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Seasonality and Breeding Success of Captive and Wild Tasmanian Devils (*Sarcophilus harrisii*)

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Abstract

The synchrony and timing of reproductive events are crucially important factors to maximize individual and offspring survival, especially in seasonal environments. To increase our understanding of the physiological basis of seasonality and the influence of associated environmental factors (maximum temperature, day length and rate of day length change associated with different latitudes) on reproduction in Tasmanian devils, we reviewed records and research data from captive facilities throughout Australia in comparison to those from a wild population study (1974 to 1987). Overall, breeding activity began 2 weeks earlier in the captive than the wild population (week 5.7 ± 0.6 versus week 7.7 ± 0.5 for devils entering into estrus during the first two week phase; n = 24 and n = 23 respectively). If the timing of reproductive activity is considered against absolute day length rather than date, both the captive and wild populations displayed similar distributions (12. 9 ± 0.7 hr versus 13. 0 ± 0.7 hr respectively; $P < 0.01$) confirming day length as a proximal cue involved in eliciting a physiological response to trigger seasonal reproductive activity regardless of location. Wild devils had a higher breeding success (75%; n = 169 versus 43%; n = 115) and larger litter size (3.4 ± 0.9 versus 2.8 ± 1.1 joeys per litter) than captive devils ($P < 0.05$). Mean maximum temperature at the onset of reproductive activity ($P < 0.05$) was higher for the captive than the wild population (28.1 ± 4.0 ^{o}C versus 22.3 ± 2.7 ^{o}C respectively). The drivers for reproductive success in captive Tasmanian devils are likely multifactorial, but our results suggest that elevated temperatures associated with shifts in breeding activity and geographical location should be examined further.

Key Words: Tasmanian devil, Marsupial, Captive Breeding, Seasonality, Day Length, Reproduction

1. Introduction

The Tasmanian devil (*Sarcophilus harrisii*) is a dasyurid (carnivorous marsupial) that is endemic to the island state of Tasmania, off the south-east corner of Australia. It is nocturnal and primarily a scavenger, the largest (6 to 10 kg) of the current extant species classified as dasyurid [1, 2]. They are considered to be solitary, only congregating to communally feed on large carcasses or to breed [1]. Although considered historically abundant during the middle and late 20th century, the devil has suffered a significant population decline over the last decade due to the fatal, transmissible facial cancer, commonly known as Devil Facial Tumor Disease (DFTD)[3, 4]. DFTD is transmitted through contact, specifically biting during agnostic encounters over carcass feeding or during mating [1, 5]. The devil lacks an appropriate immune response to the foreign cells and the development of predominantly facial tumors ensues followed by metastases or starvation through the loss of tissue, teeth and bone mass in the nasal-oral region, preventing food consumption [6-9]. Currently it is estimated that more than 80% of the natural population has disappeared due to DFTD (http://www.tassiedevil.com.au) and as a result the Tasmanian devils is currently classified as endangered by the IUCN [10].

An Insurance Population of Tasmanian devils was initiated with the first intake of 30 wild, cancer-free juvenile devils (11 males, 19 females) in early 2005. The current Insurance Population is more than ten times that size and includes breeding efforts of intensively managed captive breeding at more than a dozen mainland Australian and Tasmanian facilities and at free-range enclosures on mainland Australia, Tasmania and on Maria Island off the east coast of Tasmania (www.tassiedevil.com.au). Devils have been held and bred in captivity for over a century, yet captive breeding rates of the first 6 years of the Insurance Population (2007-2012) averaged only 38% (per communication, Australian Zoo and Aquarium Association), with the cause of reproductive failure of most of the unsuccessful pairings unknown [11, 12]. Despite low overall captive breeding success, the Insurance Population continues to increase as a result of captive breeding, increased number of wild founders and the dedication and hard work of animal care staff, researchers, volunteers and veterinarians across Australia under the direction of the Australian Zoo and Aquarium Association (ZAA) and the Department of Primary Industries, Water and the Environment Tasmania (DPIPWE).

The lifetime reproductive potential of the female Tasmanian devil is limited due to a combination of seasonal breeding and reproductive senescence typically by the age of five. Initially believed to be monestrous, we now know that the female devil is facultative polyestrous [12, 13]. Within a breeding season, a female devil has the potential of undergoing up to three estrous cycles, at intervals of approximately two months, if conception is not achieved or if pouch young are lost at or shortly after birth [12]. Although female devils have been recorded as breeding successfully as young as 1 year of age, this is limited to a small number of the devils that exceed the minimum body weight threshold that is likely needed to become a precocial breeder [14]. Generally female devils breed between the ages of 2 to 4 years, which equates to the production of a maximum of 3 litters per lifetime (although rare, females have been recorded to exceed this and produce a fourth litter in their fifth year of life). Despite giving birth to supernumerary young, a maximum of only four joeys can survive due to the presence of only four teats in the pouch [15].

Although we know the devil is a seasonal breeder, we know little about the physiological mechanisms that drive this process in this species. Length of day or “photoperiod” is the primary factor involved in regulating the timing of reproduction in most seasonal species. Melatonin is a indole amine hormone produced by the pineal gland during the dark phase of the day with variations in production associated with day length [16]. Melatonin plays a role in regulating the production of GnRH and the processing of thyroid hormones in the hypothalamus, a part of the primary pathway of the neuroendocrine control of reproduction (for a more information and a full review, please refer to

[16]). Changes in melatonin production is the primary trigger for seasonal breeders, synchronizing reproductive activity within the population as well as optimizing the timing of offspring births. Overall, gestation and lactation duration and the timing of the optimal period of food resources are related to the timing of reproductive activity for seasonal breeders and dictates the physiological response to either an increase or decrease in melatonin production beyond a critical threshold. A short-day breeder is defined as a species which becomes reproductively active as the days are getting shorter (autumn) and are in anestrus in spring and summer. A long-day breeder is defined as a species which becomes reproductively active as the days are getting longer (spring, summer) and are typically in anestrus in winter.

Mammals use environmental signals to time reproduction to take advantage of the most favorable time of year to maximize individual fitness and offspring survival. For many species, this means timing births to occur in warm seasons and when energy resources are abundant. As marsupials have short gestations and give birth to altricial, embryonic young, the optimal timing for breeding has slightly different parameters. For marsupials, it is the timing of late lactation and weaning that must coincide with optimal resource abundance as this represents both the period of greatest maternal investment and access to the necessary energy requirements for offspring survival. Photoperiod and the rate of day length change have been confirmed to be key cues for timing seasonal reproduction in marsupials [17-19]. The Tasmanian devil is classified as a short-day breeder with the onset of estrous cyclicity associated with late Austral summer and autumn [12, 13] but the specific physiology and factors associated with the timing of reproductive activity have yet to be examined.

