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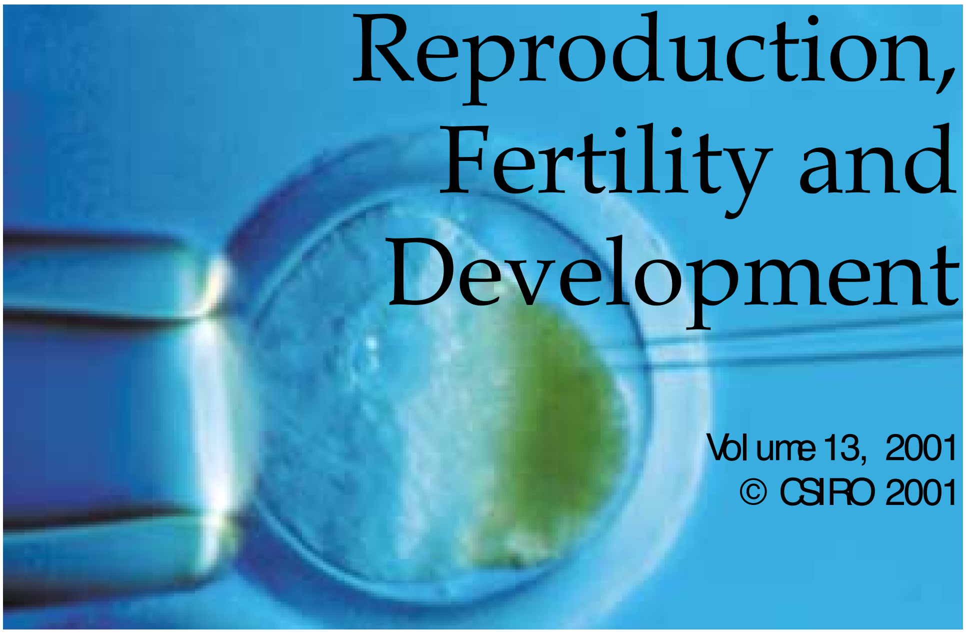

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Uncertain breeding: a short history of reproduction in monotremes

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Abstract. Although much is known about the biology of monotremes, many important aspects of their reproduction remain unclear. Studies over the last century have provided valuable information on various aspects of monotreme reproduction including the structure and function of their reproductive system, breeding behaviour, sex determination and seasonality. All three living genera of monotremes have been successfully maintained in captivity, often for long periods, yet breeding has been rare and unpredictable. When breeding has occurred, however, significant gains in knowledge have ensued; for example a more accurate estimate of the gestation period of the platypus and the incubation period for the *Tachyglossus* egg. One of the great challenges for zoos has been to understand why breeding of monotremes is difficult to achieve. Analysis of breeding successes of platypuses and short-beaked echidnas provides some insights. The evidence suggests that although annual breeding seasons are regionally predictable, individual adult females breed unpredictably, with some showing breeding intervals of many years. The reason for this variation in individual breeding intervals may be resource-dependant, influenced by social factors or may even be genetically induced. Better knowledge of factors that influence breeding intervals may improve the success of monotreme captive breeding programmes. More certainty in captive breeding is also an important issue for enterprises wishing to trade in Australian wildlife since current legislation limits export of Australian fauna for display to at least second-generation captive-bred individuals. Given their unique evolutionary position, knowledge of reproduction in monotremes needs to be gained in advance of any future population declines so that appropriate strategies can be developed to ensure their survival.

Extra keywords: breeding seasonality, comparative anatomy, echidnas, platypus.

Introduction

The living monotremes—the platypus *Ornithorhynchus anatinus*, the short-beaked echidna *Tachyglossus aculeatus* and the New Guinea echidna *Zaglossus bruijnii*—are restricted in their distribution to Australia, New Guinea, Irian Jaya and adjacent Indonesian islands (Flannery 1995; Strahan 1995). Until recently, it was assumed from their current distribution in Australia and New Guinea and limited fossil evidence from a few sites in Australia, that monotremes had evolved on the Australasian segment of the continental landmass of Gondwana (Musser 1998, 1999). However, the discovery of two monotreme teeth from late Cretaceous and early Tertiary fossil beds in Patagonia (Pascual *et al.*1992*a*, 1992*b*) confirmed a more extensive radiation of the monotremes across Gondwana. Although the nature of the relationship between fossil monotremes is still controversial, the evidence suggests that ornithorhynchids were probably the main evolutionary lineage of the monotremes, with the echidnas as a significant early branch (Archer *et al.* 1992; Musser 1998, 1999). The fossil evidence gives no indication of mode of reproduction, however, the oviparous nature of all extant monotremes suggests that oviparity was characteristic of the ancestral group from which monotremes were derived and all the extinct monotreme lineages.

Since their 'discovery' and scientific classification by Europeans in the late 18th and 19th centuries (Shaw 1792, 1799, 1800; Home 1802*a*, 1802*b*; Peters and Doria 1876), many features of the monotremes have attracted the interest and attention of science. Since Shaw's 18th century descriptions, a major focus of research of the echidna and platypus has been their mode of reproduction. The initial controversy about whether they were oviparous, ovoviviparous or viviparous took more than 85 years of scholarly, and sometimes bitter, exchanges between the main protagonists (see Griffiths 1978; Moyal 2001) to resolve. The extraordinarily succinct, but now famous, 1884 telegram by the Cambridge University-trained embryologist William Caldwell to the British Association meeting in Canada—'Monotremes oviparous, ovum meroblastic'—gives little indication of the significance of his discovery. Coincidentally, on the same day (September 2) in 1884, Wilhelm Haacke described his discovery of an egg in the pouch of an echidna to a meeting of the Royal Society of South Australia in Adelaide. He

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published his findings in the next year (Haacke 1885; Griffiths 1968; Moyal 2001). Haacke's contribution to the discovery of oviparity in monotremes has not been given the same recognition as that of Caldwell. This, perhaps, reflects Caldwell's more extensive and focused study of monotreme embryology, which was conducted over about two years. His study involved the taking of more than 1300 echidnas and many platypuses, which were returned to England with Caldwell and provided the material for the first part (Caldwell 1887) of a proposed series of publications on monotreme embryology, of which this was to be the only publication.

