

# Size, season and offspring sex affect milk composition and juvenile survival in wild kangaroos

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## Keywords

lactation; *Macropus giganteus*; maternal effects; multimodel inference; timing of reproduction; Trivers–Willard hypothesis; sex allocation; milk composition.

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## Abstract

The main component of mammalian maternal care is milk production. The composition of milk has been hypothesized to determine offspring survival, and to vary with offspring sex. Few studies, however, have examined variation in milk composition of wild mammals in seasonal environments, where environmental conditions can impact the ability of mothers to provide care. We investigated individual differences in milk composition and offspring survival in wild eastern grey kangaroos (*Macropus giganteus*). We analyzed total protein and lipid concentrations in 103 milk samples from 91 females over 2 years. In a year of low forage production, few females that lactated were in poorer condition and produced milk of lower energy content compared to females lactating in a year of high forage production. Females nursing in late winter produced milk with more lipids compared to females at the same stage of lactation in late spring, whose milk had a higher proportion of protein. The milk of larger females contained more protein than lipid, and females in better condition allocated higher proportions of protein to sons than to daughters. Increased protein concentration in milk was correlated with offspring longevity, and this effect was stronger for sons than daughters. A seasonally modulated lactation strategy enables the adjustment of milk composition to available resources. Condition-specific protein allocation in favor of sons for mothers of higher caring ability suggests adaptive sex-biased maternal care in this highly sexually dimorphic mammal.

## Introduction

Lactation is the most costly component of maternal care in mammals (Oftedal, 1985), and the prolonged dependence of offspring on milk results in ubiquitous maternal effects (Maestripieri & Mateo, 2009) with consequences for evolutionary and population ecology. Maternal traits (Georges & Guinet, 2000; Bowen *et al.*, 2001) and differential care for sons and daughters (Landete-Castillejos *et al.*, 2005; Robert & Braun, 2012) may contribute to variability in milk provisioning. Milk composition is correlated with maternal condition in pinnipeds (Iverson *et al.*, 1993; Crocker *et al.*, 2001), primates (Nommsen *et al.*, 1991; Tardif *et al.*, 2001), and ungulates (Landete-Castillejos *et al.*, 2005; Bovolenta *et al.*, 2013), but not in tamar wallabies (*Macropus eugenii*) or Columbian ground squirrels (*Urocitellus columbianus*) (Robert & Braun, 2012; Skibieli & Hood, 2015). Moreover, differences in milk provisioning affect offspring size (Mellish, Iverson & Bowen, 1999; Landete-Castillejos, Garcia & Gallego, 2001; Robert & Braun, 2012) and survival (Skibieli & Hood, 2015).

Sex-biased allocation (Trivers & Willard, 1973) is one potential source of variation in milk composition. In polygynous mammals, males have higher reproductive variance than females, therefore sons should benefit more than daughters from greater milk provisioning (Trivers & Willard, 1973). Hence, mothers with higher caring ability should favor sons, whereas mothers with lower ability should favor daughters. Some studies of milk composition have provided support for sex-biased allocation by reporting that sons received more (Landete-Castillejos *et al.*, 2005) or higher-energy milk (Powe, Knott & Conklin-Brittain, 2010) with greater lipid (Fujita *et al.*, 2012) or protein concentrations (Robert & Braun, 2012) than daughters. Other studies found no sex-related differences (Hinde, 2009; Hinde *et al.*, 2014).

Experimentally restricted diets have revealed the importance of nutrition on lactation performance (Roberts, Cole & Coward, 1985; Landete-Castillejos *et al.*, 2003; Bovolenta *et al.*, 2013). If the timing of reproduction affects milk provisioning (Landete-Castillejos *et al.*, 2001; Skibieli & Hood, 2015) then there could be large seasonal variation in maternal caring

ability in the wild. Most studies investigating maternal care through milk, however, were performed in captive (Hinde, 2009; Robert & Braun, 2012), or semi-captive (Landete-Castillejos *et al.*, 2001) environments. Hence, little is known about sex-biased milk allocation in wild mammals.