For many wildlife species, captive breeding programs are global. This means that animals are often expected to cycle and breed not only in artificial, captive conditions but in environments that often differ in latitude, longitude and altitude to their native habitat. This often means differences in absolute day length, seasonal amplitudes of day length, temperature and humidity. For example, Tasmanian devils naturally occur in the island state of Tasmania which is cooler and more southerly than any other Australian state. In the wild seasonal breeding facilitates births and weaning to occur at the optimal time for food abundance, environmental conditions and ultimately offspring survival. As food resources are rarely a factor limiting or controlling the timing of reproduction in a captive setting, endogenous circannual rhythms and photoperiod could presumably be the primary drivers of seasonality in captive animals. Despite this, very few studies have compared captive to wild populations of seasonally breeding species occurring at different latitudes to evaluate potential environmental factors influencing seasonality and breeding success in captivity. As such, there is limited information available on the relationship of altered day length, rate of change in day length, and temperature to the timing and success of estrous cycles and mating in captive environments outside of native habitats [20-23]. This review aims to examine these factors by using data gathered during previous wild and captive population research studies to gain a better understanding of seasonality and environmental factors affecting reproduction in female Tasmanian devils.

2. Methodology

The data for this project has been collated from historic breeding season records in addition to retrospective data from completed or unpublished research studies on captive and wild Tasmanian devils and evaluated against location specific environmental parameters.

2.1 Captive Breeding Population Data

Captive breeding records for healthy, adult female devils housed at 9 mainland Australia institutions were collated to represent a range of geographical locations (Table 1) over a 6 year timeframe (2007 to 2012). Captive devils included both captive born and wild caught devils and were managed and given breeding opportunities through the recommendations provided by the Australian Zoo and Aquarium Association to maximize genetic diversity as part of the Insurance Population. Records for one year old female devils were only included if they successful produced young or displayed typical signs of estrus and were observed to be mated (n = 3). Otherwise, female devils were between 2 and 4 years of age (n = 76 individuals) and total records (n = 115) included 1 to 3 years of data for each female. Data from captive female devils was sourced from a previous research study [12], supplemented by records from an unpublished research study (of co-authors T. Keeley and T. Russell) or records from individual institutions to increase study animal numbers where possible. Not all records from every year, for every institution were available for this study. Data from captive female devils included; devil's name and studbook number, year of birth, body weight (where available), institutional location, dates of the beginning of the estrous cycle (determined by signs of behavioral estrus which was followed by attempted mating or associated changes in fecal progesterone concentrations), and production of young (0 to 4). As the date of the beginning of estrus for each female may be subjected to human error, data for any estrous cycles which were not considered reliable due to a lack of fecal hormone data, missing records, or inconsistencies in relation to the known parameters of devil reproductive biology [12] were excluded from the study.

2.2 Wild Population Data

Data from a 14 year study (1974 to 1987) by co-author Dr. Leon Hughes on wild Tasmanian devils trapped in north-eastern Tasmania, in the Avoca district, were used to compare natural breeding dynamics to those of captive breeding facilities [24]. Data from healthy, wild Tasmanian devils included; body weight, reproductive status (lactating, pregnant, non-pregnant, pre-ovulatory, post-ovulatory, or pubescent), pouch size (small, functionless and undeveloped or large, functional and active), trapping date and pouch young size (head length, body weight). Female devils under 4 kg body weight with small, functionless undeveloped pouches were excluded from consideration as these were deemed to be immature devils (approximately 1 year of age). Devils between the weights of 4 to 4.9 kg were included only if they had enlarged pouches, were lactating or confirmed pregnant. All devils of a body weight of 5 kg and above were estimated to be sexually mature and were included regardless of reproductive status and pouch size. At the time of the study, methods to accurately age a devil using tooth eruption and wear had not yet been established [2] and therefore a combination of body weight and pouch development was used to distinguish immature from mature female devils.

The primary aim of the wild population study at the time was to evaluate gestation and embryonic development in the Tasmanian devil [15]. As such, trapping occurred primarily between late February and mid-May [24]. Any live trapped female devil that had an enlarged, functional pouch and body weight greater than 4 kg and was not in early lactation with viable pouch young was subjected to a laparotomy to determine reproductive status. If she was confirmed to be pre-ovulatory, post-ovulatory, pregnant, recently post-partum, or if lactating with small pouch young then the date of birth was estimated using an embryonic development or pouch young growth rate timetable [15, 24, 25]. From this, the beginning of the estrous cycle, the initiation of estrus, was estimated to be an average of 26 days prior to birth based on previous research on the length of the estrous cycle of the Tasmanian devil [12]. For both wild and captive devils, the day on which estrus started was designated a week number of the year for evaluation. Due to the method of evaluating the dates of estrus of captive and wild devils, it is hoped that evaluating this on a week basis should provide the data with an allowance of a few days either side of the specific calendar date to help reduce error due to biases from collection and calculation data.

Birth peak breadth (BPB) [20] was calculated to determine the shortest time frame in which that majority of the first estrous cycles began for the captive and wild population. We used a BPB of 80%, determined to be the shortest number of weeks in which 80% of all estrous cycles began for each locality.

2.3 Seasonal Fluctuations in Temperature and Daylight

Sunset and sunrise times and daily maximum temperatures were acquired for each study location (or the closest weather station with available data) for each year as needed from Geoscience Australia (http://www.ga.gov.au) and the Australian Bureau of Meteorology (http://www.bom.gov.au). Sunset and sunrise times were used to calculate individual day lengths and then rate of change of day length (or rate of change in photoperiod) was calculated by subtracting each successive calculation for the day length from the preceding day length to provide a value representing the difference in photoperiod from one day to the next. All values for temperature, day length and rate of day length change were averaged for each week of the breeding season (1 January to 30 June) to reduce bias associated with extreme daily temperature fluctuations. As day length did not vary between years for each location, a single year's information was used for all animals at each location. All other values were year and location dependent.

2.4 Statistics

All statistical analyses were performed using GenStat 16th Edition (VSN International Ltd., UK). A general linear model was used to test the effects of day length, rate of day length change, temperature, body weight class (3.0 to 4.9 kg versus 5 kg +), age and location on the timing (week) of the onset of estrus. Data from female devils across multiple years were assumed to be independent. As the timing of the onset of estrus may have an impact on reproductive success, we looked for a relationship between the litter size and the timing of estrus. Logistic regressions were performed using the following variables: location (captive versus wild), litter size (1, 2, 3, or 4 pouch young), week of estrus, and average weekly maximum temperature. $P < 0.05$ was considered to be significant. Data are presented as ± SD, unless otherwise noted.

3. Results

3.1 Wild Population of Tasmanian Devils

The records from the wild population study provided information for 169 adult female devils (n = 166 individuals; n = 3 sampled in two separate years)[24]. Additional records from female devils with a body weight under 4.9 kg with a small, under-developed, functionless pouch were considered pre-pubertal and not included in this study. A total of 41 females between 4 to 4.9 kg were found to be reproductively active (pregnant or lactating) confirming sexual maturity and therefore were included for analysis in this study.

Of the 169 individual records, 42 (25%) female devils were not pregnant nor had small pouch young. Of these females, seven were confirmed pre-ovulatory, within a week of ovulation and therefore were included for evaluation. Of the remaining 35, six were noted to have cystic uteri or cystic ovaries or a combination of both and one was confirmed to be at least 5 years of age (due to a capture record 4 years prior) and was therefore likely post-reproductive. Another 17 females showed signs of latent lactation suggesting the females were in the final stages of weaning young from the previous year and therefore had yet to enter into estrus. The remaining non-reproductive females (n = 12) had no indications to the possible cause of the lack of pregnancy or lactation. As the timing of estrus could not be calculated for females that were not pregnant nor carried small pouch young, these devils were not included in any further evaluations.