In the years since 1884, other significant areas of monotreme reproduction and development have been explored and described (Caldwell 1887; Hill and Gatenby 1926; Burrell 1927; Flynn and Hill 1939, 1947, Fleay 1944; Griffiths 1968, 1978; Temple-Smith 1973; Hughes and Carrick 1978; Bedford and Rivkin 1979; Carrick and Hughes 1982; Beard *et al.* 1992; Grant and Griffiths 1992; Grant and Temple-Smith 1998*a*, 1998*b*; Beard and Grigg 2000; Rismiller and McKelvey 2000; Holland and Jackson 2002). However, despite the passing of more than 200 years since monotremes were discovered by science, there are still significant gaps in our understanding about their reproduction.

This review will briefly draw together what is known about general aspects of monotreme reproduction and, in particular, knowledge that has been acquired recently. It will also examine seasonal aspects of reproduction and recent information about the breeding success of female monotremes. Finally, it will discuss breeding of monotremes in captivity and the advances this has provided for our understanding of monotreme biology.

Structure and function of the monotreme reproductive system

Knowledge about the structure of the reproductive system in male and female monotremes has been easier to acquire than information about function. The anatomical features have been described in some detail (Kolmer 1925; Hill 1933, 1941; Flynn and Hill 1939, 1947; Temple-Smith 1973; Griffiths 1978, 1984, 1999; Carrick and Hughes 1978; Bedford and Rifkin 1979; Djakiew and Jones 1981; Grant 1995; Grant and Temple-Smith 1998*a*) and there are now few areas where significant information is lacking. The monotreme reproductive system consists of a mosaic of features found in reptilian and mammalian reproductive systems, and also some that belong uniquely to the monotremes (Temple-Smith 1973; Griffiths 1978, 1999). Although the basic plan of the reproductive system is similar for all three living genera, there are interesting individual variations which provide insight into the differences in their modes of reproduction. Unfortunately, despite being brought to the attention of science more than 120 years ago (Peters and Doria 1876), most aspects of reproduction in the New Guinea long-beaked echidna are still poorly understood (Griffiths 1999). This section reviews published and unpublished data and provides some new interpretations of reproductive function in this taxon.

Anatomical characteristics

Monotremes possess hair and mammary glands, but they are clearly distinguished from other mammals by oviparity, a true cloaca (marsupials are not cloacate, but are 'monotremes' in that they possess a single external orifice for excretory and reproductive functions), filiform reptile-like spermatozoa and lactational areola without nipples. Retention of the testes in the abdomen is characteristic of all adult male monotremes and is presumably the ancestral condition for the group. Other mammals (e.g. elephants, whales, moles) are also testicondid, but since most marsupial and eutherian species have scrotal testes, the testicondid condition in these taxa is more likely to be secondarily derived.

The female reproductive system opens into the cloaca and features a urogenital sinus connected to separate left and right reproductive tracts each comprising an ovary, oviduct, uterus and cervix (Griffiths 1978, 1999; Fig. 1*b*). As in many bird species and some reptiles, only one side (the left) of the female reproductive system in the platypus is functional, but both sides are functional in the echidnas. From the view point of insemination, an interesting aspect of the anatomy of the female monotreme is the exact relationship between the urogenital sinus, the entry point of the ureters and their association with the bladder, and the position of the opening of the bladder neck into the urogenital sinus. As discussed below, this may provide evidence in support of uterine insemination in monotremes. Interestingly, despite the functional loss of one ovary, the platypus usually ovulates two ova, and sometimes three (Burrell 1927), whereas *Tachyglossus* only very rarely produces more than a single egg (Griffiths 1968, 1978). Platypus eggs, when laid, are similar in size to the echidna egg (Griffiths 1999).

As with the female, the reproductive system of male monotremes is relatively simple by mammalian standards (Temple-Smith 1973; Griffiths 1978, 1999). The intra-abdominal testes connect via efferent ducts to large, secretory epididymides, which drain individually through short vasa deferentia into the anterior end of the urogenital sinus. The canal of the urogenital sinus connects with the penile urethra following erection of the penis to convey semen to the female during copulation (Home 1802*a*, 1802*b*; Griffiths 1978). Seminal vesicles are absent from all living monotremes, and the presence of a functional prostate has been controversial. Histological observations on 'prostates' in the platypus suggested that there was no obvious seasonal cycle of activity (Temple-Smith 1973). However, in the echidna, glandular regression of the prostate outside the breeding season was observed (Jones *et al.* 1992) and experimental studies demonstrated androgen-dependence of the prostatic epithelium in this species (Jones *et al.* 1992). The possibility exists that the structure and function of this organ may differ between the platypus and echidnas. However, the monotreme 'prostate', if it exists in the same functional capacity as other mammalian groups, is unlikely to contribute significantly to the fluid component of monotreme seminal plasma. By comparison, the prostate in marsupials and the prostate and seminal vesicles in most eutherian mammals are large secretory glands, which contribute significant volumes of fluid to the seminal plasma. Finally, there is a single pair of androgen-dependent bulbourethral (Cowper's) glands, which are seasonal in their activity and secrete a clear, viscous fluid drained by single ducts into the membranous portion of the urethra, as in other mammals (Griffiths 1968, 1999; Temple-Smith 1973).