We studied milk composition in wild eastern grey kangaroos (*Macropus giganteus*) in a temperate environment. Similar to ungulates, kangaroos are large herbivores and males have higher reproductive variance than females (Fisher, Blomberg & Owens, 2002). The short gestation and prolonged lactation of marsupials, however, make kangaroos ideal for studying post-natal care (Sharp *et al.*, in press). Stage-specific changes in milk composition during marsupial lactation have been extensively studied in the congeneric tammar wallaby (Green, Newgrain & Merchant, 1980; Green, Griffiths & Leckie, 1983), with milk components changing substantially throughout lactation (Tyndale-Biscoe & Janssens, 1988). We identified determinants of milk lipid and protein, two macronutrients with high caloric density and sensitivity to maternal nutritional state (Parker, Barboza & Gillingham, 2009). We expected that females at the same stage of lactation would vary milk composition according to forage production. Male body size in kangaroos affects mating success (Rioux-Paquette *et al.*, 2015); we predicted that mothers with a higher ability to care would provide milk with higher protein content to sons than to daughters. Finally, we tested whether protein concentration at peak lactation affected offspring survival (Skibieli & Hood, 2015).

## Materials and methods

### Study area and data collection

The study area in Wilsons Promontory National Park, Victoria, Australia (38°57'S, 146°17'E) is a 1.1-km<sup>2</sup> grassland surrounding an emergency airstrip (Davis, Coulson & Forsyth, 2008), where grasses, sedges and herbs are the main forage (Davis, Forsyth & Coulson, 2010). The kangaroo population has been monitored since 2008, with annual recaptures of marked individuals (Gélin *et al.*, 2013). The climate is temperate, with mean maximum monthly temperatures ranging from 12.5°C in July to 26.8°C in February, and annual precipitation of 453–966 mm between 2012 and 2015 (Corner Inlet Station, 38°81'S, 146°19'E; [www.bom.gov.au/climate/data/](http://www.bom.gov.au/climate/data/)).

We used a pole syringe (King *et al.*, 2011) to capture females carrying pouch young aged six to 10 months from mid-winter (July) to early summer (December) in 2014 and 2015. Young were transferred to a cloth bag, sexed and their head, foot and leg lengths (mm) measured. They were marked with colored plastic ear tags (Leader Products Pty Ltd, Craigieburn, Australia). Mothers were weighed to the closest 0.25 kg and their hind leg length measured (mm). To stimulate milk letdown, oxytocin (Oxyto-Sure, Vetoquinol, concentration = 20 USP mL<sup>-1</sup>, dose = 12 µL kg) was injected intramuscularly. After 1–4 min, milk was expressed into a screw-top vial by gently stripping the teat. Samples were refrigerated for 1–4 h, and then stored at –20°C until analysis. The time of milk collection (morning, 0600–0900 h; evening, 1600–2300 h) was recorded and young were returned to the pouch. We collected

103 milk samples from 91 females. Of these, 12 and 11 were newly marked in 2014 and 2015, respectively, with colored Allflex ear tags and a flexible collar (Gélin *et al.*, 2013).

### Milk analysis

Milk samples were assayed in triplicate for total protein (Thermo Scientific-Pierce™ BCA Protein Assay Kit #23227) and lipid (Atwood & Hartmann, 1992) concentrations. The partial energy (carbohydrate content not included) calculated per unit volume was derived from lipid and protein concentrations, assuming energy values of 24.6 kJ g<sup>-1</sup> for protein and 38.1 kJ g<sup>-1</sup> for lipid (Ofstedal, 1984). At this stage of marsupial lactation, carbohydrates decrease and remain at low concentrations for the remainder of lactation (Tyndale-Biscoe & Janssens, 1988), therefore should not greatly influence milk energy. Milk yield was not quantified, and time since last suckling could not be measured because nursing occurred inside the pouch.