Of the 127 reproductively active, wild female devils, 83 were in early lactation with 1 to 4 pouch young and 44 were confirmed pregnant or within three days post-parturition through laparoscopic evaluation and evaluation during short-term captive retention.

3.2 Litter Size

Of the records from captive female devils, 41 of 115 (36%) successfully produced pouch young (2.9 ± 1.0 joeys per litter) during their first estrous cycle. Of the remaining 74 females, 62 (84%) were confirmed to have a second estrous cycle, of those 14 females (23%) were confirmed to have a third estrous cycle. A total of 6 females (6 of 62; 10% of 2nd estrous cycles or 6 of 74; 8% of females unsuccessful on the 1st estrous cycle) successfully produced pouch young on the second estrous cycle and 2 females (2 of 14; 14% of 3rd estrous cycles or 2 of 74; 3% females unsuccessful on previous cycles) produced pouch young on the third estrous cycle. For all litters produced by captive female devils regardless of estrous cycle number (n = 49 of 115; 43%), litter size was 2.8 ± 1.1 joeys per litter. As most of these litters (61%; 30 of 49) were produced by 2 year old females, the effect of age on litter size could not be determined. Of the 83 wild females observed with pouch young, litter size (3.4 ± 0.9 joeys per litter) was overall larger than those of captive female devils ($P < 0.05$). Wild devils had a higher percentage of litters with the maximum number of joeys (4) than captive females and a lower percentage of litters with the minimum number of joeys (Fig. 1; $P < 0.05$). Although only a limited number of single pouch young litters (n = 14) were present within the total dataset, these females tended to have a larger body weight (mean 6.0 kg) than those with litters of three or four pouch young (mean 5.2 kg)($P < 0.05$) but were similar to those with two pouch young (mean 5.6 kg)($P > 0.05$).

3.3 Timing of Seasonal Initiation of Breeding Activity

Body weight class (3 to 4.9 kg versus 5 kg +) in wild devils had no relationship to the timing of the breeding activity but in captive devils, smaller females came into their first estrus later than larger females (10.0 ± 3.6 versus 8.0 ± 1.9 weeks respectively; $P < 0.05$). For captive females (as age data was not available for wild devils), body size was linked to age as smaller females tended to be younger females (2 years of age). No weight data was available for the 1 year old devils but these three devils all came into estrus late in the season (week 14 to 19).

3.4 Environmental Conditions Associated with the Initiation of Breeding Activity

Day length and rate of day length change varied with geographical location (Table 2). The earliest date for the initiation of the first estrous cycle in a captive female devil was the 23 January (2010), with a total of 8 females entering into estrus during the last quarter of January (24 to 31 January) and another 15 females during the first quarter of February (1 to 7 February). This included females from all localities with a range of ages 2 to 4 years. Breeding success of this first wave of females for this estrous cycle was 29% (7/24). Conversely, the earliest date for the initiation of the first estrous cycle in a wild female devil was 4 February (1981), the only female coming into estrus during the first quarter of February. Four wild female devils entered into estrus during the second quarter of February (8 to 14 February) and another 18 during the third quarter of February (15 to 21 February). Overall, reproductive activity was initiated 2 weeks earlier (Table 2) in captive than wild devils with the first two week phase of devils entering into estrus during week 5.7 ± 0.6 or week 7.7 ± 0.5 for captive and wild devils respectively ($P < 0.05$) but the day length at which this occurred was the same (13.7 ± 0.2 hr and 13.8 ± 0.2 hr respectively, $P > 0.1$). Reproductive activity of captive devils overall (Table 2) occurred earlier than wild devils ($P < 0.05$) but day length average was still similar ($P > 0.1$). In the wild, reproductive activity was more synchronized than in captivity when evaluated by date (week) and the BPB was 4 and 6 weeks respectively (Fig. 2a). Figure 2b demonstrates that seasonality in female devils has a strong correlation to day length ($P < 0.01$), with both the captive and wild populations demonstrating a similar distribution of females entering into estrus if considered against day length, standardized for the weekly day length intervals found in Tasmania, the devil's natural habitat. Rate of day length change was not a predictor of the timing of the onset of breeding activity in female devils ($P > 0.1$).

The latest date for the initiation of the first estrous cycle in a captive female devil was 9 May (2008) with another 3 females entering into their first estrous cycle of the year between 29 April and 7 May. All of the captive female devils which entered into their first estrous cycle late in the season (n = 10), week 14 or later (29 March or later), were either 1 or 2 years of age, this being their first year of sexual maturity. Four of these (2 years of age) for which body weights were available were all under 4 kg body weight at the beginning of the breeding season. For wild females (n = 11) entering into their first estrous cycle of the year during a similar timeframe (29 March or later), age could not be determined but these females had an average body weight of 5.8 ± 1.1 kg.

Mean maximum daily temperature was higher for the captive population than for the wild population (Table 2; $P < 0.01$). There was no correlation with maximum weekly temperature and the timing of the onset of the breeding season. For captive female devils, litters sizes of only 1 pouch young were correlated with higher weekly temperatures than those with litter sizes of 4 pouch young ($P < 0.05$) but data was limited. No relationship between temperature and litter size was observed in the wild population ($P > 0.1$).

4. Discussion

For global captive breeding programs for threatened and endangered wildlife, the gold standard is to try to replicate natural conditions, including environment and social context, within the restrictions of artificial housing to ensure the retention of natural behaviors and to optimize animal health and breeding success. To do this, a thorough understanding of the species within their natural environment is essential. This study examined differences in environmental conditions between wild devils in their natural habitat of Tasmania to those held in captive breeding centers throughout Australia to increase our understanding of location (latitude and longitude) specific factors associated with seasonality and reproduction.

4.1 Photoperiod

Photoperiod and rate of change of day length and their ability to control seasonality has been examined in a number of marsupials, including some of the smaller dasyurids [17-19]. All Antechinus are semelparous with males dying shortly after the brief, remarkably synchronous breeding season [26]. The timing of this highly synchronized breeding season has been confirmed to be controlled by a combination of endogenous circannual rhythm, photoperiod and rate of change of photoperiod [18, 27]. The results from our study demonstrate that in the wild, most Tasmanian devils breed over a 4 week period in late summer, early autumn. When the timing of the first estrous cycle for females within the captive population is considered against day length instead of date, the results show a similar distribution as the wild population. This confirms that seasonality in female devils is associated with day length, during the period of decreasing day length. This physiological response suggests it is likely that it is changes in melatonin production during decreasing day length to a critical threshold day length that initiates this response and triggers reproductive activity.