A fascinating, but poorly studied, feature of the male reproductive system in monotremes is the penis. In the platypus and *Tachyglossus*, although the basic organization of the penis is similar, some features of penile structure and adornment are unique and probably reflect important functional divergence. The flaccid penis of monotremes is retracted into a preputial sac, which develops from a diverticulum of the ventral cloacal surface (Griffiths 1968, 1978; Temple-Smith 1973). Erection protrudes the penis through the cloacal opening before mating. The position, shape and size of the erect monotreme penis have not been described. However, in order for intromission to occur in the known mating positions of the platypus and echidna (Strahan and Thomas 1975; Rismiller and Seymour 1991; Beard *et al.* 1992; Hawkins and Fanning 1992; De-La-War and Serena 1999), the penis would need to be reflected into a

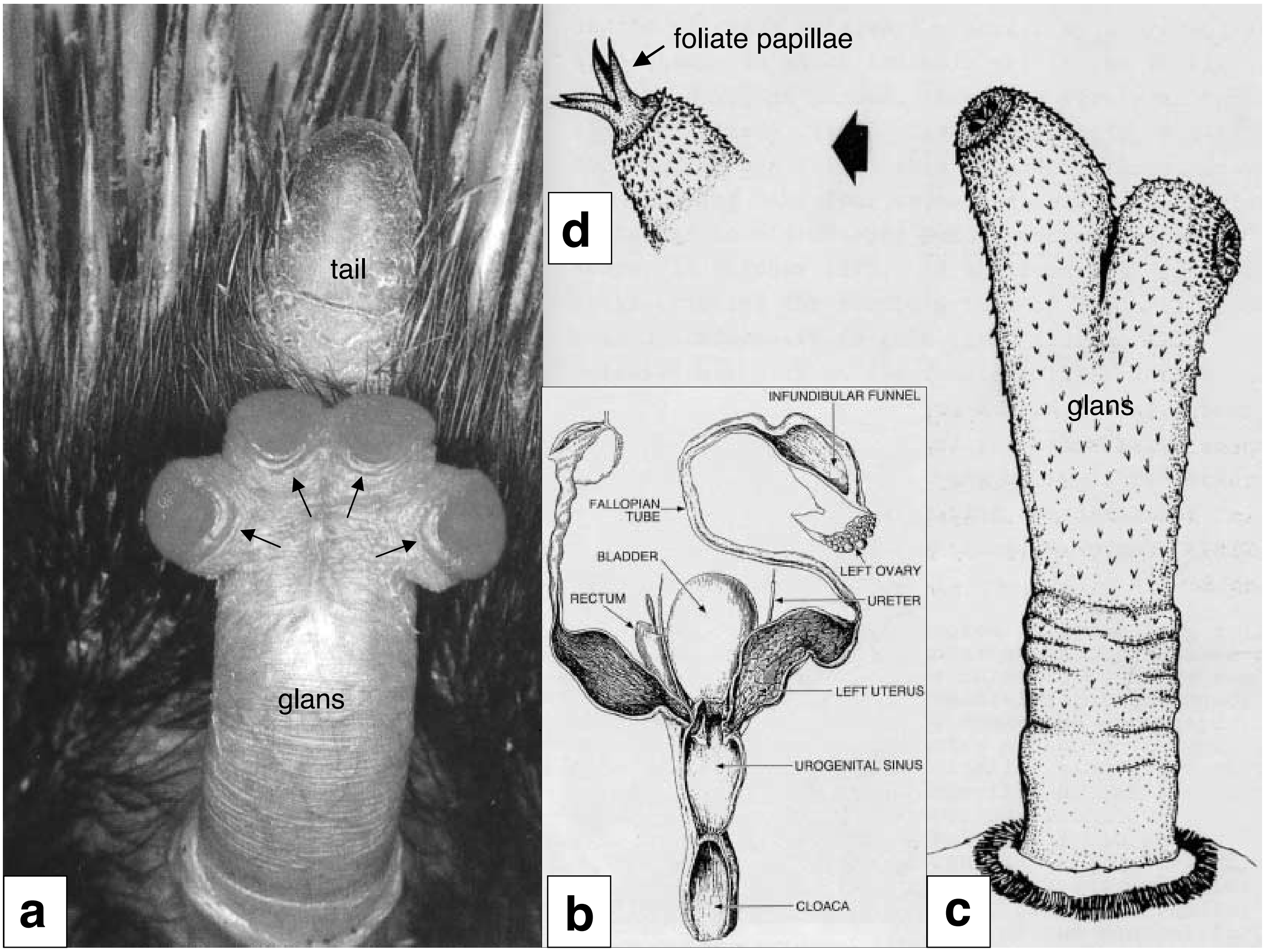

Fig. 1. (*a*) Extruded penis of the echidna, *Tachyglossus aculeatus*, showing the symmetrical 'quadripartite anemone-like' appearance (arrows) of the glans (photo credit and permission, G. Grigg). (*b*) Female reproductive system of the platypus *Ornithorhynchus anatinus* showing the relationship between the bladder, urogenital sinus and the cloaca and the non-functional right ovary which is a characteristic of this species (with permission, M. Griffiths). (*c*) Diagram of the extended penis of the platypus showing characteristic bifid asymmetry of the glans and the two laterally positioned, retracted rosettes. (*d*) Erected left rosette showing the group of four foliate papillae. (*c* and *d*, from Temple-Smith 1973.)

forward position on erection. The configuration of the male reproductive system, in particular the glans penis and its adornments, and the female reproductive tract in the platypus and echidna provide evidence that semen is deposited in the cervical canal or directly into the uterus rather than the urogenital sinus.