### Explanatory variables

Environmental, maternal and offspring characteristics could affect milk composition. The maternal characteristics we considered were leg length and relative body condition, measured as the ratio of observed body mass to mass predicted by an OLS regression of log female mass on log leg length (Le Cren, 1951). Age was unknown for 73% of mothers and hence was not included in analyses. The offspring characteristics we considered were sex and age. Age was the mean of estimates from sex-specific growth models for head, foot and leg lengths (Poole, Carpenter & Wood, 1982) and was included in analyses to account for increases in both lipid and total protein concentrations in milk (Green *et al.*, 1980, 1983). Environmental variables included year of sampling, sampling date and birthdate of young, the two latter characteristics representing seasonal variables. Birthdate was expressed as Julian day, with 1 August as day 1, because the peak of births occurs during spring and early summer (60% were born in November–January, 9% were born in May–October). Year of sampling indirectly considered forage production, that was not seasonally predictable. Forage production was measured in January, April, July and October by harvesting palatable vegetation from 50 systematically distributed, 0.79-m<sup>2</sup> circular wire-mesh exclusion cages. We calculated forage production between estimated birthdate and milk sampling date for each mother-young pair assuming constant daily vegetation growth between measures.

We examined how milk composition explained offspring survival using age of juveniles in days at their last sighting. We searched for marked young on most days from late July to early December, and again in early March. Maximum longevity was 550 days (18 months), the minimal age of weaning (Poole, 1975).

### Model development and analysis

We first evaluated the sources of variation in milk composition by considering multiple hypotheses. We tested sex-biased allocation by including interactions between maternal

characteristics and offspring sex. We considered an alternative hypothesis, environmental modulation of milk provisioning at annual and seasonal levels, by including year and offspring birthdate in analyses, and interactions between year and both maternal condition and offspring sex were tested. Preliminary analyses indicated a curvilinear relationship between milk macronutrients and age of young, therefore age was included as a quadratic term. A total of 19 hypotheses were modeled using linear mixed regressions (Table 1; Appendix S1), and

three response variables describing milk composition, total protein and lipid concentrations, and the lipid-to-protein ratio (LPR), were separately investigated. High LPR indicates a greater proportion of lipids than proteins, and low LPR indicates greater protein content regardless of milk solids concentrations. Using LPR accounts for the confounding effect of milk volume diluting macronutrients (Hinde, 2009).

We formulated 11 hypotheses to explain the effect of milk composition on offspring longevity (Table 1; Appendix S1),

**Table 1** Parameters used in linear mixed models fitted to assess determinants of milk composition (*m*) and effects of milk composition on offspring longevity (*Y*) in Wilsons Promontory National Park, Australia, in 2014 and 2015

