). It is unknown if the mother was still feeding the juveniles milk at this stage as the behaviour of the animals was not viewed in the nest, nor was it determined if the mother was still lactating. We found four occasions where weaning occurred after emergence, in three cases this was within 1 – 5 days, and in one outlying case, 42 days. However, as we determined this from the last time the mother was with the offspring in the burrow, it was possibly much earlier. A wild juvenile was found with both milk and macroinvertebrates in its cheek pouches after emergence, suggesting that weaning can occur after emergence in the wild (Grant, 2007). In the closely related echidna, weaning was also found to occur over approximately 2 weeks, as the young emerged from the maternal nursery burrow and foraged before abandoning it entirely (Morrow & Nicol, 2013). Most mammals will gradually transition their offspring from milk onto solid food (Langer, 2008). However, there are some species that abruptly wean young, such as elephant seals (*Mirounga leonina*) where the mother leaves to feed at sea after a few weeks of intensive lactation (Arnbom, Fedak, Boyd, & McConnell, 1993).

The advanced growth stage of a juvenile platypus at emergence suggests an abrupt weaning strategy. The exact timing of weaning is likely to be an adaptive technique which the mother can alter based on the individual needs of the offspring around the time of emergence.

All juveniles in our study reached their adult weight at approximately eight to nine months of age which is earlier than from wild studies where juveniles were found to be 88 – 99% of the adult length by 10 -11 months of age (Grant & Temple-Smith, 1983). The juveniles in our study may have reached their maximum size earlier than in the wild due to their *ad libitum* diet in captivity, the
provision of nesting boxes, the lack of competition from other platypuses and other environmental hazards that wild juveniles are likely to experience.

Knowledge about maternal care is important for understanding the basic survival strategies for a species. This study has demonstrated the high investment of time and energy in maternal care by female platypuses to produce well developed juveniles at the time of weaning. The method used to monitor the nesting chamber via video surveillance in captivity will improve the overall management and welfare of breeding animals, but could also potentially be used to monitor development in other species that use burrows. While much remains unknown about maternal care in platypuses, similar studies on wild platypuses would greatly enhance our knowledge and also confirm whether the behaviours occurring in captive animals are also similar to their behaviour and development in the wild.

**Acknowledgments**

This research was funded by Healesville Sanctuary, the Holsworth Wildlife Research Endowment and the Winifred Violet Scott Trust. Special thanks to the platypus keepers, particularly Sarah Boyd, at Healesville Sanctuary for their assistance attaching the transmitter and reviewing video footage.
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Chapter 7 Burrow use by juvenile platypuses in their natal home range
Chapter 7

Burrow use by juvenile platypuses in their natal home range

Abstract

Little is known about the way juvenile platypuses use habitat after emergence from the maternal nesting burrow and during dispersal. The aim of this study was to determine the pattern of burrow use by juvenile platypuses in their natal home range and whether there were any associations between burrows and vegetation. Between March 2015 and March 2017, seven juvenile platypuses were captured along a 3 km stretch of stream at Badger Creek, Victoria and fitted with radio-transmitters. The locations of animals in their burrows were recorded each day for the duration of transmitter attachment (range: 14 - 132 days). Collectively, the juveniles used 74 different burrows, with each juvenile using 11 ± 2 burrows. Overall, 65% of burrows (48) were used only once, 22% (16) were categorised as moderate-use (between 2 – 9 times) and 13% (10) were used frequently (> 10 times). No juveniles dispersed during the monitoring period, which suggests that they remain at their natal site at least until the sub-adult stage. While we found some association with the pattern of burrow use and particular vegetation communities, vegetation did not appear to be a strong factor driving burrow site selection. The use of many different burrows by juvenile platypuses is likely to be an advantageous behaviour and a strategy to improve their chances of survival. Multiple burrow use may allow juveniles to avoid competition with conspecifics, exposure to ectoparasites and develop shelter-seeking behaviour. Juvenile platypuses remained in their natal home range, where conditions are likely to be good if they support breeding, while completing their growth and development prior to dispersal.

Introduction

Juveniles of many species experience ontogenetic shifts in their ecology (McHugh, Allen, Barleycorn, & Wells, 2011; Werner & Gilliam, 1984). Mammals,
in particular, change from a milk diet to an adult diet at weaning, while also changing their habitat use (McHugh et al., 2011). When juveniles emerge from nests or burrows they must learn how to forage and avoid predators and, as they develop towards maturity, also need to find their own shelter (McHugh et al., 2011). While extended maternal care is common in many mammalian species, juveniles may also compete with adults for resources. For example, southern elephant seals (*Mirounga leoninae*) exhibit intra-specific competition for food, and juveniles will forage in smaller areas and make shorter and more frequent trips out to sea than adults (Field, Bradshaw, Burton, Sumner, & Hindell, 2005).

Platypuses are semi-aquatic, semi-fossorial, egg laying mammals that use burrows they dig in river banks for shelter and to breed. These burrows have been classified into two types (Burrell, 1927). Resting burrows, which are used extensively by both adults and juveniles throughout the year, are simple in structure and are primarily used for sleeping and shelter during the day. These burrows are typically used by a single individual at a time, although there are records of two animals sharing a single burrow, or the same burrow may be used by different individuals at different times (Serena, 1994). The nesting burrow is more complex in structure and is only used by a female platypus during the breeding season to lay her eggs and rear her young until independence. Females lay eggs in spring each year (Burrell, 1927). The eggs are estimated to be incubated by the female for 10 days and the young remain in the nesting burrow, during lactation, for the first four months of their life (Chapter 6; Griffiths, 1978). They emerge from the nesting burrow in late summer and enter the water to commence feeding on the adult diet of aquatic invertebrates (Grant, Griffiths, & Temple-Smith, 2004). Juveniles are well developed at this time and can be up to 70% of their adult weight and 87% of their adult length at this age (Grant & Temple-Smith, 1998a). There is no data available to indicate whether or not wild females continue to share their burrows with their young after they emerge. Captive breeding studies have recorded juveniles staying in the same burrow for up to 42 days post-emergence, but in 90% of cases the young did not return to the nesting burrow or the same resting place as the mother beyond 5 days after emergence (see Chapter 6). The dispersal of juvenile platypuses from their natal home range is still poorly understood. Recapture rates are low for platypuses in
general, so it is difficult to determine if individuals have dispersed from their natal area, perished or if they are still present and eluding recapture (Grant et al., 2004; Griffiths, Kelly, & Weeks, 2013). Further, because platypuses shed their transmitters after relatively short periods (Otley, Munks, & Hindell, 2000; Serena, 1994), it is difficult to monitor the movements of juveniles for sufficient time to record dispersal.

Ideal platypus habitat has been described as “a river or stream with relatively steep earth banks consolidated by the roots of native plant species with foliage overhanging the banks. The river or stream itself should contain a diversity of instream habitats, including aquatic vegetation and logs, and comprise of a series of distinct pools of less than 5m in depth, with little sand accumulation, and separated by cobbled riffle areas” (Grant & Temple-Smith, 1998b). Riparian vegetation is thought to provide cover for platypuses on the water’s surface, influence water temperature, provide organic matter which forms the basis of the food web and provide security around burrow entrances (Grant & Temple-Smith, 1998b). Vegetation and fallen trees along river banks prevent erosion and give the soil stability which is important for burrowing (Buchanan, 1991). There are many factors affecting the stability of a burrow such as the type and moisture content of the substrate, the presence of plant roots and heavy rock fragments (Brandt, Parrish, & Hodges, 1995; Stokes & Boersma, 1991). Overhanging vegetation, stream flows, undercut banks, steepness and consolidated soil are important habitat characteristics for platypuses (Serena & Grant, 2017; Serena, Thomas, Williams, & Officer, 1998). Platypus burrows are also positively associated with rocky ledges, natural debris, fallen logs and uprooted trees (Serena et al., 1998). However, the structural components and vegetation communities around burrow sites have not been described. These are likely to be important as different plant groups will use water differently, have different root systems in terms of providing soil stability and provide varying levels of cover. Such information is important to inform habitat protection and restoration programs aimed at conserving platypuses across their distribution.