The glans penis in both species terminates in unusual specializations. In the echidna, the glans is incipiently bifid and symmetrical, with each half having a rounded surface bearing two epidermal rosettes which produce a 'quadripartite anemone-like appearance' (Griffiths 1978; Fig. 1*a*). The surface of the penis appears smooth and the terminal branches of the penile urethra pass through the epidermal rosettes of the glans. The platypus also has a bifid penis, but, in contrast to *Tachyglossus*, it has a dense cover of rear-facing keratinized epidermal spines across most of its surface and the glans is asymmetrical, with the left side much larger than the right (Temple-Smith 1973; Fig. 1*c*). The glans terminates in a pair of rosette-like structures, one on each side, which in the flaccid state are retracted (Fig. 1*c*), but which presumably on erection protrude laterally from the glans (Fig. 1*d*). Each rosette comprises a group of foliate papillae, usually four on the left (Fig. 1*d*) and three on the right, through which the terminal branches of the penile urethra pass (Temple-Smith 1973; Carrick and Hughes 1978) and the semen would flow during ejaculation.

Differences in the symmetry of the female reproductive system between the platypus and the echidna exactly match the differences in symmetry of the penis in males of these two species. In *Tachyglossus*, the symmetrical glans of the penis (Fig. 1*a*) complements the functional symmetry of the female reproductive system, whereas in the platypus, enlargement of the left side of the glans (Fig. 1*c*) aligns with the functional (left) side of the female reproductive tract (Fig. 1*b*) in the mating position. Three lines of evidence support our suggestion that insemination is intrauterine in monotremes. First, the poor representation of male accessory glands in monotremes suggests that ejaculate volume is likely to be small, a feature of some other species such as the camelids (see Skidmore *et al.* 2001), which are intrauterine inseminators. Although the cauda epididymidis appears to be more highly secretory in monotremes than in other mammals (Temple-Smith 1973; Carrick and Hughes 1978; Jones *et al.* 1992), it is unlikely to contribute significantly more volume to the semen. Second, the alignment of the glans adornments in the platypus and echidna complement the structure of the female tract and offer the potential for these adornments to enter or closely abut the cervical canal to deposit semen directly into the cervix and the uterus. Third, the position of the neck of the bladder adjacent to the cervices in female monotremes (Griffiths 1999; Fig. 1*b*) suggests that depositions of semen into the urogenital sinus are at risk of being flushed out during any act of micturition that occurs after insemination.

Gestation

Broome (1895) estimated the gestation period in *Tachyglossus* as approximately 27 days. However, three recent field studies have provided a shorter estimate of gestation length in *Tachyglossus*. Griffiths (1999) cited 21–23 days, and Beard and Grigg (2000) and Rismiller and McKelvey (2000) provided estimates of 20–23 days and 23 ± 1 day respectively. In the platypus, gestation length is still controversial. Griffiths (1999) suggests that minimum length was at least 9 days. However, a recent estimate of 15–21 days, from captive breeding of the platypus at Healesville Sanctuary (Holland and Jackson 2002), more closely approximates the recent estimates for short-beaked echidnas.

Whether monotremes show a condition similar to brooding behaviour has not been considered. The few females that have been studied in the period leading to successful breeding have shown stereotyped periods of nest-building and nest-attachment not unlike that seen in birds. Where detailed records of captive platypuses are available, there is evidence from some females during years when breeding has not been successful, that nest-building and early nesting behaviour are not readily distinguishable from those years when breeding has been successful (Fleay 1980; Holland and Jackson 2002). Attempts at breeding in captive platypuses have often resulted in nest-building and egg-laying, but failure of the eggs to develop (Fleay 1980). Whether these failures are the result of inadequate conditions in the nesting chamber for development and hatching or from infertile or unfertilized eggs needs further examination.

Incubation, pouch and incubation burrow

All extant monotremes construct burrows for protection of the female and young during the breeding period (Burrell 1927; Griffiths 1978; Rismiller 1992; Beard and Grigg 2000). In the platypus, this usually consists of a burrow, which can be more than 8 m in length (Burrell 1927; Temple-Smith 1973), terminating in a spherical nesting chamber (Burrell 1927; Temple-Smith 1973; Fleay 1980). Before egg-laying, as confirmed in the wild and in captivity, the female drags large amounts of wet vegetation into the incubation chamber. This vegetation is thought to provide, in the absence of a pouch, a level of humidity required to prevent desiccation of the eggs and the neonates during incubation and the early postnatal period (Burrell 1927; Grant 1995). In short-beaked echidnas, the female develops a pouch or incubatorium before egg-laying into which the egg is laid directly from the protruded end of the cloaca. Development of a pouch has not been confirmed in *Zaglossus bruijni*, but Griffiths (1978) obtained circumstantial evidence from information volunteered by 'villagers, living in the mountains of Papua… that the female carries its young in a pouch'. There is no evidence that echidnas gather vegetation to line and humidify their incubation burrows, in fact in many areas of its range in Australia it would be difficult, if not impossible, for short-beaked echidnas to find suitable vegetation. The humidity developed inside the temporary pouch of the echidna, augmented perhaps by the natural humidity of the burrow in which the female remains from late gestation until the first few days following hatching (Beard and Grigg 2000), protects the egg and neonate from desiccation during the incubation period. In *Tachyglossus*, the young is carried in the pouch for 45 to 50 days and then deposited into a plugged nursery burrow where it remains until it is weaned at about five and a half months of age (Beard and Grigg 2000). In an evolutionary sense, comparing the semi-aquatic and terrestrial lifestyles of the platypus and echidnas, the development of a temporary echidna-like pouch to carry up to three pouch young would seem incompatible with the semi-aquatic lifestyle of the platypus. What is not clear is whether a pouch is an acquired structure in the evolution of echidnas or if this is a plesiomorphic character in the monotreme lineage, which has been lost in the platypus and perhaps other ancestral, but now extinct, semi-aquatic ornithorhynchids such as *Obduradon dicksonii*.