Overall, captive devils housed at latitudes which are lower (geographically more northern to Tasmania), exhibit a phase shift and extension of the first estrous cycle window. For the highly synchronized breeding season of the brown antechinus, it has been shown that a phase delay of the natural photoperiod by two months delays the timing of the breeding season in accordance with the delay [19]. The authors hypothesize that presumably it is the rate of day length change that plays a critical role in the control of reproduction in the Antechinus [19]. Conversely, our results suggest that perhaps the progression to a critical day length, with a specific direction of day length change, is important in the control of reproduction in devils but is not necessarily rate dependent. The rate of change of day length is dependent on the geographical location, specifically altitude and latitude, and therefore each rate of change of day length would also be related to the very specific day length of that geographical location. If day length remains static and the rate of change decreases, this can shift or extend the breeding activity. We have demonstrated that differences in magnitude of the rate of change of day length did not change the absolute day length at which reproductive activity occurred for devils at differing geographical locations, but extended the period of time over which this occurred. This extension of the window in which the first estrous cycle occurs appears to be due to an extension of the number of days over which the same day lengths occur naturally in Tasmania. An expansion of the period over which reproductive activity occurs may lead to a less synchronous breeding season. For the devil, this is unlikely to be a concern in regards to synchronizing male and female reproduction as mature male devils produce sperm throughout the year [28]. However, for species such as the Antechinus where males die shortly after breeding, the period of breeding must be well synchronized and occur over a short period; lengthening the period in which females enter estrus may decrease breeding success if males have exhausted their sperm reserves or are already disappearing from the population.

With different Antechinus species inhabiting the same geographical area, there is also a differential response to rate of day length change associated with body weight, with larger species breeding earlier, ensuring the isolation of reproductive activity between sympatric species [18]. Although we did not observe a correlation with body weight and the first wave of females to enter into estrus in wild devils, we did note that in captive devils, small females tended to breed later in the season, supporting the hypothesis that body weight thresholds may also factor into individual seasonal variation. For Antechinus it is likely it is a very specific, population dependent relationship between rate of change in day length and absolute day length, in addition to endogenous circannual rhythm and body size, that synchronizes and times reproductive activity [17, 18, 29]. For devils, the rate of change of day length does not appear to be a factor in the timing of reproductive activity but there does appear to be an interaction between an independent endogenous circannual rhythm and day length. Southern Queensland, the lowest latitude of the captive locations, and its proximity to the equator means that day length peaks at 13.9 hr at the summer solstice. If day length was the only trigger for initiating reproductive activity, devils housed in Queensland would be expected to be the first to breed, which is not the case. In fact, the BPB of females housed in Queensland in relation to day length occurs in the latter half of the BPB of wild and captive devils suggesting an endogenous circannual rhythm exists to help regulate photoperiod triggers for seasonal reproductive activity in female devils, increasing the synchrony of breeding.

4.2 Latitude

Latitude has been shown to have a pronounced effect on the timing of reproductive patterns in opossum species (*Didelphis*)[21]. When examining results from multiple *in situ* studies over a 14^{o} latitude change (30^{o} to 44^{o} N) in the Virginia opossum (*Didelphis virginiana)*, mean litter size decreased as the location neared the equator and the duration of the breeding season shifted towards an earlier onset [21]. These results parallel the trend seen with devils with decreased litter size and earlier onset of the breeding season in captive devils compared to their more southerly wild counterparts over a similar range in latitude change. White-eared (*Didelphis albiventris)* and big-eared opossum (*Didelphis aurita)* are also found across a large latitude range (5 to 34^{o} S and 19 to 27^{o} S respectively) but had less consistent trends through locations possibly due to a closer proximity to the equator overall [21]. Overall across Didelphid species, it appears that the duration of the breeding season decreases with an increase in latitude, with some of those closest to the equator displaying year-round breeding [21]. This is likely due to decreased variation in day length and temperature, the closer to the equator a species inhabits, which also may decrease the potential seasonal effects of food resources [21]. These authors suggest that higher latitudes increase environmental restraints, decreasing the breeding season or shifting it, to time offspring weaning with periods of food abundance. As such the females must optimize their fecundity by increasing mean litter size to ensure as many offspring survive as possible, which is extremely important at the extremes of the ranges where adult survival across years is low [21]. This could also be a possible alternative hypothesis for devils, to explain the disparity between average litter size between the captive and wild population. If survival rates for both adults and young are lower in the wild, this may favor larger litter sizes to maximize the chance of genetic perpetuation. In seasonal environments, the timing and synchrony of reproduction can be considered crucially important factors for fitness and survival.

Whilst we try to simulate captive conditions to those of the wild as best as possible, including habitat and diet, there are obvious constraints associated with captivity, including limitations on housing design and availability. Species such as the dibbler (*Parantechinus apicalis*) are monoestrous with females coming into estrus over a short period in the Austral late summer, early autumn [30]. Dibblers are naturally found on one of three islands off the western coast of Australia near Jurien Bay (-30.297 S, 115.042 E) about 200 km north of Perth (-31.952 S, 115.859 E) or in the Fitzgerald River National Park (-33.948 S, 119.615 E) approximately 500 km southeast of Perth [30]. An early study showed that 3 male and 3 female island dibblers translocated to Melbourne (-37.814 S, 144.963 E) prior to becoming sexually mature, housed under natural daylight conditions of the area for three years, all achieved sexual maturity (producing sperm or completing estrous cycles confirmed by changes in body weight and vaginal cytology) but the timing of reproductive activity within and between sexes was overall asynchronous and all attempts to breed were unsuccessful [22]. It is possible that translocation prior to sexual maturity in this species was enough to alter their photoperiod history, further compounding the disruption in photoperiod cues associated with a different rate of photoperiod change and absolute day length to that of their natural habitat. In Antechinus, if exogenous melatonin is administered before the Antechinus reaches sexual maturity, desynchronization of the breeding season occurs [31]. Over half the captive devils examined in this study were wild born (52%; 60 of 115) and were not removed from Tasmania until a few months prior to their first breeding season or after the age of 2 years. Generally, devils are moved between locations as adults so it is unknown if relocation over a large range of latitude prior to sexual maturity would disrupt their photoperiod history enough to disturb reproductive activity and breeding success.

4.3 Temperature

Temperature and rainfall may also be proximate cues for the timing of seasonal reproduction but because of the lack of predictability and the potential for sharp differences over short distances (especially rainfall), these factors often lack strong predictive values [16]. In extreme years, when overall temperatures over extended periods are higher than average, combined with lower than average rainfall, this may cue forthcoming drought conditions which may have a negative impact on reproduction [16]. However, the timing needed to have a significant effect in a marsupial may differ from eutherians as the primary maternal investment is in lactation not pregnancy therefore the occurrence of estrous cycles may not be inhibited but the successful weaning of offspring may be. The timing and occurrence of an estrous cycle and the successful production and retention of pouch young in a dasyurid is likely influenced by not only photoperiod but a combination of other proximate cues related to perceived energy availability and competition for food resources (population density). Captivity ensures consistent food resources but environmental cues may still trigger innate perceptions that less favorable conditions are developing (eg. drought), that the population density is high (proximity to other devils) or alternatively higher temperatures may induce physiological stress (through elevated pressure on thermoregulatory physiology), all potentially detrimental to breeding success; requiring future research.