Understanding the habitat use of young animals is as important for conservation as understanding requirements for suitable breeding habitats. Juvenile platypuses show naïve, exploratory behaviour (J. Thomas, pers. obs.),
and while this may be a risk in the presence of predators, such behaviour may allow juveniles to explore, adapt and cope with new environments. For many species, juvenile dispersal is critical for genetic diversity, resource distribution and regulation of the population (Greenwood, 1980). There are many factors influencing and driving the dispersal of vertebrates from their natal home range. Individuals may choose to disperse on their own through an innate behaviour or they may be forced out by others (reviewed in Greenwood, 1980). Dispersal may be driven by density or resource dependence, when a population reaches its carrying capacity (Massot & Clobert, 1995; Pasinelli, Schiegg, & Walters, 2004). However, there are also advantages to delayed dispersal from the natal home range until sexual maturity, by remaining in a familiar environment with access to its resources (Kokko & Ekman, 2002).

The aims of this study were to determine 1) the number of burrows used by juvenile platypuses in their natal home range 2) the pattern of burrow use after their emergence, 3) if juveniles dispersed and 3) if there were any associations between particular vegetation and the burrows.

Methods

Study site

This study was conducted along approximately 3 km of Badger Creek, (also known as Coranderrk Creek), between Don Road and Koo Wee Rup Road, Badger Creek, Victoria. The majority of the site is contained within the grounds of Healesville Sanctuary (Zoos Victoria) and the adjoining Coranderrk Bushland (see results). The stream has mature riparian forest vegetation along most of its length within the study site.

Trapping and Tagging

Twelve fyke nets were set up along the creek at approximately 600 m intervals, for 1 – 2 nights in the last week of March in each of 2015, 2016 and 2017. These dates were selected to maximise the chance of capturing juveniles based on emergence estimates from other populations, and also data from a local captive breeding program (J. Thomas pers. obs.; Grant et al., 2004; Holland & Jackson, 2002). Pairs of nets were set in the afternoon with one net facing upstream and the other facing downstream. Nets were secured in place with
rocks and timber plant stakes and monitored regularly from 21:00 to 06:00 h, with no more than four hours between checks. Any platypuses captured were removed from the net and placed into a calico bag and taken back to a nearby vehicle for processing. Each platypus was microchipped for future identification (Trovan Unique ID100 FDX-A). Animals were weighed to the nearest gram (Pesola spring balance 2 kg, Switzerland) and checked for spur development. Animals were classified as juveniles using their weight and spur class (as per Temple-Smith, 1973). Each juvenile was fitted with a 13 g, 40 mm x 18 mm x 10 mm (l x w x h), VHF core radio-transmitter (model V2G 152C; Sirtrack, New Zealand). A patch of fur the size of the transmitter was shaved down to the skin on the animal’s rump. Transmitters were then fixed in place using a fast setting adhesive (Selley’s Quick Fix Supa glue) and the surrounding fur smoothed around the transmitter so it sat flush with the animals lower back. Platypuses were processed and released into the stream at the site of capture. The nets were closed/removed from the creek by 06:00 h each morning.

Radio-tracking and identification of burrow locations

Each platypus was radio-tracked daily using an Ultra digital receiver and a handheld 3-element Yagi antenna (Sirtrack, New Zealand), during the day to their resting burrow for the duration of transmitter attachment (Table 7.1). The burrow locations were recorded using a GPS (eTrex Vista HCx, Garmin). If the location was within 1 m of a previously recorded burrow it was considered to be the same burrow. Every animal was radio-tracked until the transmitter fell off, at which time transmitters emitted the ‘motionless’ signal that was programmed to occur after 24 hours of no movement. The battery life was estimated to be up to six months, however all transmitters fell off before their batteries became flat. Attachment duration for each animal tracked is provided in Table 7.1. Burrow use by individual juveniles were classified into “single-use”: those burrows used only; “moderate-use”: burrows used between 2 - 9 times; or “frequent-use”: burrows used by the same animal 10 times or more.

Vegetation surveys

We surveyed the vegetation associated with 46 burrows (out of 74 possible burrows = 68% of all burrows located) to determine the local scale vegetation characteristics that may lead to site selection for burrows. The method was based
on that described in Threlfall, Harrison, Van der Ree, Williams, and Jones (2015). A 10 m x 5 m quadrat was established aligning to a best fit with edge of the creek and with the burrow fix in the centre. If more than one burrow fell within the same quadrat, the centre was repositioned between the locations. A total of 30 quadrats, encompassing 46 burrows, were assessed. Each quadrat was split into transects 0.5 m apart. The vegetation that was present at 0.2 m intervals along each transect and up to 1 m high, was scored by category (Table 7.2). A list of plant species and the strata level these species occupied (i.e. ground, middle or upper) was recorded for each quadrat. We also recorded whether plants were locally native or exotic species. Fallen logs, rocks, organic matter and bare ground, were also recorded. Structures such as cement drains, footpaths and bulky rubbish were also recorded into a single category – artificial structures.

**Statistics and analysis**

Burrow GPS data were mapped using ArcGIS 10.5 (Esri Incorporated). Results are presented as the mean ± standard error of the mean (SE). Analysis of variance (ANOVA) was used to compare means between burrow types and vegetation communities using SYSTAT (Systat software, San Jose, CA). $P$ values of less than 0.05 were considered significant. If significance was found between burrow types, a Bonferroni pairwise comparison was used to determine where significance occurred.

**Results**

**Number of juveniles captured and fitted with transmitters**

Seven juveniles captured from 2015 – 2017 were monitored via radio-tracking. This included four juvenile females and three juvenile males that were tracked for 14 – 132 days (Table 7.1). Four animals were tracked in the first year, followed by two in the second year and one in the third year of the study.
Table 7.1: Sex, mass, transmitter attachment and monitoring periods for juvenile platypuses in this study. “Burrow range” = the distance along the stream between the two furthermost burrows used by that individual, with all other burrows falling within that range.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Sex</th>
<th>Mass at capture (g)</th>
<th>Date of transmitter attachment</th>
<th>Transmitter attachment days</th>
<th>Number of days located</th>
<th>Total number of burrows used</th>
<th>Burrow range (km)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Female</td>
<td>600</td>
<td>31/3/2015</td>
<td>80</td>
<td>71</td>
<td>19</td>
<td>1.26</td>
</tr>
<tr>
<td>B</td>
<td>Male</td>
<td>500</td>
<td>31/3/2015</td>
<td>43</td>
<td>42</td>
<td>9</td>
<td>1.33</td>
</tr>
<tr>
<td>C</td>
<td>Female</td>
<td>500</td>
<td>31/3/2015</td>
<td>37</td>
<td>35</td>
<td>7</td>
<td>1.55</td>
</tr>
<tr>
<td>D</td>
<td>Male</td>
<td>750</td>
<td>31/3/2015</td>
<td>132</td>
<td>107</td>
<td>17</td>
<td>0.76</td>
</tr>
<tr>
<td>E</td>
<td>Male</td>
<td>750</td>
<td>20/3/2016</td>
<td>14</td>
<td>14</td>
<td>4</td>
<td>0.65</td>
</tr>
<tr>
<td>F</td>
<td>Female</td>
<td>510</td>
<td>20/3/2016</td>
<td>34</td>
<td>32</td>
<td>14</td>
<td>2.26</td>
</tr>
<tr>
<td>G</td>
<td>Female</td>
<td>750</td>
<td>20/3/2017</td>
<td>110</td>
<td>101</td>
<td>4</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Burrow use