Incubation and suckling frequency

Beard and Grigg (2000) have shown that female echidnas in south-eastern Queensland spent two to three weeks in a plugged 'incubation' burrow, maintaining a normal and stable body temperature from the last few days of gestation, through incubation and including the first few days after hatching. The single young was carried in the female's pouch for 45–50 days, and reached a weight of approximately 200 g before being transferred to a different plugged 'nursery' burrow. During the early stages of lactation, the female visited regularly, every six days at first, gradually increasing in frequency to about every four days before the visits ceased and, presumably, the newly independent young emerged at a calculated five and a half months of age.

The only information on incubation and suckling frequency in the platypus has been obtained from recent observations on captive breeding (Holland and Jackson 2002). These observations indicate that, although food intake rises to a maximum in late lactation indicating the high-energy requirements of lactation, the duration of daily visits to the nesting burrow by the female platypus declined in the later stages of lactation.

Seasonal breeding

Little is known about seasonal breeding activity of the long-beaked echidna in New Guinea. The only data available that provide any indication of seasonality are from six testicular samples examined by Griffith (1978). These showed changes in testicular activity that suggested that males entered breeding condition in late June, reached a peak of reproductive activity in July and showed significant signs, from a single sample, of testicular regression in October. Reproductive tracts and, in particular, follicular development of two female *Zaglossus* taken in July and October appeared to support seasonal breeding in this species (Griffiths 1999).

There is clear evidence of reproductive seasonality in the platypus and *Tachyglossus*. In the platypus, there is a north–south cline in the start of the breeding season. Breeding appears to be earlier in Queensland and New South Wales and later in the southern limits of their distribution in Victoria and Tasmania (Temple-Smith 1973; Griffiths 1978; Handasyde *et al.* 1992; Grant 1995; Jabukowski *et al.* 1998; New *et al.* 1998; Bethge 2001). However, the difference in the start of the breeding season appears to be only about two months across this cline and the evidence for Queensland is at present rather anecdotal. At Eungella in southern Queensland, mating has been observed during late July and August in consecutive seasons (D. Parer, personal communication). This is about a month earlier than the peak breeding season on the Barnard, Shoalhaven and Murrumbidgee Rivers in New South Wales (Temple-Smith 1973; Griffiths 1978; Jabukoski *et al.* 1998; New *et al.* 1998). However, captive platypuses at Burleigh Heads in southern Queensland showed mating and nest-building activities that were not always different from similar breeding events for captive platypuses in Victoria (Fleay 1980). Whether this is an effect of captive conditions, and in particular access to regular sources of food during winter before the breeding season, remains to be explored.

In males, the breeding season is marked by significant increases in testicular and accessory gland weights (Temple-Smith 1973; Griffiths 1978), and in the weights of other androgen-dependent glands such as the cervical scent glands and the crural (venom) gland (Temple-Smith 1973). Large increases in plasma and faecal androgens (Handasyde *et al.* 1992; New *et al.* 1998) and plasma cortisol (New *et al.* 1998) before and during the breeding season are associated with increased aggressive behaviour in males (Temple-Smith 1973). Large increases in plasma and faecal progesterone have also been observed in female platypuses (Jabukowski *et al.* 1998) during the breeding season. These coincide with significant changes in the mass of the functional left ovary and in both uteri (Temple-Smith 1973). Although seasonal changes are observed in both uteri, the functional left uterus becomes considerably larger during the breeding season than its non-functional counterpart (Temple-Smith 1973). These data suggest two immediate possibilities, or a combination of both. The first possibility is that the cells and tissues of the right uterus are not able to respond to the seasonal stimulatory affects of steroid hormones in the same way as the left uterus. Second, the right uterus may have developed a smaller and more restricted blood supply than the left, which results in a reduced exposure of the right uterus to steroid hormones in the peripheral circulation. A third possibility is that there is a significant direct effect on the left uterus from hormones secreted by the Graffian follicles and corpora lutea of the seasonally active ipsilateral ovary.

In the short-beaked echidna, breeding occurs mostly during the period from late winter to late spring (late June to mid-September). The timing of the breeding season appears to be remarkably consistent in all regions where seasonal breeding data has been collected (Griffiths 1968 1978; Beard *et al.* 1992; Rismiller and McKelvey 1996; Beard and Grigg 2000). This applies even in echidna populations that have been studied in southern Tasmania and the Snowy Mountains of New South Wales where echidna populations live above the winter snowline (Beard *et al.* 1992). In the Snowy Mountains, the echidnas arouse from hibernation during the coldest part of the year to mate. In contrast to short-beaked echidnas in other parts of Australia (see Rismiller 1992; Rismiller and McKelvey 1996, 2000), male Snowy Mountains echidnas have never been observed to form up into mating trains behind oestrous females as a prelude to mating (Beard *et al.* 1992). This suggests that a different mechanism for mate selection may be operating in this population, since studies of echidna mating trains on Kangaroo Island have proposed that mating trains enable larger males to gain access to oestrous females and exclude smaller males from mating (Rismiller 1993, 1999). Since only one male in the mating train has been observed to mate with the female, there is no possibility of sperm competition in this species. This suggestion also fits with the observation that there is no obvious sexual dimorphism between male and female echidnas (Griffiths 1978). However, considering the high rate of extra-pair matings in many species of birds and mammals, the progeny of field-matings need to be DNA-tested to confirm the observed paternity.

A recent radiotelemetry study of echidnas in southeastern Queensland (Beard and Grigg 2000) found that, as in other study sites in Australia, mating occurred mostly in July and August. Echidnas in this region had the ability to conceive successfully a second time within the one season, in response to the loss of the first young and may also be able to attempt breeding each year. Unlike the platypus, the evidence for seasonal reproductive activity in female echidnas is derived from observations of mating and the incidence of females with eggs and pouch young. No detailed study has yet been made of seasonal changes in the components of the female reproductive system (Griffiths 1999). Similarly, there is as yet no published study of seasonal changes in androgens, oestrogens or progesterone in echidnas. However, there is a noticeable seasonal change in mass and spermatogenic activity of the testis in echidnas, with the testes large and actively producing spermatozoa in late winter through to early spring and small, regressed and quiescent from late spring through to early autumn (Griffiths 1978). These observations suggest that, as in the platypus, significant seasonal changes in androgens and the mass of secondary sex glands also occur in sexually active male short-beaked echidnas.