Model	Description
<b>Milk composition</b>	
<i>m</i> {.	Milk composition varies randomly (i.e. null model)
<i>m</i> { <i>a</i> + <i>a</i> <sup>2</sup> }	Milk composition varies curvilinearly with age of young ( <i>a</i> )
<i>m</i> {bd}	Milk composition varies linearly with birthdate of young (bd)
<i>m</i> { <i>C</i> }	Milk composition varies linearly with maternal relative condition ( <i>C</i> )
<i>m</i> { <i>L</i> }	Milk composition varies linearly with maternal leg length ( <i>L</i> )
<i>m</i> { <i>S</i> }	Milk composition varies with offspring sex ( <i>S</i> )
<i>m</i> {yr}	Milk composition varies with years of sampling (yr)
<i>m</i> {all}	Milk composition varies additively with all predictor variables
<i>m</i> { <i>S</i> × <i>C</i> }	Milk composition varies with an interaction between offspring sex and maternal relative condition
<i>m</i> { <i>S</i> × <i>L</i> }	Milk composition varies with an interaction between offspring sex and maternal leg length
<i>m</i> {yr + bd}	Milk composition varies additively with year of sampling and birthdate of young
<i>m</i> {all + <i>S</i> × <i>C</i> }	Milk composition varies additively with all predictor variables and an interaction between offspring sex and maternal relative condition
<i>m</i> {all + <i>S</i> × <i>C</i> + <i>S</i> × yr}	Milk composition varies additively with all predictor variables and an interaction between offspring sex and both maternal relative condition and year of sampling
<i>m</i> {all + <i>S</i> × <i>L</i> }	Milk composition varies additively with all predictor variables and an interaction between offspring sex and maternal size
<i>m</i> {all + <i>S</i> × <i>L</i> + <i>S</i> × yr}	Milk composition varies additively with all predictor variables and an interaction between offspring sex and both maternal size and year of sampling
<i>m</i> {all + yr × <i>C</i> + <i>S</i> × yr}	Milk composition varies additively with all predictor variables and an interaction between year of sampling and both maternal relative condition and offspring sex
<i>m</i> {all + <i>S</i> × <i>C</i> + <i>C</i> × yr}	Milk composition varies additively with all predictor variables and an interaction between maternal relative condition and both year and offspring sex
<i>m</i> {all + <i>S</i> × <i>C</i> + <i>C</i> × yr + <i>S</i> × yr}	Milk composition varies additively with all predictor variables and two-way interactions between offspring sex, year and maternal relative condition
<i>m</i> {all + <i>S</i> × <i>C</i> × yr}	Milk composition varies additively with all predictor variables and a three-way interaction between offspring sex, year and maternal relative condition
<b>Offspring survival</b>	
<i>Y</i> {.	Offspring longevity varies randomly (i.e. null model)
<i>Y</i> { <i>a</i> }	Offspring longevity varies linearly with age of young ( <i>a</i> )
<i>Y</i> {lip}	Offspring longevity varies linearly with milk lipid concentration (lip)
<i>Y</i> {prot}	Offspring longevity varies linearly with milk protein concentration (prot)
<i>Y</i> { <i>C</i> }	Offspring longevity varies linearly with maternal relative condition ( <i>C</i> )
<i>Y</i> { <i>S</i> }	Offspring longevity varies with offspring sex ( <i>S</i> )
<i>Y</i> {yr × bd}	Offspring longevity varies with an interaction between year of sampling and birthdate of young
<i>Y</i> {all}	Offspring longevity varies additively with all predictor variables
<i>Y</i> { <i>S</i> × lip}	Offspring longevity varies additively with all predictor variables and an interaction between offspring sex and milk lipid concentration
<i>Y</i> { <i>S</i> × prot}	Offspring longevity varies additively with all predictor variables and an interaction between offspring sex and milk protein concentration
<i>Y</i> { <i>S</i> × lip + <i>S</i> × prot}	Offspring longevity varies additively with all predictor variables and an interaction between offspring sex and both milk lipid and protein concentrations

including offspring sex and interactions between sex and macronutrients. Age of young at capture was included in models because it was correlated with longevity ( $r_p = 0.20$ ,  $P < 0.001$ ). Maternal condition was included because it is linked to juvenile survival in agile wallabies (*Macropus agilis*) (Bolton, Newsome & Merchant, 1982). We also considered birthdate and an interaction between birthdate and year.

To establish the relative strength of evidence for each candidate model, we performed model selection using Akaike's Information Criterion corrected for sample size ( $AIC_c$ ) (Burnham & Anderson, 2002) for the four candidate sets. Models were ordered according to  $\Delta i$  values relative to the model with lowest  $AIC_c$ . Akaike weights ( $w_i$ ), indicating the probability that a model was the best in the candidate set (Burnham & Anderson, 2002), were computed for each model. A model had significant support if  $w_i \geq 0.05$ . We then performed multimodel inference to estimate averaged effect sizes; parameter estimates for variables that best described variance in milk composition and offspring longevity had 95% confidence intervals that did not overlap zero (Burnham, Anderson & Huyvaert, 2011).

All models included maternal identity as a random variable to account for the use of non-averaged triplicates. We used the *lmer* function in the *lme4* package (Bates et al., 2014) with R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria) to fit models to the data, and the *aictab* function in the *AICcmodavg* package (Burnham et al., 2011) to obtain  $AIC_c$  values, perform model averaging and estimate model parameters.

## Results

### Proximate milk composition

We analyzed 103 samples, 78 from 2014 and 25 from 2015 (Table 2). Among females marked in previous years, 49% (70 of 144) carried pouch young in 2014 and 11% in 2015 (15 of 131) ( $X^2 = 44.48$ , d.f. = 1,  $P < 0.0001$ ). Young in 2014 were estimated to be 7 days older at capture, on average, than in 2015 (range: 196–284 days; Table 3). The sampling period was 37 days longer in 2014 (31 July to 5 December) than in 2015 (29 July to 29 October). Estimated birthdates spanned 5 months in 2014 (23 November 2013 to 29 April 2014) and 4 months in 2015 (5 December 2014 to 29 March 2015).