During this study, 402 burrow fixes were collected (one per day per animal) and 74 different burrow sites identified over 3 km of the creek. The maximum number of days a platypus was recorded using the same burrow was 53 days. The mean number of different burrows used per animal was 11 ± 2 (Table 7.1). Burrow entrances were rarely identified due to density of the surrounding vegetation on the creek bank. The majority of animals were located underground less than 0.5 m from the stream edge.

The mean maximum range of burrows used by each individual was 1.2 ± 0.2 km along the stream (Table 7.1). The mean distance between each burrow was 160.3 ± 24.3 m with no significant difference between each animal (\( F_{6, 48} = 0.61, P = 0.72 \); Figure 7.1). The closest burrows used by one animal was 7.3 meters apart. There was no significant difference between range of distance of burrows in relation to year or sex (\( F_{2, 4} = 0.72, P = 0.54 [\text{year}] ; t = 1.14, \text{d. f.} = 4.66, P = 0.31 [\text{sex}] \)). For most individuals the number of burrows used increased with time: the longer the animal was radio-tracked, the more burrows it used (Figure 7.2). However, the rate of increase was faster for some animals and slower for others. The sequential locations of burrows used by each animal are presented in Figure 7.3.
The number of times that juveniles used different burrows varied (pooled data). Overall, 48 burrows (65%) were classified as single use, 16 as moderate-use (22%; between 2 – 9 times) and 10 (13%) were used frequently (used > 10 times). Frequent-use burrows were occupied for between 16 – 53 days. The mean number of single-use burrows per animal was 7 ± 2, moderate-use 2 ± 1 and frequent-use 1 ± 0. The distribution of each burrow use type along the stream is presented in Figure 7.4.
Figure 7.2: Sequential increases in the number of burrows used over time by individual juvenile platypuses monitored during this study. The black lines and open symbols are females and the grey lines with closed symbols are males.

Single-use burrows were the most common (50 – 88% of burrows used by 6/7 animals). However, in one individual, 75% of the burrows used were frequent-use burrows (Figure 7.5).

For six juveniles the most number of days were spent in frequent-use burrows (50 – 95%); the remaining juvenile spent the most days in moderate-use burrows (54%). On average, 66% of days were spent in a frequent-use burrow, 19% of days in a moderate-use burrow and 15% of days in a single-use burrow.
Figure 7.3: Pattern of burrow use along Badger creek by individual juvenile platypuses as they were sequentially used each point represents a burrow. A) Female A in 2015, B) Male B in 2015, C) Female C in 2015, D) Male D in 2015, E) Male E (grey) and Female F in 2016, F) Female G in 2017.
Figure 7.4: Map of the location of burrow types. Large circles = frequent-use burrows, medium circles = moderate-use burrows, small circles = single-use burrows. The length of stream between the two open stars indicates the trapping area. The length of stream between the two open circles indicates the location all juveniles were captured.

Figure 7.5: Percentage of fixes for each animal in each burrow type. Light grey = single-use burrows, dark grey = moderate-use, black = frequent-use burrows. A-G indicate data for each individual juvenile monitored.
Vegetation

The average number of points at which vegetation was present/measured per quadrat was 873 ± 38, with a range of 589 - 1262. The number of measures per quadrat was higher for those that had more vegetation across different strata. There was no significant difference between the number of measures at a burrow site and burrow use type ($F_{2, 45} = 2.12, P = 0.13$).

Across all burrows, 39% of records were for locally native plant species, 29% were exotic plant species and 32% were non-plant material including rocks, logs, organic matter and artificial structures.

There was no significant difference between the number of plant species that were in different strata levels (ground, middle or upper story) and burrow use types ($F_{2, 46} = 1.18, P = 0.32$). Ground cover was the most heavily represented category, followed by middle and upper story vegetation.

Plant species richness

Ninety-four plant species were identified across all sites. Fifty-three of these (56%) were locally native to the region and 40 were exotic. The most common plant types across all burrow sites were ground ferns, exotic scramblers and exotic tufted graminoids (Figure 7.6). The native species present in the most quadrats were forest hound’s-tongue (*Austrocynoglossum latifolium*) and tall sedge (*Carex appressa*), both in 59%, and austral mulberry (*Hedycarya angustifolia*) and hazel pomaderris (*Pomaderris aspera*), both in 46% of quadrats. The most prevalent exotic species were annual veldtgrass (*Ehrharta spp.*) in 78%, wandering trad (*Tradescantia fluminensis*) in 70%, and creeping buttercup (*Ranunculus repens*) in 54% of quadrats.

Four burrows were in artificial structures, which included three cement stormwater drains and one under a boardwalk.
Vegetation by burrow-use type

The mean occurrence of each vegetation category for each burrow-use type is presented in Table 7.2. All burrows had a similar occurrence of lichen, large herbs, ground ferns, trees and exotic tufted graminoids, with no significant difference found (Table 7.2). Frequent-use burrows were associated with the most logs, organic matter, native tufted and non-tufted graminoids, tree ferns, small – medium native shrubs and exotic small herbs. Moderate-use burrows were associated with the most paths/ artificial structures, exotic scramblers and ground ferns. Single-use burrows were associated with the most bare ground, rocks, exotic non-tufted graminoids and large native shrubs. There was a significant difference between the amount of large native shrubs and rocks present between burrow types \((F_{2,45} = 3.55, P = 0.037\) and \(F_{2,45} = 5.21, P = 0.009\), respectively), with more of these around single use burrows than frequent and moderate-use burrows. When comparing the vegetation around single use burrows with the two other burrow types, the post hoc test showed there were significantly more rocks around single use burrows than moderate and frequent.
burrows ($P = 0.04$ and $0.05$, respectively). There was no difference with any of the other classified plants and burrow use types.

There was a significant difference between the amount of native vegetation compared with exotic vegetation between burrow types ($F_{2, 37} = 3.29$, $P = 0.05$), with moderate-use burrows containing significantly more than single-use burrows ($P = 0.01$; Figure 7.7). There were similar amounts of exotic and non-plant vegetation around all burrow types.

Considerable variation was found between vegetation categories and burrow locations that included both native and exotic vegetation types (Figure 7.8). There was no significant difference between any vegetation community and burrow type (grass: $F_{2, 45} = 1.40$, $P = 0.26$; ground fern: $F_{2, 45} = 2.79$, $P = 0.07$; herb: $F_{2, 45} = 0.9$, $P = 0.92$; scrambler: $F_{2, 45} = 0.59$, $P = 0.56$; shrub: $F_{2, 45} = 1.25$, $P = 0.30$; tree: $F_{2, 45} = 0.01$, $P = 0.99$; tree fern: $F_{2, 45} = 1.92$, $P = 0.16$).
Table 7.2: Mean, SE and p values for each vegetation classification that was measured against each juvenile platypus burrow type. Frequent (n = 4): burrow used 10 or more times; Moderate (n = 15): burrows used 2 - 9 times; Single (n = 29): burrow used once only, within the monitoring period. * denotes statistical significant where $P < 0.05$.