Frequency of breeding

As with most aspects of their reproduction, nothing is known about the breeding frequency and success of individual female long-beaked echidnas in New Guinea. In the platypus and short-beaked echidna, however, there is now convincing evidence to show that few adult females breed successfully in consecutive seasons. There is also considerable variation between females in their time of first breeding and their breeding success following the transition from sub-adult status to sexually mature adults.

Rismiller and McKelvey (2000) recorded the breeding frequency of wild female short-beaked echidnas (*Tachyglossus aculeatus multiaculeatus*), the fate of their offspring, and the recruitment of sub-adults into a population over a 7-year period on Kangaroo Island, off the coast of southern Australia. They reported that females mated only once with a single male from a mating train of males in each breeding season and all females that were observed to have mated produced a single fertile egg about 23 days later. The proportion of females from the population that bred each year exceeded 50% only once during the study and was usually between 10 and 30%. Breeding success differed between individuals and years. Seventeen different females produced 22 hatchlings between 1990 and 1996, with the total number of young produced each season varying from 1 to 9. Of the 17 young produced, only 8 were known to have successfully weaned and the new sub-adults found in the study site generally matched the number of young known to have been produced and survived each year.

Grant *et al.* (1983) also provided evidence that female platypuses do not breed each year in the wild. They showed that only 64% of females captured in the breeding season over a number of years were lactating and that lactating females were most numerous in December, about one-third to a half of the way through the breeding season in that region. A lower incidence of females lactating later in the breeding season suggests that some females might lay and hatch eggs, but fail to raise the nestlings successfully. This hypothesis has not yet been tested. In a longer-term field study in the upper reaches of the Shoalhaven River in New South Wales on the breeding frequency of female platypus (T. Grant and P. Temple-Smith, unpublished), similar observations and results were obtained to those of Rismiller and McKelvey (2000) in *Tachyglossus*.

As part of their study, Rismiller and McKelvey (2000) monitored for the first time the breeding success of individual female echidnas over the 7-year period. Their observations clearly show that, although possible, it is uncommon for sexually mature female echidnas to breed in successive years (Fig. 2). Some females bred for two years in succession, but most showed gaps of more than two years between successful breeding. A similar observation has recently been made for individual female platypuses in the wild (T. Grant and P. Temple-Smith, unpublished observations) and also for Koorina, the female platypus that has now bred twice in captivity at Healesville Sanctuary (Holland and Jackson 2002) (Fig. 3).

Captive breeding

All three genera of montremes have been successfully kept in captivity. Both species of echidnas have been known to survive under captive conditions for periods exceeding 30 years, which for short-beaked echidnas compares favourably with the sparse records of their longevity in the wild (more than 45 years) (Griffiths 1968; Rismiller 1999). Twenty-two years is the longest recorded captive lifespan for the platypus, and platypuses in the wild are known to live at least 16 years for females and 8 years for males (T. Grant and P. Temple-Smith, unpublished data). However, although all three monotreme species have been maintained successfully in captivity by various zoos and wildlife parks, captive breeding has been successful only in the platypus and *Tachyglossus* (Fleay 1944, 1980; Dobroruka 1960; Augee and Gooden 1995; Olney and Fisken 1997; Holland and Jackson 2002).

Captive breeding of echidnas has not provided much additional information about reproduction in this species than is now know from studies in the wild. This is partly because breeding echidnas in captivity has been haphazard, with keeping staff often only realizing that breeding has occurred with the serendipitous finding of an egg or pouch young in the incubatorium or by the observation of a dead pouch young in the enclosure (Olney and Fisken 1997). The husbandry requirements for successfully breeding echidnas in captivity are still unknown.

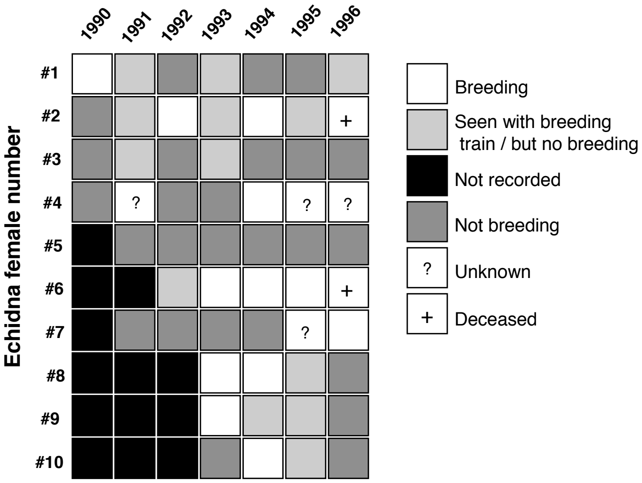

Fig. 2. Annual breeding success of 10 adult female short-beaked echidnas over a period of 7 years. Only two of the females were known to have bred in consecutive years. Data from Rismiller and McKelvey (2000).

Captive breeding in the platypus has also been rare, with young being successfully raised to weaning only three times in captivity (Fleay 1944, 1980; Holland and Jackson 2002; L. Fisk, personal communication), all at Healesville Sanctuary in the state of Victoria. However, because of difficulties in collecting accurate and useful field data on platypus breeding, these events and associated observations on captive breeding behaviour of pairs that did not breed (Hawkins and Fanning 1992) have contributed significantly to our understanding of many aspects of reproduction and development in this species. The contributions from studies of captive platypuses include mating behaviour sequences, more accurate estimations of gestation length and the incubation period, observations on the collection and use of nesting material and time required for nest-building, food requirements during lactation, growth and development of nestlings and swimming and feeding behaviour of juveniles following emergence from the breeding burrow.