The overall breeding success rate of the captive devils from the records used in this study was only 43% which was similar to the overall success rate of the captive breeding program (38%) over the period from which records were obtained (Australian Zoo and Aquarium Association records). The first wave of females to come into estrus in captivity had only a 29% success rate of producing viable young despite 50% (12 of 24) being 2 years of age (25% of which produced young), the prime age of breeding for a female Tasmanian devil. Within our captive dataset, the majority of pouch young were produced during the first estrous cycle (84%) and we presume that most of the litters observed in wild devils were also of the first estrous cycle. If critical day length is a driver for timing reproductive activity in female devils and thereby shifts the beginning of the breeding season by a few weeks in captivity due to an earlier obtainment of the appropriate day length, this also causes the breeding season to occur over periods of maximum temperature that are significantly higher than those of their natural environment. The island state of Tasmania has a cool temperate climate providing cooler temperatures than the mainland states of Australia due to is geographical location off the south-east coast of Australia. It is possible that higher average temperatures and heat waves, often experienced at least once per season in many parts of mainland Australia may have a negative impact on the production of pouch young or litter size in captive devils. If heat is perceived to be associated with drought and the potential for decreased food resource or causes physiological stress, some devils may defer reproduction until the next year. Periods of drought, common in Australia, have been shown to have a detrimental effect on breeding success of marsupials [32-34]. For a species such as the Antechinus, it doesn’t have the luxury of delaying reproduction until the next breeding season as males have only a single opportunity to breed due to its semelparous nature, therefore a decline in breeding success can have catastrophic effects on the population. Drought conditions have been shown to decrease both number of female agile antechinus successfully producing pouch young as well as litter size [32]. As the energetics of pregnancy in a marsupial are low due to short gestations and the birth of embryonic young, adverse environmental conditions may not suppress the estrous cycle or pregnancy but may increase the loss of pouch young at or soon after birth due to increasing energetic demands of lactation [33]. This may be another potential factor associated with lower reproductive success and reduced litter size in captive devils housed in mainland Australia.

4.4 Timing of Reproductive Success

Reproductive failure in captive Tasmanian devils is likely to occur at one of two critical time points during the breeding season. The first critical stage is at conception, failure most likely related to either mate incompatibility resulting in a lack of mating or male infertility. Despite low genetic diversity, breeding rates reported for wild devil populations are generally good suggesting that unlike other species like the cheetah, decreased male fertility associated with reduced genetic diversity is unlikely in the devil [14, 15, 35-37]. A recent study of captive devils showed that 61% of unsuccessful breeding attempts were with male devils with proven fertility (production of pouch young)[12] suggesting that male infertility is unlikely to be the primary factor in failed pairings. Mate incompatibility is difficult to determine if obvious signs of aggression are not observed. Although mating is not always observed in captivity due to the devil’s nocturnal nature and a lack of infrared video surveillance at most facilities, pairings are considered “good” if affiliative behaviors are observed (eg. denning together) or the male displays mate-guarding. Devils are separated when aggression is noted but a lack of aggression does not necessary confirm an optimal pairing if mating is not observed.

Reproductive failure could also occur at or shortly after birth. Conception rates are high in Tasmanian devils (75% of females from the examined wild population were pregnant or had small pouch young) and even if a large percent of embryos fail to complete maturation, the large number of eggs ovulated (on average 30 to 60 per ovulation) increases the chances that joeys are born [15]. In many of the captive devil pairings, mating was observed yet the female failed to produce young. Pouch checks are generally conducted at least 2 to 3 weeks after predicted birth, therefore for unsuccessful females, it is unknown if joeys are born but lost at or shortly after birth. If loss at or shortly after birth is the stage at which most breeding attempts fail, we have yet to be able to identify specific social or environmental factors that are causing this to occur and therefore have been unable to improve overall captive breeding success. It is possible that a shift in the timing of birthing events or geographical location to hotter weather may influence the retention of some or all of the joeys born. Our results confirm that captive devils are exposed to hotter temperatures than their native Tasmania and temperature has a negative correlation to the production of young and litter size. Why some females may be more sensitive than others to elevated temperatures is unknown.

Population studies suggest that Tasmanian devils in the wild are more likely to produce pouch young every year within their reproductive lifespan than captive devils. Our results indicate that in captive devils, few females produced young on a second or third estrous cycle (less than 10%). It is possible that these estrous cycles are less fertile, producing less oocytes for fertilization; potentially detrimental as embryonic developmental failure is high (R. L. Hughes, unpublished data), or there may be other endogenous factors at play. In the wild, having pouch young on a second or third estrous cycle may have a negative impact on the reproductive potential for the following year if lactation (250 to 280 days) and weaning delays the timing of reproductive activity within the next breeding season, perhaps even deferring it completely. This may not be a suitable trade-off for young devils but for a female in her last year of reproduction, it would be beneficial to reproduce late rather than not at all. Another potential consequence of late breeding in the wild would be that at weaning, offspring would be smaller and younger than other joeys born of the same year and may be less competitive for food resources, decreasing the survival especially if population density is high. Regardless, individual variability and physiology still limits the number of devils which undergo subsequent estrous cycles and successfully produce young if earlier breeding is unsuccessful.

4.5 Conclusions

How much flexibility does the Tasmanian devil have in its life history? It appears to be not as much as we had hoped. Captive breeding efforts are continuously improving, yet captive breeding rates remain much lower than those observed in the wild. Although we can not rule out cryptic female mate choice as a factor, it is unlikely to account for all of the unsuccessful pairings. Anecdotal reports suggest that captive breeding within facilities in Tasmania can also be variable, so although temperature may be a factor in mainland Australia, there are still likely multifactorial aspects of captivity which are impacting breeding success that we have yet to elucidate. We hope that the many points of discussion outlined throughout provide future avenues to explore other possibilities.

With wildlife species, it is not always possible to collect blood samples frequent enough to monitor hormones such as melatonin to gain information about seasonal reproductive physiology, especially for endangered species for which handling is restricted. With this study, we demonstrate that comparison of historic breeding records between captive and wild devils was sufficient to gain valuable information on the seasonal regulation of reproduction in this large dasyurid species. Species which exhibit seasonal patterns of reproduction may benefit from the study and evaluation of ecological factors in play at the onset of the breeding season or onset of sexual maturity to better understand the relationship between an animal and its environment. Photoperiod is a predictive cue for seasonal reproduction in the Tasmanian devil and alters the timing of reproduction in accordance with geographical location.

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References

[1] Pemberton D, Renouf D. A field study of communication and social behaviour of the Tasmanian devil at feeding sites. Australian Journal of Zoology. 1993;41:507-26.

[2] Pemberton D. Social organization and behaviour of the Tasmanian devil, *Sarcophilus harrisii* [Ph.D. Thesis]. Hobart: University of Tasmania; 1990.

[3] Lachish S, Jones M, McCallum H. The impact of the disease on the survival and population growth rate of the Tasmanian devil. Journal of Animal Ecology. 2007;76:926-36.

[4] Hawkins CE, Baars C, Hesterman H, Hocking GJ, Jones ME, Lazenby B, et al. Emerging disease and population decline of an island endemic, the Tasmanian devil *Sarcophilus harrisii*. Biological Conservation. 2006;131:307-24.