<table>
<thead>
<tr>
<th>Vegetation type</th>
<th>Frequent</th>
<th></th>
<th>Moderate</th>
<th></th>
<th>Single</th>
<th></th>
<th>F ratio</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bare ground</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>15</td>
<td>4</td>
<td>1.92</td>
<td>0.15</td>
</tr>
<tr>
<td>Bryophyte/Lichen</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>0.34</td>
<td>0.71</td>
</tr>
<tr>
<td>Ground fern</td>
<td>88</td>
<td>28</td>
<td>159</td>
<td>50</td>
<td>57</td>
<td>19</td>
<td>0.64</td>
<td>0.53</td>
</tr>
<tr>
<td>Large herb (exotic)</td>
<td>28</td>
<td>16</td>
<td>35</td>
<td>13</td>
<td>36</td>
<td>7</td>
<td>0.07</td>
<td>0.93</td>
</tr>
<tr>
<td>Large herb (native)</td>
<td>31</td>
<td>26</td>
<td>42</td>
<td>18</td>
<td>45</td>
<td>9</td>
<td>0.16</td>
<td>0.85</td>
</tr>
<tr>
<td>Large shrub (exotic)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>NA</td>
</tr>
<tr>
<td>Large shrub (native)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td>3</td>
<td>3.55</td>
<td>0.04*</td>
</tr>
<tr>
<td>Log</td>
<td>37</td>
<td>11</td>
<td>16</td>
<td>4</td>
<td>24</td>
<td>4</td>
<td>0.38</td>
<td>0.69</td>
</tr>
<tr>
<td>Medium shrub (exotic)</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2.17</td>
<td>0.13</td>
</tr>
<tr>
<td>Medium shrub (native)</td>
<td>27</td>
<td>10</td>
<td>14</td>
<td>4</td>
<td>15</td>
<td>4</td>
<td>0.90</td>
<td>0.41</td>
</tr>
<tr>
<td>Non-tufted (exotic)</td>
<td>1</td>
<td>1</td>
<td>16</td>
<td>16</td>
<td>14</td>
<td>14</td>
<td>0.08</td>
<td>0.92</td>
</tr>
<tr>
<td>Non-tufted (native)</td>
<td>65</td>
<td>26</td>
<td>63</td>
<td>22</td>
<td>20</td>
<td>8</td>
<td>2.65</td>
<td>0.08</td>
</tr>
<tr>
<td>Organic matter</td>
<td>172</td>
<td>49</td>
<td>168</td>
<td>35</td>
<td>135</td>
<td>18</td>
<td>1.46</td>
<td>0.24</td>
</tr>
<tr>
<td>Path/man-made structure</td>
<td>8</td>
<td>8</td>
<td>37</td>
<td>25</td>
<td>27</td>
<td>10</td>
<td>0.18</td>
<td>0.84</td>
</tr>
<tr>
<td>Rock</td>
<td>3</td>
<td>1</td>
<td>28</td>
<td>9</td>
<td>62</td>
<td>9</td>
<td>6.10</td>
<td>0.01*</td>
</tr>
<tr>
<td>Scrambler (exotic)</td>
<td>36</td>
<td>22</td>
<td>100</td>
<td>38</td>
<td>84</td>
<td>16</td>
<td>0.58</td>
<td>0.57</td>
</tr>
<tr>
<td>Scrambler (native)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0.14</td>
<td>0.87</td>
</tr>
<tr>
<td>Small herb (exotic)</td>
<td>18</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>10</td>
<td>2</td>
<td>1.74</td>
<td>0.19</td>
</tr>
<tr>
<td>Small herb (native)</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>0.20</td>
<td>0.82</td>
</tr>
<tr>
<td>Small shrub (Exotic)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.34</td>
<td>0.71</td>
</tr>
<tr>
<td>Small shrub (native)</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2.59</td>
<td>0.09</td>
</tr>
<tr>
<td>Tree</td>
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<td>19</td>
<td>32</td>
<td>11</td>
<td>34</td>
<td>8</td>
<td>0.05</td>
<td>0.95</td>
</tr>
<tr>
<td>Tree fern</td>
<td>74</td>
<td>72</td>
<td>52</td>
<td>22</td>
<td>20</td>
<td>7</td>
<td>3.08</td>
<td>0.06</td>
</tr>
<tr>
<td>Tufted (exotic)</td>
<td>88</td>
<td>43</td>
<td>70</td>
<td>21</td>
<td>118</td>
<td>20</td>
<td>1.23</td>
<td>0.30</td>
</tr>
<tr>
<td>Tufted (native)</td>
<td>168</td>
<td>105</td>
<td>53</td>
<td>29</td>
<td>43</td>
<td>13</td>
<td>2.45</td>
<td>0.10</td>
</tr>
<tr>
<td>Water</td>
<td>18</td>
<td>6</td>
<td>19</td>
<td>5</td>
<td>20</td>
<td>4</td>
<td>0.17</td>
<td>0.84</td>
</tr>
</tbody>
</table>
Figure 7.8: Mean (±SE) occurrence of vegetation types, both native and exotic, around each burrow type (frequent-, moderate- and single-use burrows). Black = frequent-use burrows, dark grey = moderate-use burrows, light grey = single-use burrows. Open circles = data points.

Discussion

This is the first study to have monitored how juvenile platypuses use burrows in their natal home range after emergence from the maternal nesting burrow. Juveniles used multiple burrows within a small range in three ways; frequently, moderately or on a single occasion. While they spent most of their time in frequent-use burrows, the majority of burrows used were single-use. The vegetation along the bank did not appear to be a strong driving factor in burrow site selection of juvenile platypuses in our study.

Natal home range length determined from burrow spacing

The home range lengths of juvenile platypuses, 0.7 – 1.7 km, were previously found to be significantly smaller than adult home ranges of 2.9 – 7.3 km (Serena et al., 1998; Serena, Worley, Swinnerton, & Williams, 2001). The streams in that study were similar to our study site, being tributary streams approximately 30 km from the Yarra River. While not directly comparable, as our study recorded stationary burrow fixes instead of active fixes, each platypus in our study used burrows that were located within a range of 0.49 – 2.26 km of...
stream, which is similar to previous findings (Serena et al., 1998). The pattern of movement between the burrow locations shows that most of the juveniles returned to a central stretch of the creek, rather than progressively moving along it in a single direction. This suggests they were not yet dispersing from their natal range. A previous study that recorded the dispersal of a single juvenile, found dispersal occurred in a single night, after the animal had spent its time in a similar home range (Serena, 1994). Juveniles may occupy a smaller territory or home range than adults for various reasons, including to reduce encounters with conspecifics, to build fitness and to develop survival skills such as foraging, finding shelter and predator avoidance, in a small familiar area that has a good provision of resources (Berube, Dunbar, Rützler, & Hayes, 2012). Adult male platypuses produce venom seasonally in response to elevating testosterone levels, commencing when juveniles have been emerged for approximately 3 months (Temple-Smith, 1973). Males also spatially separate along the stream at this time where they are likely to be establishing exclusive territories for the breeding season (Gust & Handasyde, 1995). We found that burrows used by the same animal were close together with an average distance of 160 meters apart, and two burrows were as close together as 7 meters. Newly emerged juveniles would need to be cautious of breeding males and may find it advantageous to occupy a small and familiar area of the creek.