Monitoring of food intake by the female platypus Koorina, which bred in 1998/1999 at Healesville Sanctuary (Holland and Jackson 2002) showed a slow initial rate of feeding after egg-laying, which then sharply rose 20 days later (Fig. 4). Around the time of emergence of the two nestlings from the natal burrow, food consumption by the female had reached a peak that correlated closely with the high energy demands of late lactation. At this time, about 140 days after the eggs are thought to have hatched, maternal daily food intake had reach approximately 900 g (90–100% of bodyweight). Typical food consumption by

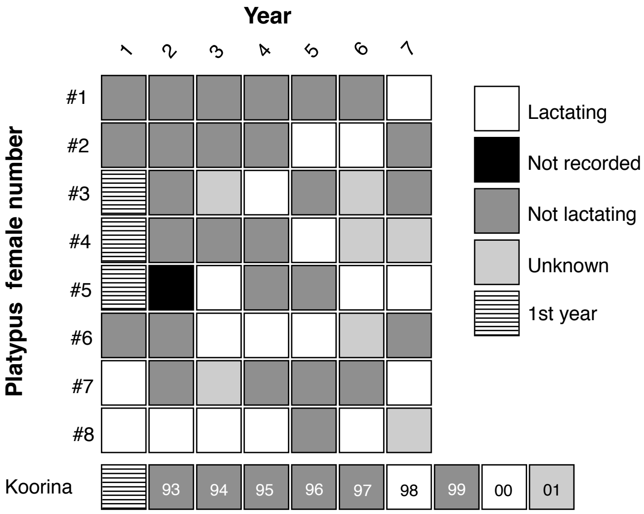

Fig. 3. Incidence of lactation in 8 adult female platypuses during the breeding season in the upper Shoalhaven River over periods of up to seven years (T. Grant and P. Temple-Smith, unpublished observations). With the exception of number 8, only females numbers 2, 5 and 6 were known to have lactated in successive years. The remaining females showed intervals of between 1 and 6 years between lactation. Lactation was tested for by an intramuscular injection of oxytocin. The captive breeding record of Koorina (Healesville Sanctuary) is also shown.

Koorina at the corresponding time of year in a year when she was not breeding was approximately 200–300 g per night or 20–30% of her bodyweight (1000 g). Similar observations have been made on captive platypuses (15–28% of bodyweight per night; Krueger *et al.* 1992) and for wild platypuses by Munks *et al.* (2000) who estimated that platypuses consume 19%–28% of their body mass in food per night. Bethge (2001) has recently suggested a similar estimate for captive non- breeding platypuses.

These daily food intakes require further investigation for wild lactating female platypuses. The notion that for a few weeks, at the least, during late lactation each female platypus requires a daily intake of about 1 kg of benthic organisms seems difficult to accept, especially in some river sections with resident populations of 6–8 platypuses. If three of the female platypuses are in late lactation, a resident population of 8 animals would be expected to need about 4.5 kg of benthic organism each day or 31.5 kg per week. However, perhaps under field conditions, platypuses both lactating and non-lactating may be able to survive on less than predicted from the captive animals. This hypothesis warrants a much more detailed study of the field condition and dietary intake requirements of lactating and non-lactating platypuses in relation to the distribution, density and availability of benthic organisms. Competition for food by wild platypuses and the inability of some breeding females to consistently obtain the large quantities of benthic organisms needed to maintain lactation, especially in mid- to late lactation, may be an important factor regulating the successful reproduction of individual female platypuses in the wild. Indeed access to suitable food resources may influence the individual breeding success of wild females each year, and may regulate the numbers of wild females that are able to breed successfully in particular riverine and lake habitats. However, captive studies clearly show that access to food is not the only factor controlling reproductive success. Captive females with access to high-quality food resources are still unable to breed successfully year after year.

Knowledge about changes in food intake has been used in two subsequent breeding seasons by keeping staff at Healesville Sanctuary as an indicator of breeding success. In 1999/2000, Koorina, the female that had bred in 1998/1999, did not show an increase in food intake after she had shown nest-building and early nesting behaviour similar to that in the 1998/1999 season, and she was subsequently shown not to have bred. However during the 2000/2001 breeding season, changes in the female's behaviour and food intake, which were similar to those in the 1998/1999 breeding period, suggested that the female had successfully bred again. This was subsequently confirmed by the emergence of a healthy young male about 140 days later in April 2001.

Observations from captive breeding have challenged long-held dogmas about the breeding biology and behaviour of platypuses. It had been previously concluded from inadequate field data that females remained in the nesting chamber from just before egg-laying until the neonates were