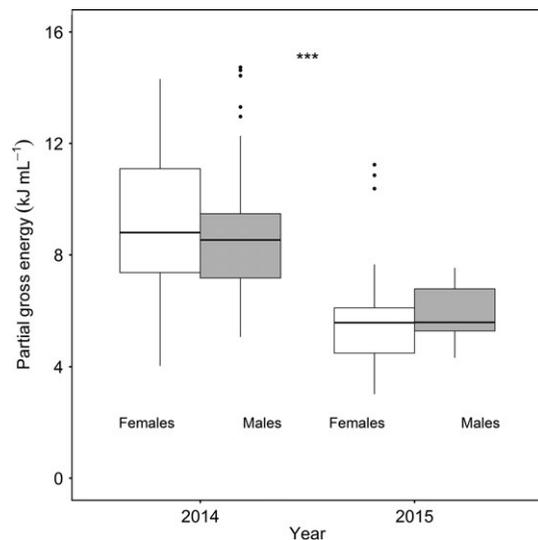
Forage production was 48% lower in 2015 than in 2014 (Table 3), and maternal condition (Table 3) and partial energy (Fig. 1) were also higher in 2014 than in 2015. Maternal body condition increased in spring (Table 4). Further examinations of relationships between explanatory variables (Tables 3 and 4)

**Table 2** Number of milk samples assayed during a 2-year study of eastern grey kangaroos at Wilsons Promontory National Park, Australia

	Year		Total
	2014	2015	
Daughters	35	16	51
Sons	43	9	62
Total	78	25	103

**Table 3** Mean  $\pm$  SD values and two-way analysis of variance by year of sampling and sex of offspring for variables recorded for each milk sample collected at Wilsons Promontory National Park, Australia, in 2014 and 2015

	Year		Offspring		Year x Offspring							
	Mean values		Mean values		Mean values							
	2014	2015	F	P	F	P						
	174 $\pm$ 56	119 $\pm$ 41	58.66	<0.001	160 $\pm$ 45	0.018						
Lipid concentration (mg mL <sup>-1</sup> )	90.4 $\pm$ 22.4	48.9 $\pm$ 8.5	235	<0.001	82.4 $\pm$ 26.9	1.64	0.201	1	0.893	1	2.349	0.126
Protein concentration (mg mL <sup>-1</sup> )	47 $\pm$ 35	41 $\pm$ 27	2.25	0.135	44 $\pm$ 32	0.47	0.492	1	0.949	1	0.949	0.331
Date of sampling (1 = 29 July)	175 $\pm$ 41	175 $\pm$ 33	0.005	0.944	175 $\pm$ 37	0.00	0.988	1	1.849	1	0.880	0.175
Pouch young birthdate (1 = 1 August)	1.007 $\pm$ 0.018	0.998 $\pm$ 0.011	10.02	0.002	1.007 $\pm$ 0.017	2.87	0.092	1	0.880	1	0.880	0.349
Maternal relative condition	529 $\pm$ 14	525 $\pm$ 15	3.48	0.063	528 $\pm$ 13	0.12	0.733	1	1.704	1	1.704	0.192
Maternal leg length (mm)	233 $\pm$ 19	226 $\pm$ 16	7.55	0.006	230 $\pm$ 17	0.84	0.362	1	0.746	1	0.746	0.388
Age of pouch young at capture (days)	154 $\pm$ 15	80 $\pm$ 18	1191	<0.001	142 $\pm$ 30	6	<0.001	1	0.327	1	0.327	0.568
Total forage production during lactation (g m <sup>-2</sup> )												



**Figure 1** Partial gross energy of eastern grey kangaroo milk by year and offspring sex. Energy derived from lipids and proteins was significantly lower ( $F = 8.93$ , d.f. = 1,  $P < 0.001$ ) in 2015 than in 2014 but was independent of offspring sex ( $F = 0.049$ , d.f. = 1,  $P = 0.83$ ).