In our study, juveniles persisted in their natal home range for the duration of radio-tracking, which was until they were at least 10 months of age. They are still considered to be juveniles at this stage as they do not become sexually mature until approximately two years of age (Grant et al., 2004; Temple-Smith, 1973). They are also not fully grown until approximately 12 – 18 months of age (Grant, 2007). Dispersing juveniles can often end up in poor habitat (Van Horne, 1983), so by remaining in the natal home range, which presumably contained good resources as it supported successful breeding, they can increase their chance of survival by completing development in habitat with adequate food resources (McNew & Woolf, 2005). This is also during the time of the year when conditions are most difficult, with low air and water temperatures, increased rainfall and steam levels, and associated reductions in the abundance of aquatic food resources. Mortality rates can be high in animals under one year of age and
higher during dispersal (Bekoff, 1977). Animals that disperse closer to their age of maturity can have a higher rate of survivorship as they have gained more experience with age (Bekoff, 1977). In the Shoalhaven River, 13% of juvenile female platypuses were recaptured in the same area up to one year of age, 19% of those were recaptured after more than two years and two individuals remained as adults and bred in the area (Grant, 2004). Most juvenile males (86%) were not recaptured after the first year, which suggests they dispersed at this time. However, low re-capture rates for platypuses make it difficult to determine if an animal dispersed or if it evaded capture (Griffiths et al., 2013). In the closely related Tasmanian short-beaked echidna (Tachyglossus aculeatus setosus), juveniles also remain in the natal home range for their first year (Morrow & Nicol, 2013). Our data are consistent with these findings and suggest that juvenile platypuses spend considerable time in their natal home range, potentially until maturity or until they have reached adult size.

**Use of multiple burrows**

Multiple burrows or dens may be used by animals as a strategy for predator avoidance by having less predictable behaviour, reduce ectoparasite loads and to defend territories against, or avoid, conspecifics (Lindenmayer, Pope, & Cunningham, 2004; Loureiro, Rosalino, Macdonald, & Santos-Reis, 2007; White, Ralls, & Garrott, 1994). We found that the juvenile platypuses monitored in our study used between four and 19 burrows each in a 3 km stretch of stream. Adult platypuses also use multiple burrows (Gust & Handasyde, 1995; Serena, 1994). At our site, a study conducted almost 30 years prior and over a similar time frame found 67 platypus burrows that were used by 15 animals (Serena, 1994). While in that study both adult and juvenile platypuses were radio-tracked (for between 8 – 85 days), they similarly each used between one and 18 burrows and the longer the animal was tracked, the more burrows were used. We found the rate of increase in the number of burrows used varied between individuals. We radio-tracked one female for 110 days during which she used only four burrows, compared with another animal that was radio-tracked for 14 days and also used four burrows. While it would appear platypuses continue to find and use new burrows regularly over time, the rate at which they do this varies. This is likely to be an advantageous behaviour for animals living in a regularly
changing habitat, such as streams, which are exposed to variable water levels through seasonal flooding.

Platypuses, like many mammals, can be infested with ectoparasites such as fleas, ticks and mites (Munday, Whittington, & Stewart, 1998). Ectoparasites are commonly acquired through direct contact with an infested animal, but can also be acquired from the environment (e.g. ticks; Wall & Shearer, 2001). One way for animals to manage the spread of ectoparasites is to use multiple burrows, in which case the health benefits may outweigh the cost of construction. The European badger (*Meles meles*) and Brants’ whistling rat (*Parotomys brantsii*) actively manage their ectoparasite burden by moving burrows when the load exceeds their tolerance level, every 1 – 2 days (Butler & Roper, 1996; Roper, Jackson, Conradt, & Bennett, 2002). They also avoid burrows that were recently used by conspecifics, which reduces the chance of encountering an infestation (Butler & Roper, 1996). Our data is consistent with the idea that use of multiple burrows may be a strategy to help reduce ectoparasite loads in juvenile platypuses.

Although little is known regarding the major predators of platypuses, anecdotal reports suggest that the introduced red fox (*Vulpes vulpes*) is one of their main predators (J. Thomas pers.obs.; Serena, 1994). For this terrestrial predator, the most vulnerable position for a platypus is shallow water and on the bank. Having less predictable emergence behaviour can make it more difficult to be located (Lindenmayer & Meggs, 1996). Changing burrows regularly could assist wild platypuses in avoiding predators.

**Frequency of burrow use**

As in our study, platypuses have previously been found to use a high number (42%, n = 57) of single-use burrows (Serena et al., 1998). Despite most of the burrows being single-use, we also found that juveniles spent most of their time in frequent-use burrows and the least amount of time in single-use burrows. While we were not able to determine if the juveniles were creating these burrows or if they used burrows created by other animals, it seems likely that juveniles would also attempt burrow construction to develop this skill. We found most of the juveniles were resting in burrows close to the water’s edge (~0.5 m).
Previously, adult and sub-adults platypuses were found resting within 1 – 2 m of the edge (Serena, 1994) and one juvenile found resting in the entrance to a burrow of an adult (M. Serena, pers. comm.). This suggests that juveniles may not rest as far back, create shorter burrows or use burrows or scrapes created by another platypus/species.

Other mammals, such as armadillos (Dasypodidae), also use burrows for a single time (Reichman & Smith, 1990). They will dig a new burrow each night when either the energy costs of creating it are low or the benefits to the behaviour are high (Reichman & Smith, 1990). For most animals, digging a burrow is a costly behaviour in terms of time and energy expenditure (Ebensperger & Bozinovic, 2000). However, the costs of a burrow can be minimised if it does not contain a nest, is not used to cache food, is simple in design and used frequently throughout their life (Reichman & Smith, 1990). Investing quality into the construction of a burrow can reduce energy costs long term if it improves survival and therefore reproductive success (Reichman & Smith, 1990). Pocket gophers (Thomomys bottae) can use up to 3400 times as much energy to dig one metre as it does to walk that far (Vleck, 1979). Burrows constructed in soil with a high moisture content, such as that along a river bank, have lower energy costs associated with their construction than those dug into soil that is hard and dry (Reichman & Smith, 1990). While platypuses expend more energy walking on land than swimming, they only use half as much energy for these activities as semiaquatic eutherians of a similar size (Bethge, Munks, & Nicol, 2001). Therefore, digging a burrow may not be as energetically costly for platypuses as it is for eutherians.

While creating a new burrow has potentially high energy costs associated with its construction, the use of another animals burrow carries the risk of encountering a con-specific or predator (Reichman & Smith, 1990). Some species, such as kangaroo rats (Dipodomys spectabilis), will continue to reside in the natal burrow for several months after weaning, some will stay until maturity and some inherit the burrow from their mother (Jones, 1984). Juveniles could minimise the risk of encountering a con-specific by continuing to use the maternal nesting burrow where they were reared or by using burrows created by their mother. Some mammals will pass their burrows onto their young (Reichman &
Smith, 1990). We found that most (five out of seven) juveniles had one frequent-use burrow, and two individuals had two and three frequent-use burrows. This would suggest the frequent-use burrows contained characteristics, such as being well constructed or secure, which were favoured by juvenile platypuses.