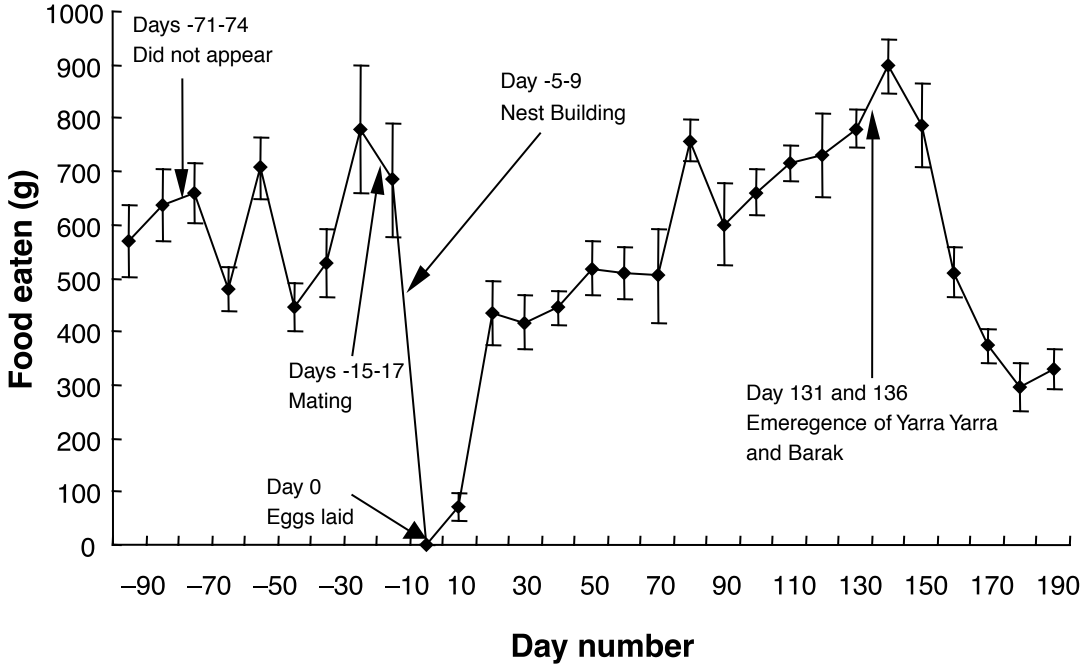

Fig. 4. Average food consumption of breeding female during the 1998/1999 breeding season. Standard error bars are shown. Before Day –4 the food consumption is for the male and female, both of which had access during this time the same feeding area. The values used are based on the average consumption over 10-day intervals (with permission, Holland and Jackson 2002).

old enough to suckle (Burrell 1927). It seemed inconceivable that once incubation had commenced, and before hatching, that the female, as sole carer of the progeny, would be able to leave the eggs (Griffiths 1978). Fleay's observations in 1943, however, suggested that about six days into the incubation period the female exited the nesting burrow and return to the water for a brief time before continuing the incubation. This observation has been confirmed from observations made during the recent captive breeding success at Healesville Sanctuary in which the female left the nest on Days 5, 7 and 10 for about 30 min (Holland and Jackson 2002). Questions that remain are how and where does the female deposit the eggs or early neonates during these early excursions from the natal burrow and how, when she returns, does she reposition the eggs or neonates to continue brooding them? These questions will only be answered with the successful observation of breeding females with nestlings inside the nesting chamber.

Conclusion

Because of their interesting evolutionary relationships, their unique features and their fascinating biology, monotremes deserve greater attention than they have received in the past. Although they have been known to science for more than 200 years, there are fundamental aspects of monotreme reproductive biology and indeed many other features of their general biology that still await detailed study. This perhaps reflects the infrequent breeding success of all monotremes in captivity and, for the platypus and *Zaglossus* in particular, the difficult nature of collecting meaningful data from field studies. However, the development of novel molecular techniques using DNA are beginning to provide new ways of examining population genetics and parentage in monotremes, especially the platypus (Gemmell *et al.* 1992, 1995). New generations of field equipment to log movements and activities and enable more accurate location of individuals and their movements will also greatly contribute to our understanding of the biology of this important group of mammals.

The Short adage 'we cannot conserve until we comprehend' (Short 1985) should be an inspiration for institutions and funding bodies to continue to accept that field studies are an essential activity for studying the biology of monotremes. Interpretations of new molecular directions for science, especially in relation to conservation, are still dependent on accurate and current knowledge of species and their habitats. Zoos also need to recognize and affirm their commitment to a significant role in species research both *ex situ* and *in situ.* For example, the past and recent observations at Healesville Sanctuary of captive breeding events of the platypus demonstrate the value of a scientific approach. Knowledge from the two recent platypus breeding events serve to underscore the important contribution that zoos and wildlife organizations can make to the understanding and interpretation of life history events of species that are extremely difficult to witness in their natural environments.

The lucrative potential export market for platypuses provides commercial incentives for breeding platypus in captivity by some private wildlife enterprises, as well as some zoos. More certainty in captive breeding of monotremes is an important issue for those enterprises wishing to trade in some Australian native species, including the platypus, since current government requirements limit the export of these species to individuals that are at least second generation bred in captivity. This requirement brings into focus the question of what is captive breeding and how much husbandry and management of a platypus population is required to satisfy government regulations. This debate remains to be had, but pressure to clarify what is captive breeding and what are captive-bred platypuses will grow with the increase in desire by organizations to add significantly to their income from trading in platypuses. That platypuses have already been successfully transported for display to the US at least three times in the past, using wild individuals brought into captivity (see Fleay 1980), will only increase the pressure on government to clarify these requirements.

Finally, a recent revision of the genus *Zaglossus* has described three contemporary species, including *Zaglossus bruijnii*, and five subspecies of *Zaglossus bruijnii* (Flannery and Groves 1998). This analysis of mostly museum specimens and collection data urgently needs confirmation from field studies of *Zaglossus* across its distribution. Also, the development and implementation of a conservation action plan for the genus *Zaglossus*, which contains the only endangered monotremes, is now an even higher priority.

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The measure of Roger Short's talent is not just found in his writings, brilliant as these are, but especially in his presentations and conversations, which are universally appealing. He has the gift of being able to transcend age and intellect and deliver powerful messages equally to his peers and the public alike with disarming simplicity. Always challenging, motivating and fascinating, his presentations usually have a bold link with the unexpected and an unforgettable line or compelling aphorism or adage (e.g. 'We cannot conserve unless we comprehend' or 'Big balls, bad boys'). Roger you have enticed many by your enthusiasm and oratory to your changing fields of interest and set them on courses to new and exciting careers. You are a great mentor and a generous distributor of information and ideas. Congratulations on a stunning career so far—*quo vadis*? *Peter Temple-Smith*.

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