[5] Pearse AM, Swift K. Transmission of devil facial-tumour disease. Nature. 2006;439:549.

[6] Loh R, Bergfeld J, Hayes D, O'Hara A, Pyecroft S, Raidal S, et al. The pathology of Devil Facial Tumor Disease (DFTD) in Tasmanian devils (*Sarcophilus harrisii*). Veterinary Pathology. 2006;43:890-5.

[7] Pyecroft S, Pearse A, Loh R, Swift K, Belov K, Fox N, et al. Towards a case definition for devil facial tumour disease: What is it? EcoHealth. 2007;4:346-51.

[8] Siddle HV, Kreiss A, Eldridge MDB, Noonan E, Clarke CJ, Pyecroft S, et al. Transmission of a fatal clonal tumor by biting occurs due to depleted MHC diversity in a threatened carnivorous marsupial. Proceedings of the National Academy of Science. 2007;104:16221-6.

[9] Woods GM, Kreiss A, Belov K, Siddle HV, Obendorf D, Muller HK. The immune response of the Tasmanian devil (*Sarcophilus harrisii*) and the Devil Facial Tumour Disease. EcoHealth. 2007;4:338-45.

[10] IUCN. IUCN Red List of Threatened Species. Gland, Switzerland: IUCN Species Survival Commission; 2008.

[11] Roberts MG. The keeping and breeding of Tasmanian devils (*Sarcophilus harrisii*). Proceedings of the Zoological Society of London 1915;85:575-81.

[12] Keeley T, McGreevy PM, Fanson BG, Masters K, O’Brien JK. The reproductive cycle of the Tasmanian devil (*Sarcophilus harrisii*) and factors associated with reproductive success in captivity. General and Comparative Endocrinology. 2012;176:182-91.

[13] Guiler ER. Observations on the Tasmanian devil, *Sarcophilus harrisii* (Marsupialia: Dasyuridae) II. Reproduction, breeding and growth of pouch young. Australian Journal of Zoology. 1970;18:63-70.

[14] Lachish S, McCallum H, Jones M. Demography, disease and the devil: life-history changes in a disease-affected population of Tasmanian devils (*Sarcophilus harrisii*). Journal of Animal Ecology. 2009;78:427-36.

[15] Hughes RL. Reproduction in the Tasmanian devil *Sarcophilus harrisii* (Dasyuridae, Marsupialia). In: Archer M, editor. Carnivorous Marsupials. Sydney: Surrey Beatty and Sons; 1982. p. 49-63.

[16] Hazlerigg D, Simonneaux V. Seasonal regulation of reproduction in mammals. In: Plant TM, Zeleznick AJ, editors. Knobil and Neill's Physiology of Reproduction. 4th ed. London: Elsevier Inc.; 2015. p. 1575-603.

[17] Dickman CR. Effects of photoperiod and endogenous control on timing of reproduction in the marsupial genus *Antechinus*. Journal of Zoology London. 1985;206:509-24.

[18] McAllan BM, Dickman CR, Crowther MS. Photoperiod as a reproductive cue in the marsupial genus *Antechinus*: ecological and evolutionary consequences. Biological Journal of the Linnean Society. 2006;87:365-79.

[19] McAllan BM, Joss JMP, Firth BT. Phase delay of the natural photoperiod alters reproductive timing in the marsupial *Antechinus stuartii*. Journal of Zoology London. 1991;225:633-46.

[20] Zerbe P, Clauss M, Codron D, Lackey LB, Rensch E, Streich JW, et al. Reproductive seasonality in captive wild ruminants: implications for biogeographical adaptation, photoperiodic control, and life history. Biological Reviews of the Cambridge Philosophical Society. 2012;87:965-90.

[21] Rademaker V, Cerqueira R. Variation in the latitudinal reproductive patterns of the genus *Didelphis* (Didelphimorphia:Didelphidae). Austral Ecology. 2006;31:337-42.

[22] Woolley P. Reproductive pattern of captive Boullanger Island dibblers, *Parantechinus apicalis* (Marsupialia: Dasyuridae). Wildlife Research. 1991;18:157-63.

[23] Marneweck DG, Ganswindt A, Rhodes S, Bellem A, Bryant J, Wielebnowski N, et al. Reproductive endocrinology of zoo-housed aardwolves. Acta Theriol. 2013;58:223-32.

[24] Hughes RL, Keeley T. Population dynamics of Tasmanian devils in north-eastern Tasmania 1974 -1987. The Australian Mammal Society Conference. Melbourne, Australia 2014.

[25] Hughes RL, Keeley T. The reproductive biology and embryonic development of the Tasmanian devil. The Australian Mammal Society Conference. Sydney, Australia 2013.

[26] Bradley AJ. Stress, hormones, and mortality in small carnivorous marsupials. In: Jones M, Dickman C, Archer M, editors. Predators with pouches: the biology of carnivorous marsupials. Sydney: CSIRO Press; 2003. p. 250-63.

[27] Dickman CR, Hayssen V, King DH. Effects of seasonal reversal of photoperiod on the reproductive rhythm of a small marsuipal Journal of Zoology London. 1987;213:766-8.

[28] Keeley T, McGreevy PM, O'Brien JK. Characterization and short-term storage of Tasmanian devil spermatozoa collected post-mortem. Theriogenology. 2011;76:705-14.

[29] McAllan BM, Geiser F. Photoperiod and the timing of reproduction in *Antechinus flavipes* (Dayuridae: Marsupialia). Mammalian Biology. 2006;71:129-38.

[30] Mills HR, Bradshaw FJ, Lambert C, Bradshaw SD, Bencini R. Reproduction in the marsupial dibbler, *Parantechinus apicalis*; differences between island and mainland populations. General and Comparative Endocrinology. 2012;178.

[31] McAllan BM, Westman W, Joss JMP. The seasonal reproductive cycle of a marsupial, *Antechinus stuartii*: effects of oral administration of melatonin. General and Comparative Endocrinology. 2002;128:82-90.

[32] Parrott M, Ward SJ, Temple-Smith P, Selwood L. Effects of drought on weight, survival and breeding success of agile antechinus (*Antechinus agilis*), dusky antechinus (*A. swainsonii*) and bush rats (*Rattus fuscipes*). Wildlife Research. 2007;34:437-42.

[33] Tyndale-Biscoe CH. Australian marsupials - to cherish and to hold. Reproduction, Fertility and Development. 2001;13:477-85.

[34] Renfree MB, Shaw G. Reproduction in Monotremes and Marsupials. Encyclopedia of Life Sciences. 2001:1-5.

[35] Jones ME, Paetkau D, Geffen E, Moritz C. Microsatellites for the Tasmanian devil (*Sarcophilus laniarius*). Molecular Ecology Notes. 2003;3:277-9.

[36] Jones ME, Paetkau D, Geffen E, Moritz C. Genetic diversity and population structure of Tasmanian devils, the largest marsupial carnivore. Molecular Ecology 2004;13:2197-209.