**Vegetation**

We found no strong evidence to suggest that specific vegetation types along the river bank were important for burrows sites. Riparian vegetation has long been considered a critical component of platypus habitat, as the plant roots act to consolidate the river bank which is necessary to create stable burrows and also provide overhanging vegetation to conceal entrances from predators (Grant & Temple-Smith, 1998b). Although we found a higher association between frequent-use burrows with tree ferns, fallen logs, native grasses, organic matter and native shrubs, single-use burrows were commonly associated with bare ground, rocks, exotic grasses and large native shrubs. The single-use burrow sites were significantly associated with more exotic vegetation than the moderate-use burrow sites. Exotic vegetation is positively associated with soil disturbance and with a loss of native species (McIntyre & Lavorel, 1994). This might suggest that the single-use burrows may have been in poorer quality sites. Grasses are typically good at stabilising soil as they have a fibrous root system which is more dense than a branching root system that occurs in trees and shrubs (Buchanan, 1991). As all of our burrow sites contained large amounts of both native and exotic grasses, we suggest that this contributes to bank stability and therefore a good burrow site. We found all burrow types associated with similar amounts of vegetation cover at all strata levels, suggesting that the locally available habitat was generally well vegetated. Further study should be conducted to compare burrow use at a site where vegetation density along the bank is low.

The ability of juvenile platypuses to routinely find new burrows within a night suggests they are highly adaptive, a behaviour that may have an important function in a changing environment. This may be particularly advantageous when living in streams that change in their structural characteristics when exposed to variable autumn rainfall that can cause rapid flooding, or when living in the natal home range where encounters with con-specifics could be high. Persisting in the
natal home range until maturity potentially provides juveniles with the best available habitat to complete their growth and learn to forage, and might therefore improve their chances of survival after dispersing.

**Acknowledgements**

We thank every volunteer who came out trapping on the overnight surveys. Thank you to Marty White for assisting with the design and collection of data on the vegetation surveys and species identification in the quadrats. Thanks to Melody Serena for discussing and comparing the findings from this study with her own work. This study was funded by Healesville Sanctuary, Holsworth Wildlife Trust and Winifred Violet Scott Trust. It was conducted under Zoos Victoria Animal Ethics Committee approval ZV12014.
References


Temple-Smith, P. (1973). *Seasonal breeding in the platypus, with special reference to the male.* (PhD thesis), Australian National University, Canberra.


Appendix 1: Classification of plant species present in quadrats (n = 94) and their prevalence

<table>
<thead>
<tr>
<th>Native or Exotic</th>
<th>Scientific name</th>
<th>Common name</th>
<th>Category</th>
<th>Strata level</th>
<th>Percentage occurrence in quadrats</th>
</tr>
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<tbody>
<tr>
<td>Native</td>
<td>Austrocynoglossum latifolium</td>
<td>Forest Hound’s-tongue</td>
<td>Scrambler</td>
<td>Ground</td>
<td>58.7</td>
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<tr>
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<td>Ground</td>
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<td>Growth Form</td>
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<td>----------------------------------</td>
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<td>Ground</td>
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<td>Silky Oak</td>
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Chapter 8 General discussion
Chapter 8

General discussion

My study has established the enormous investment of time and energy that is required for the complete cycle of reproduction in female platypuses, *Ornithorhynchus anatinus*. This includes a complex series of behaviours from the first encounters during the mating period, through to burrow construction, lactation and the dispersal of juveniles.

My key findings are:

- The energy intake of captive platypuses varied seasonally, with the least kilojoules consumed during the breeding season and the most kilojoules consumed in the post-breeding season. Crayfish made the largest contribution to the diet, year round, - these are highly nutritious and energy rich. While platypuses demonstrated preference for the less mobile prey items that were offered, no selection was shown seasonally (Chapter 3).

- Captive female platypuses control encounters with males until they are ready to mate by using three evasive strategies: avoidance, flight and resistance. Females avoided encounters by switching to a more diurnal activity pattern which reduced activity overlap with the male and also spent less time active than the male. They used a flight response when encountering a male, by leaving the pond immediately if he was present and also using resistance, by leaving the majority of the courtship encounters first and also having a preliminary courtship behaviour where no physical contact is required with the male (Chapter 4).

- The nesting burrows created by female platypuses in captivity varied in length and structure between years, but ultimately provided a concealed and secure space for the rearing of young. The burrows were composed of narrow tunnels, ‘pugs’ of backfilled earth, dead ends and multiple entrances that lead to a compacted nesting chamber. Females spent an average of 8 hours collecting nesting material, in particular wet mat-rush leaves, which were carried to the nesting chamber in her tail (Chapter 5).
I used cameras to monitor a captive female platypus and her nestlings in their burrow. The average length of gestation was 16 days, followed by an incubation and lactation period of 128 days. The female initially spent most of her time with the eggs/nestlings in the burrow and decreased that time as the nestlings developed. Her energy intake doubled during the final month of lactation, where weaning appeared to occur as an instantaneous event (Chapter 6).

In the wild, emerged juvenile platypuses persisted in their natal home range during their first year and used multiple burrows within a short length of stream relative to adults. Some of their burrows were used frequently, some moderately and a large number were used on a single occasion. There was no evidence to suggest that vegetation influenced the location of juvenile burrow sites (Chapter 7).

Breeding investment

My data suggests that breeding for the female platypus is costly, in terms of both time and energy. Captive females started altering their behaviour around May to avoid males. They then invested time in courtship and mating behaviours during September and commenced nest building during a short gestation, when energy intake in the diet was at its lowest. They lactated and provided maternal care for four months, to have newly emerged and independent offspring in February. At this time their energy intake was twice that of a non-lactating female. Juveniles in the wild then persisted in the natal home range, consuming resources until after May. This suggests a minimum 12 month investment in the rearing of young from one breeding season. In the wild, a female platypus does not breed every year, however the reasons for this are unknown (Grant, Griffiths, & Temple-Smith, 2004). This is also true for another monotreme, the short-beaked echidna (Rismiller & McKelvey, 2000). Temple-Smith and Grant (2001) have speculated the inconsistency in breeding may be resource dependent, genetically induced or from social organisation (density). However, it is difficult to answer this question as it is also unknown at which point breeding for these animals has failed (i.e. at mating, nesting, incubation, lactation or chose not to participate). I found that breeding had failed in the captive animals at various
points including failure to mate, failure to nest build/construct the burrow, failure immediately after egg laying (embryonic development in utero) and failure at the end of incubation (embryonic development in the egg). The reasons for failure in each case were unknown. It appears this may be common for the monotreme breeding cycle. Inconsistent breeding frequencies could provide advantages to the population by allowing the female more time to replenish body condition, freeing up resources within a territory until juveniles disperse. If females produce an average of one juvenile per year that survived to adulthood, from approximately two years of age through to 20 years of age (Grant et al., 2004), 18 animals would be recruited to the population over her lifetime. A recruitment rate this rapid could very quickly create overpopulation in the environment. Population density may also be a significant factor in breeding success. Population density may be expressed in food availability and opportunity for females to replenish body condition rapidly after the breeding season. This may be a signal that prevents females from recovering sufficiently to breed the following year. Overall, this may improve the survival of the juveniles and adults through a slow rate of reproduction. A full cycle of breeding is an energetically demanding process for a female platypus.