provided additional evidence of the lack of bias in sampling. Average age of young, sampling date and birthdate were not different according to offspring sex (Table 3). Time of day did not affect protein concentrations (Welch two-sample  $t$ -test:  $t = -0.42$ , d.f. = 261.02,  $P = 0.68$ ), but lipid concentrations were higher in the morning than in the evening (Welch two-sample  $t$ -test:  $t = 2.84$ , d.f. = 207.46,  $P = 0.005$ ). On average, evening samples were collected later in the season (Welch two-sample  $t$ -test;  $t = -2.26$ , d.f. = 258.58,  $P = 0.02$ ).

## Determinants of milk composition

### Lipid and protein

The concentrations of protein and lipids varied substantially (24.04–151.50 mg·mL<sup>-1</sup> and 46.76–316.10 mg·mL<sup>-1</sup>, respectively), with significant year effects (Table 3). Detailed distributions, model selection and multimodel inference summaries for these macronutrients are provided in Appendices S2 and S5.

### Lipid:protein ratio

Because sampling date and birthdate of young were highly correlated (Table 4), models fitted with each variable yielded similar results. We report models using birthdate because they had greater strength of evidence than those including sampling date (Likelihood-ratio test:  $X^2 = 7.11$ , d.f. = 1,  $P < 0.001$ ). There was support ( $w_i \geq 0.05$ ) for three models explaining LPR (Table 5). Model averaging (Table 6) suggested that LPR was best explained by negative interactions between maternal condition and both year and offspring sex (Fig. 2a,c), and by negative effects of birthdate (Fig. 2b) and maternal size (Fig. 2d). Detailed multimodel inference summaries are provided in Appendix S3.

### Juvenile survival

Longevity estimates were accurate and precise for young that died before mid-December because we located every marked individual almost daily. We excluded 12 late-born pouch young from the analyses to avoid underestimating longevity because they were last seen alive when we left the study area. Most young disappeared before permanent pouch emergence and only 11 (10%) survived to 550 days (2014: six females, two males; 2015: two females, one male). Mean longevity was  $321 \pm 97$  (range: 201–550) days. Two models had significant support (i.e.  $w_i \geq 0.05$ ; Table 7). Model-averaged estimates (Table 6) confirmed that offspring longevity increased with maternal condition and lipid concentration (Fig. 3a), but increased with protein concentration only for sons (Fig. 3b). Finally, longevity decreased with birthdate and was lower in 2015 than in 2014 (Table 6). Detailed multimodel inference results are presented in Appendix S4.

## Discussion

Forage productivity and milk composition of eastern grey kangaroos differed markedly between years. The substantial annual and seasonal differences in forage production during lactation in the 2 years of the study were associated with differences in maternal condition and milk composition, that we attribute to much lower forage production in 2015. Milk composition was further explained by offspring sex through an interaction with

**Table 4** Pearson's product-moment correlation coefficients between maternal, pouch young and environmental variables characterizing eastern grey kangaroo milk samples ( $n = 103$ ) collected at Wilsons Promontory National Park, Australia, in 2014 and 2015

	Pearson's product-moment correlation coefficients ( $r_p$ )									
	Age of pouch young	$P$	Sampling date	$P$	Birthdate	$P$	Maternal relative condition	$P$	Maternal leg length	$P$
Sampling date (1 = 29 July)	-0.06	0.306	-	-	-	-	-	-	-	-
Birthdate (1 = 1 August)	-0.53	<0.001	0.87	<0.001	-	-	-	-	-	-
Maternal relative condition	0.07	0.209	0.28	<0.001	0.19	<0.001	-	-	-	-
Maternal leg length (mm)	0.16	0.006	-0.17	<0.004	-0.22	<0.001	-0.13	0.027	-	-
Total forage production during lactation (g·m <sup>-2</sup> )	0.48	<0.001	0.15	<0.010	-0.11	0.059	0.19	<0.001	0.12	0.033

**Table 5** Model selection summary for the effects of maternal characteristics, age and sex of pouch young, birthdate and year on lipid-to-protein ratio in eastern grey kangaroo milk