[37] Crosier AE, Marker L, Howard JG, Pukazhenthi B, Henghali JN, Wildt DE. Ejaculate traits in the Namibian cheetah (*Acinonyx jubatus*): influence of age, season and captivity. Reproduction, Fertility and Development. 2007;19:370-82.

Table 1. The specific location and number of Tasmanian devils examined in this study. Devils housed in areas within a short distance (less than 200 km) of each other were analysed together to maximize numbers per Australian state where needed. Maximum daily temperatures were measured at each given weather station and averaged to provide weekly maximum temperatures for evaluation.

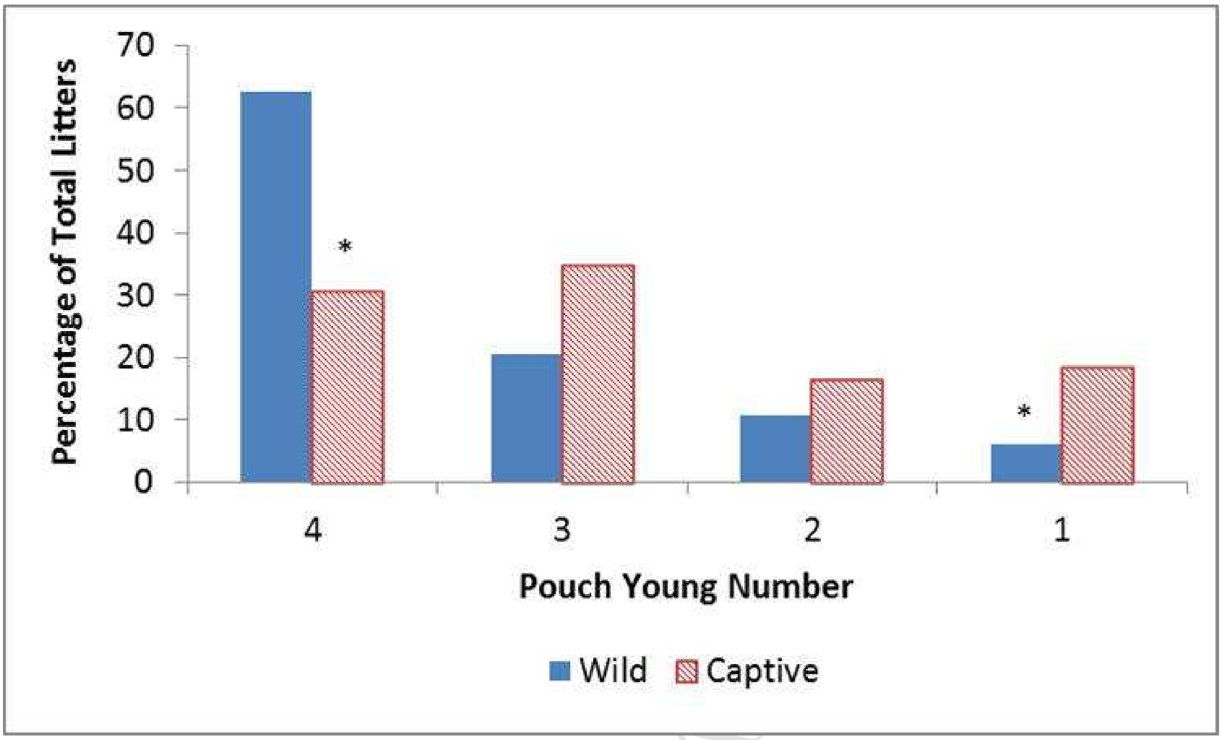
Locality	Number of Records	Years	Location	Weather Station	Latitude	Longitude
Tasmania (Wild Population)	169	1974 to 1987	Avoca District	91022	- 41.78	147.72
Southern Queensland	17	2007 to 2012	Australia Zoo	40284	- 26.86	152.96
			Dreamworld	40764	- 27.86	153.31
			Currumbin Wildlife Sanctuary	40764	- 28.14	153.49
Victoria	23	2009 to 2012	Healesville Wildlife Sanctuary	86383	- 37.65	145.52
			Ballarat Wildlife Santuary	86383	- 37.56	14.87
South Australia	18	2008 to 2012	Monarto Zoo	24584	- 35.12	139.14
New South Wales (Inland)	29	2008 to 2011	Taronga Western Plains Zoo	65070	- 32.22	148.58
New South Wales (Coastal)	28	2008 to 2011	Taronga Zoo	66037	- 33.87	151.21
			Australian Reptile Park	66037	- 33.43	151.34

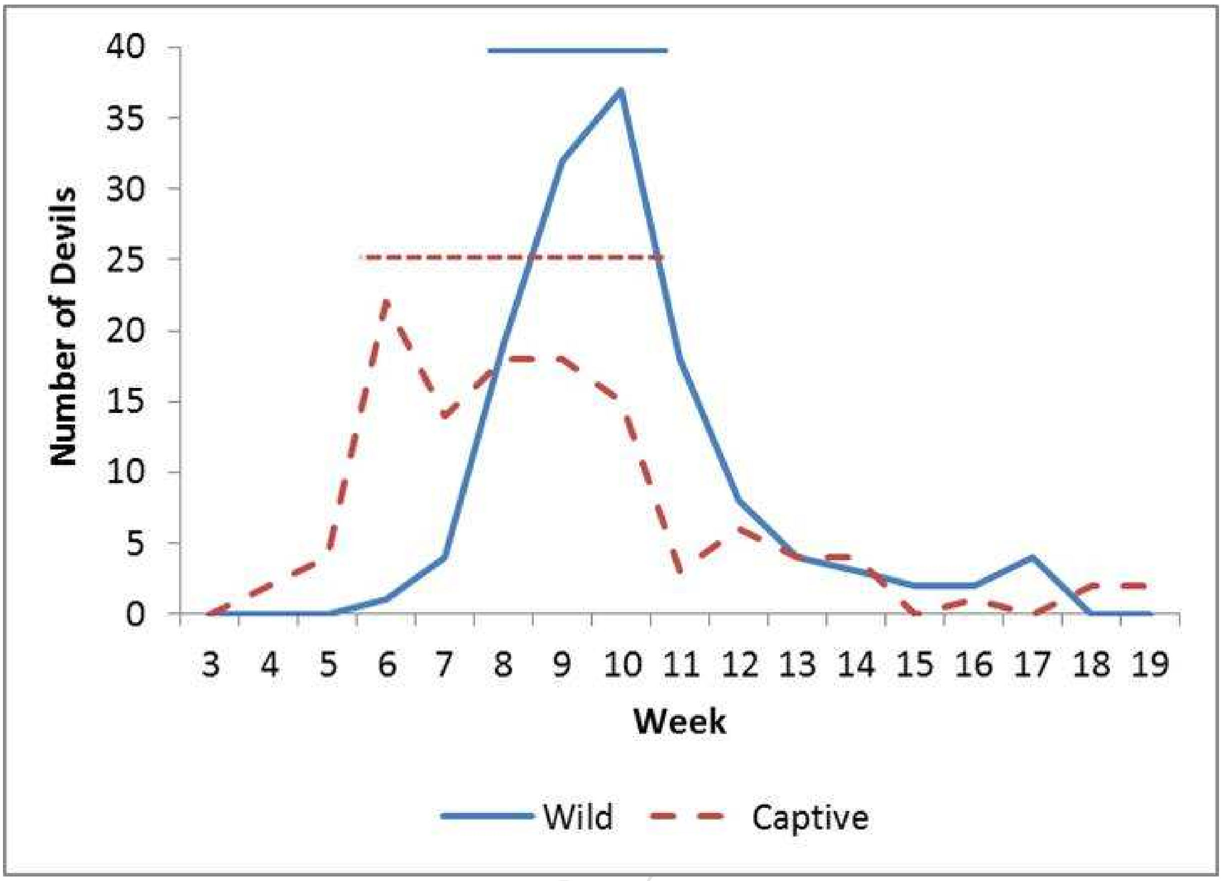
Table 2. Summary of the mean values for the timing of the beginning of first estrus (week), rate of day length change (min/d), day length (hr) and maximum temperature (^{o}C). All factors were evaluated as weekly averages or the week in which the beginning of estrus was confirmed or calculated to occur. Data is ± SD. * Denotes statistical significance in difference (*P* < 0.01) between the captive and wild populations.