Female platypuses use adaptations, in terms of behaviour, as a strategy to integrate the incompatibilities of their breeding requirements/physiology with their environment. They leave dependent young that are not able to survive in their aquatic environment, in a nesting burrow – this provides security and the right microclimate that other mammals such as echidnas or marsupials provide in the environment of a pouch. Further, as mammals, they use lactation to provide offspring with a consistent supply of the nutrients that are required for their growth and development (Peaker, 2002). To a large extent, this supply is provided to young somewhat independently from resources available in the environment (i.e. the ability to store nutrients as fat (Peaker, 2002), which can be mobilised at a later time) and where the dependent young are not able to obtain or consume food in the adult habitat. In the wild, tail fat stores of adult female platypuses increase in the period leading up to the breeding season and are then at their lowest during lactation (Grant et al., 2004; Handasyde, McDonald, & Evans, 2003). This suggests the use of body fat for energy. I found that the lactating
female made a significant time investment in the early stages of lactation, where feeding was restricted and she likely relied primarily on her body fat stores. However, in the later stages when time with the young decreased, the female increased her consumption of dietary kilojoules which, combined with the mobilisation of body fat, provided for the additional energy demands associated with late lactation. Changing energy requirements for females as lactation progresses is common among marsupials. This caters for the changing milk composition and therefore changing energy demands of the offspring (Green, Merchant, & Newgrain, 1988; Griffiths, Green, Leckie, Messer, & Newgrain, 1984). This provides the energy required for the rapid growth of platypus nestlings within the safety of the nesting burrow. I found that weaning appeared to occur quickly in captivity, as the nestlings were emerging to the water for the first time. This suggests emergence was the end of maternal care. Emerged juveniles in a wild population then persisted in their natal home range after maternal care had ended. Juvenile beavers (*Castor canadensis*) will similarly stay in the natal home range to assist in the rearing of siblings and territory defence. This maximises their lifetime inclusive fitness and improves their chance of establishing territories after dispersal (McNew & Woolf, 2005). Juvenile beavers living in poor quality habitat were more likely to disperse earlier than those in high quality habitat. Juvenile platypuses are likely to be developing survival skills, in terms of acquiring food and shelter, by staying in the home range of their mother which presumably was a high quality habitat able to meet her energy demands during lactation.

Mammals will maximise their lifetime reproductive fitness by using slow to fast life history strategies that balance life span, recruitment and survival (Partridge & Harvey, 1988). The platypus appears to exhibit a slower life history strategy, being quite long-lived (Grant, 2004) and making a high investment in a small number of offspring which would typically contribute to a high survival rate. While it is clear that platypuses breed relatively slowly, it is unknown how many juveniles survive into the sub-adult and adult stage (Bino, Grant, & Kingsford, 2015; Serena, 1994). This has been difficult to determine in wild populations as recapture rates are low. The absence of individuals from a population, particularly juveniles, could indicate they have dispersed, eluded capture or perished (Grant et al., 2004). Further research is needed to assess the survival of juveniles and
provide evidence that a slow breeding strategy for the platypus is effective in delivering high recruitment and survival to platypus populations.

**Recommendations for management of wild populations**

The high investment in breeding by female platypuses suggests that high quality habitat is important for the long term persistence of this species in the wild. One important feature of a high quality habitat for platypuses is the stream-side vegetation. The roots from the vegetation consolidate earth banks providing stability for burrows (Grant & Temple-Smith, 1998). The availability of suitable locations for the nesting burrow in the habitat, is critical for a female platypus during the breeding season as it is where she will raise her offspring. Plant roots can also provide some protection from high water flow regimes to minimise flooding over summer when dependant young could drown if burrows are flooded (Bino et al., 2015). The vegetation overhanging the stream is likely to provide cover and protection from predators when platypuses are on the water’s surface (i.e. where courtship and mating behaviours are performed over long durations). Aquatic plants such as Eel grass (*Vallisneria spiralis*), benthic algae and floating pondweed (*Potamogeton tricarinatus*) in the stream provide the basis for the food web (Klamt, Davis, Thompson, Marchant, & Grant, 2016). This vegetation is habitat for the aquatic invertebrates on which platypuses feed and also the nesting material needed for establishing the appropriate environmental condition in the nesting chamber of the breeding burrow for successful reproduction.

High production of benthic invertebrates are necessary for females to reach sufficient body condition to breed and almost certainly for juveniles to survive during the period after emergence from their natal burrow when they need to learn to forage effectively and find suitable burrows in the immediate habitat. Previous estimates of stream productivity suggested that 13.1 g DW m\(^{-2}\) year\(^{-1}\) of macroinvertebrate prey could support 22 – 45 platypuses within a 1.5 km reach of stream in NSW (Marchant & Grant, 2015). The daily energy intake I found in captive platypuses suggests that the lower estimate of 22 platypus in a stream of this productivity is more likely. While captive animals are likely to have lower energy requirements than wild animals as they have smaller areas to move over
and reliable access to food, the energy intake they chose to consume under ‘optimal’ food conditions, is likely to be similar to their wild counterparts in a high quality habitat. However, from my finding that the energy intake of a captive female in late lactation was considerably higher than other periods of the year, I predict that the macroinvertebrates required per adult female would be approximately twice as high during the final two months of lactation as at other times in the year.

**Recommendations for captive management**

*Captive diet*

My study found that freshwater crayfish and mealworms were the highest value and most nutritious prey items offered in the current diet for captive platypuses at Healesville Sanctuary. I found they consumed up to 20% of their body mass in food daily, and that the *ad libitum* diet, containing a variety of invertebrates, offered throughout this study maintained the animals in good to optimal condition without any individual becoming overweight. In zoos, the artificial diet offered can often meet the nutritional needs of non-breeding mammals, but not meet the needs of breeding females. For example, numbat (*Myrmecobius fasciatus*) breeding females require a pure termite diet, while non-breeding animals are maintained on a specialised custard. (Cooper, 2011). Further, while some of our adult females bred successfully on this diet, they sometimes also failed to breed on this diet and, particularly in the past, have been notoriously difficult to breed in captivity, suggesting there still may be room for improvement. Captive platypuses also demonstrated preference for the less mobile prey in their diet. The captive diet has limited species diversity compared to that of wild platypus (Marchant & Grant, 2015). Providing additional prey species that show different mobility patterns would present animals with additional behavioural enrichment (foraging and sensory). This may not only improve maintenance and breeding success of platypuses in captivity but also further the commitment of modern zoos, to the continuous improvement of the welfare of animals in their collections. My recommendation from the results of this captive diet study is to maintain an *ad libitum* feeding regime into the future for the Healesville platypuses and also for platypuses in other captive facilities.
However, this diet could also benefit from improvement through the provision of additional wild macroinvertebrates, such as dragonfly nymphs, produced commercially to improve prey species diversity for behavioural enrichment.

**Housing and facilities for breeding**

The main features of the housing for breeding platypus in captivity, should consider providing a large area to cater for the needs of female platypuses to control interactions with males. I found that the ability of the female to use avoidance strategies around the male platypus was critical during the breeding season. A large exhibit, as detailed in this thesis (Chapter 2), should include multiple ponds for both feeding and movement to ensure that females have enough space to feed alone and also to successfully evade a male if she chooses to. Ponds can provide additional cover through partially submerged logs (which were also used for courtship and mating) and vegetation along the pond edges. Multiple burrow sites should also be provided to allow females the choice to avoid the male. The inability of a female platypus to use avoidance behaviour when housed with a male could lead to death, injury or poor welfare.