Model ID	LL	K	AIC <sub>c</sub>	$\Delta_i$	$w_i$
<b><i>m</i> {all + <i>S</i> × <i>C</i> + <i>C</i> × yr}</b>	<b>-251.08</b>	<b>12</b>	<b>527.24</b>	<b>0.00</b>	<b>0.52</b>
<b><i>m</i> {all + <i>S</i> × <i>C</i> + <i>C</i> × yr + <i>S</i> × yr}</b>	<b>-250.43</b>	<b>13</b>	<b>528.11</b>	<b>0.87</b>	<b>0.34</b>
<b><i>m</i> {all + <i>S</i> × <i>C</i> × yr}</b>	<b>-250.17</b>	<b>14</b>	<b>529.79</b>	<b>2.55</b>	<b>0.15</b>
<i>m</i> {all + <i>S</i> × <i>C</i> + <i>S</i> × yr}	-276.57	12	578.22	50.98	0.00
<i>m</i> {all + yr × <i>C</i> + <i>S</i> × yr}	-276.95	12	578.97	51.73	0.00
<i>m</i> {all + <i>S</i> × <i>C</i> }	-281.18	11	585.27	58.03	0.00
<i>m</i> {all + <i>S</i> × <i>L</i> + <i>S</i> × yr}	-301.37	11	625.64	98.40	0.00
<i>m</i> {all + <i>S</i> × <i>L</i> }	-303.96	11	630.82	103.58	0.00
<i>m</i> {all}	-310.36	10	641.47	114.24	0.00
<i>m</i> {yr + bd}	-320.27	7	654.93	127.69	0.00
<i>m</i> { <i>S</i> × <i>C</i> }	-325.20	8	666.89	139.65	0.00
<i>m</i> {bd}	-339.28	4	686.69	159.45	0.00
<i>m</i> { <i>S</i> × <i>L</i> }	-340.14	8	696.76	169.52	0.00
<i>m</i> { <i>a</i> + <i>a</i> <sup>2</sup> }	-346.53	5	703.27	176.03	0.00
<i>m</i> { <i>S</i> }	-360.84	4	729.82	202.58	0.00
<i>m</i> {yr}	-362.49	4	733.11	205.88	0.00
<i>m</i> { <i>L</i> }	-362.97	4	734.07	206.83	0.00
<i>m</i> {.}	-364.33	3	734.73	207.50	0.00
<i>m</i> { <i>C</i> }	-364.31	4	736.76	209.53	0.00

LL, log-likelihood; K, number of estimated parameters; AIC<sub>c</sub>, Akaike's Information Criterion corrected for small sample size;  $\Delta_i$ , difference between model AIC<sub>c</sub> and the lowest AIC<sub>c</sub> in the model set;  $w_i$ , Akaike model weight.

Models are ranked in descending order of support, and models with significant support ( $w_i \geq 0.05$ ) are in bold.

**Table 6** Model-averaged parameter estimates explaining variation in lipid-to-protein ratio in eastern grey kangaroo milk and offspring longevity, using standardized variables (reduced and centered to the mean)