Locality	Number of Records	Week	Rate of Day Length Change (Minutes/Day)	Day Length (Hr)	Maximum Temperature (^{o}C)
New South Wales (Inland)	29	9.1 ± 3.4	1.8 ± 0.2	12.8 ± 0.7	29.7 ± 3.7
Southern Queensland	17	9.0 ± 2.0	1.6 ± 0.1	12.7 ± 0.4	29.1 ± 1.2
South Australia	18	6.9 ± 1.5	2.0 ± 0.2	13.5 ± 0.3	30.0 ± 5.0
New South Wales (Coastal)	28	10.2 ± 3.8	2.0 ± 0.1	12.6 ± 0.9	25.5 ± 3.1
Victoria	23	8.3 ± 2.2	2.3 ± 0.2	13.2 ± 0.6	26.8 ± 3.9
Tasmania (Wild)	134	10.2 ± 2.2*	2.8 ± 0.1*	13.0 ± 0.7	22.3 ± 2.7*
Captive Overall	115	8.8 ± 3.0*	2.0 ± 0.3*	12.9 ± 0.7	28.1 ± 4.0*
Tasmania (Wild) - First 2 week phase	23	7.7 ± 0.5**	2.6 ± 0.1**	13.8 ± 0.2	25.0 ± 1.6**
Captive Overall - First 2 week phase	24	5.7 ± 0.6**	1.8 ± 0.2**	13.7 ± 0.2	32.3 ± 4.7**

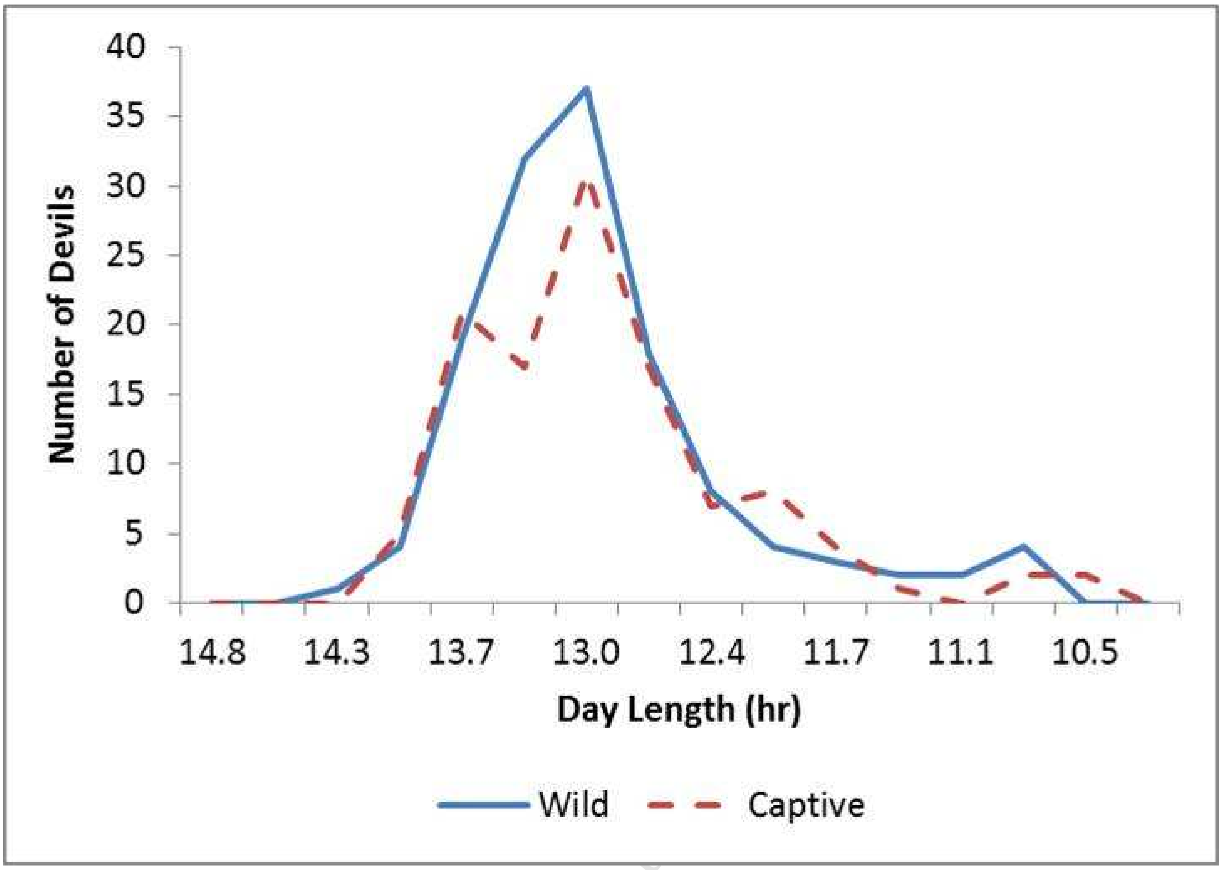
Figure 1. Litter size distributions for wild and captive female devils. * Denotes statistical significance in difference between the percentage of total litters that include 1 to 4 pouch young between captive and wild female devils ($P < 0.05$).

Figure 2a. The timing, date by week intervals, of the beginning of the estrous cycle for wild (n = 134) and captive (n = 115) devils. Lines indicate the period over which 80% of the birth peak breadth occurs. 2b.The timing, by day length, of the beginning of the estrous cycle for wild and captive devils. The weekly day length intervals are standardized to those which naturally occur in Tasmania.


Percentage of Total Litters
70
60
50
40
30
20
10
0
*
*
4
3
2
1
Pouch Young Number
Wild
Captive



40
35
30
25
20
15
10
5
0
Number of Devils
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
Week
Wild
Captive


Number of Devils
40
35
30
25
20
15
10
5
0
14.8
14.3
13.7
13.0
12.4
11.7
11.1
10.5
Day Length (hr)
Wild
Captive