Breeding females should be provided with the opportunity to construct their own nesting burrows in earth. While this proves more challenging in trying to monitor animal health (as females in earth burrows become inaccessible), it provides the added welfare benefits of allowing the animal to create the appropriate conditions within the burrow that the eggs and young will require for successful development. The burrowscope, as described in Chapter 6, provides us with the ability to monitor these animals in a natural burrow. The soil in the tanks should be provided at a depth greater than 0.5 m; this exceeds the average depth I found in burrows constructed by captive females in my study. The soil should be maintained with a high moisture content to simulate a stream bank, and be planted with native plants such as grasses and ferns, to promote soil stability.

**Monitoring breeding animals**

Breeding animals should be housed in conditions that allow exposure to natural photoperiod to allow physiological changes to occur in response to changing seasons (i.e. seasonal increases in testosterone in males; Temple-
Smith, 1973). The animals should be allowed to choose their activity cycle, whether it be nocturnal or diurnal. Experience in the field and captivity shows that platypuses will be nocturnally active for most of the year; this is the common pattern of activity in the wild (Gust & Handasyde, 1995; Serena, 1994) and also in captivity if animals are free to do so (Hawkins, 1998; pers. obs.). So as to not disturb the animals sleep cycle during routine captive monitoring, I recommend the animals in the breeding facilities be monitored via CCTV video surveillance using cameras placed above the ponds. Footage should be reviewed daily to ensure females are having adequate time to feed alone and that they are able to evade the male platypus if required. Courtship and mating behaviour can also be observed to allow keeping staff to know when nesting material should be provided and when the male platypus should be removed. This will ensure the female has sufficient space and time for nesting, without needing to be evasive. This approach will also free up food resources during early lactation, when her activity levels are reduced.

A variety of fresh vegetation for nesting material, of varied thickness and structure, should be provided on the water’s surface. Keeping staff need to consider including leaves from species such as *Poa enciformis* and *Lomandra longifolia*, eucalyptus leaves, stringy bark from eucalyptus trees and reeds such as *Phragmites australis*. Providing material in close proximately to the nesting burrow will likely increase the female’s chances of successfully carrying vegetation into the nesting burrow without dropping it *en route*.

The amount of food consumed by the lactating female should be carefully monitored and each type of food item increased as required. This increase is likely to occur after the first eight weeks of lactation. The female should have *ad libitum* food supplied to meet the large energy demands of lactation. A lactating female will consume at least double the amount of food of a non-lactating female in the final month before juveniles emerge from the burrow; this increase in the quantity of food provided needs to be taken into account to ensure that breeding is successful.

After emergence, the female and juveniles should be given access to several feeding areas and most importantly, multiple burrow spaces for resting.
My observations in the wild have demonstrated that juveniles regularly use many different burrows. This is likely to be an important learning experience for them and providing captive juveniles after emergence with sufficient space for this is probably very important. This will also allow the female the choice to feed and rest away from her young. As juveniles were found persisting in the natal home range in the wild until at least one year of age, to properly mimic wild conditions, they should ideally be kept within the captive breeding facility until at least the start of the next breeding season (August). However, I do not consider that removing them prior to this time would necessarily create poor welfare outcomes for these animals. Moving them soon after emergence to another housing facility would also allow them to become familiar with the environment and develop their foraging and shelter seeking skills as they would in the natal home range. Transfers of juveniles to other institutions should ideally occur when they have reached one year of age; this timing would fit closely with the approximate age of dispersal of juveniles in the wild.

Overall the failure of adult females to breed every year appears to be a naturally occurring pattern in monotremes (Grant et al., 2004; Rismiller & McKelvey, 2000). It is possible that this is the result of the large amount of time and energy invested in breeding in a single year and may be influenced by body condition and/or population density (i.e. persisting juveniles). Based on this, I suggest that captive females should not be allowed to breed every year. Thus alternating annually the selection of females to be housed in the breeding facility is recommended providing that their condition is optimal for breeding.

**Future directions**

This is the first study, since the limited descriptions of naturalist Harry Burrell (1927) and the records of Healesville Sanctuary director David Fleay (1944), to observe and describe in detail the behaviour and basic foundations of platypus breeding biology throughout the cycle from courtship to juvenile dispersal. The difficulty in observing the reproductive behaviour of this species in the wild has limited the ability of researchers to establish studies to collect useful information from the wild. The changing role of zoos in recent years a new
focus on conservation, developing high standards of animal welfare within captive collections and a greater emphasis on conducting scientific research into their management and breeding programs (Gray, 2015), has provided new opportunities to study platypuses in a captive setting. This includes unique opportunities to observe how animals behave in different conditions, how they behave in controlled environments and also to observe the types of behaviours that could occur in wild animals, so that we can better develop field studies to answer pertinent questions on their biology.

While my research has described many aspects of platypus breeding biology, there are still areas of their reproduction that require further investigation in captive breeding programs. For example, my research found no specific difference in any breeding parameter that explained why young were successfully bred in some years and not in others. This makes improvements to the success of captive breeding programs difficult as it is not clear why some females failed to breed. Further studies are now needed to identify and investigate factors during the breeding season that result in breeding failure.

The current captive diet for platypuses appears to meet their nutritional requirements for longevity, good body condition and breeding success. However, the species offered in captivity are different and less diverse to those detected in the wild diet (i.e. caddisflies, mayflies and dragon/damselflies; Marchant & Grant, 2015); these aquatic macroinvertebrates are not able to be purchased commercially or easily bred. As the nutritional content of many wild prey species is unknown, further studies need to examine how closely the current captive diets replicate the nutritional composition of the wild diet.

There is still further work required to fully understand platypus breeding behaviour and breeding success. Courtship is typically a process of mate choice and sexual selection, where animals are able to assess and select particular traits that will favour the survival of their future offspring (Clutton-Brock & McAuliffe, 2009). While I found that courtship was partly used by females to control breeding encounters, the particular qualities they may be assessing through the swimming patterns is unknown as the female had access to only one male each breeding season. Understanding if and how females are assessing particular traits in the
male may help to improve future captive breeding success by ensuring that males that are able to express those desirable traits are selected for breeding. Additionally, obtaining more data on the microclimate in the nesting burrow, for example temperature and humidity, would also allow us to understand how to provide suitable conditions for successful incubation, hatching and development of the nestlings in the burrow environment. While my study was able to monitor breeding behaviour over many years, I was only able to study two females, which means we need to be cautious in interpreting the data. Although the cost of maintaining platypuses in captivity is significant, there is a need to conduct further studies to record the behaviour of more individuals. This may be achieved more readily by a larger multiple site study using animals from different captive facilities, however the challenge in such a study would be standardising the captive conditions between facilities.

As both camera trapping and radio-tracking technology improves, more reliable and less labour intensive methods of remote monitoring of wild platypuses will be available. Recently, microchip readers were deployed along streams to detect the movement of pit-tagged platypuses long term (Macgregor, 2015). GPS transmitters of suitable size are also becoming more available and in the near future we should be able to deploy these on wild platypuses. Ultimately we will gain the most insightful understanding of platypus breeding behaviour, by developing technologies that allow us to monitor them more accurately and effectively in the wild. Such knowledge would assist in improving captive breeding programs to ensure that zoos are able to maintain self-sustaining captive colonies that do not require regular introduction of wild caught animals; this approach is consistent with the philosophy and strategies of the modern zoo (Gray, 2015).
References


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Title:
Breeding biology of the platypus (Ornithorhynchus anatinus)

Date:
2018

Persistent Link:
http://hdl.handle.net/11343/217187

File Description:
Breeding biology of the platypus (Ornithorhynchus anatinus)

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