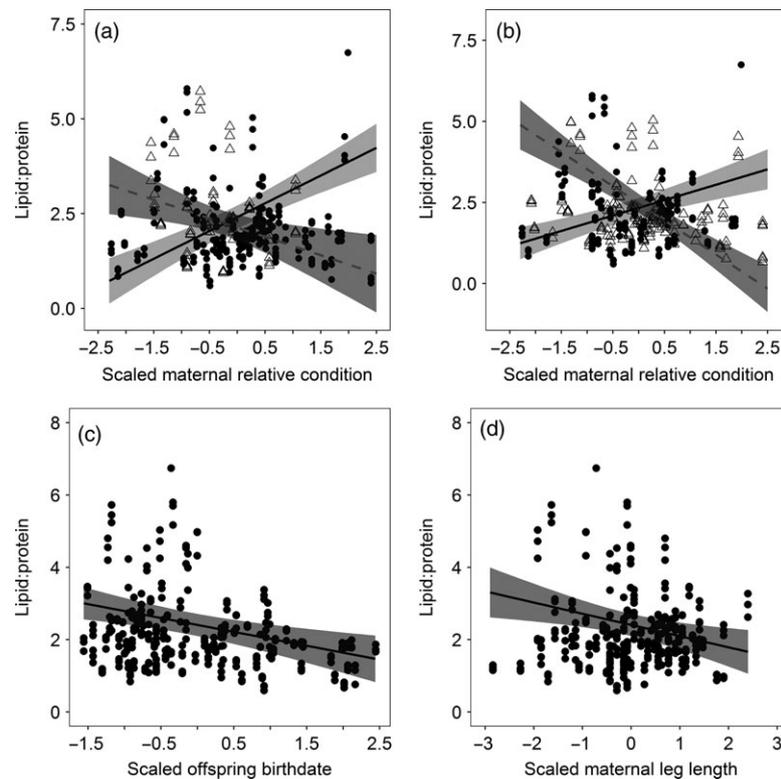
Terms	Parameter value	95% confidence interval
<b>Lipid:protein</b>		
Age of young	-0.23	-0.34, -0.13
Year (reference = 2014)	-0.06	-0.26, 0.13
Sex of offspring (reference = female)	0.20	-0.07, 0.46
Maternal relative condition	0.16	-0.03, 0.35
Maternal leg length	-0.31	-0.52, -0.10
Offspring birthdate	-0.38	-0.61, -0.16
Year × sex	0.41	-0.24, 1.07
Maternal condition × sex	-1.46	-1.78, -1.14
Maternal condition × year	-1.23	-1.53, -0.92
<b>Offspring longevity</b>		
Age of young	-5.64	-13.19, 1.91
Year (reference = 2014)	-15.38	-28.43, -2.32
Maternal relative condition	52.98	44.65, 61.32
Offspring birthdate	-23.15	-29.24, -17.06
Offspring sex	-35.51	-43.42, -27.59
Lipid concentration	18.99	12.13, 25.85
Protein concentration	45.37	30.72, 60.02
Lipid × sex	15.66	-0.87, 32.20
Protein × sex	36.97	19.95, 53.99

maternal condition, as mothers in better condition allocated more protein to sons than to daughters. Interestingly, larger mothers in our study area tend to have more sons (Le Gall-

Payne, Coulson & Festa-Bianchet, 2015), and during our study they produced milk with more protein than smaller mothers. Moreover, milk composition had a direct and sex-dependent effect on offspring survival.

Forage production during lactation in 2015 was half of that in 2014, and mothers in 2015 were in poorer condition and produced lower-energy milk than mothers in 2014. Similarly, food-restricted red deer hinds (*Cervus elaphus*) produced less milk, containing less lipids, protein and glucose, than control hinds (Landete-Castillejos *et al.*, 2003), and female tamar wallabies increased food intake to avoid metabolizing fat reserves as lactation progressed (Cork, 1991). The concomitant differences in milk composition and forage production suggests that eastern grey kangaroos relied at least partly on current nutrition to meet lactational requirements. Lactating kangaroos increase their bite rate and midday foraging time compared to non-lactating females (Gélin *et al.*, 2013). Increasing foraging effort may not, however, compensate for the costs of lactation when forage is scarce (Parker *et al.*, 2009). Low forage production likely lowered protein intake, but females that maintained relatively high condition in the poor year (2015) produced high-protein milk, suggesting that they were able to buffer against forage scarcity.

Milk composition was also affected by birthdate. At mid lactation, early-born kangaroos received more milk lipids than proteins, independently of maternal condition. For temperate herbivores, dietary protein might not be enough for milk production before the onset of spring vegetation growth (Taillon, Barboza & Côté, 2013). Also, mothers of early-born pouch young face both low food abundance and high thermoregulatory costs because of cooler temperatures in late winter and



**Figure 2** Determinants of milk lipid-to-protein ratio in eastern grey kangaroos. (a) Model-averaged effect of scaled maternal relative condition moderated by year (a proxy of forage production). 2014: closed circles, solid line; 2015: open triangles, dotted line. (b) Model-averaged effect of scaled maternal relative condition as a function of offspring sex. The relationships between scaled maternal relative condition  $\times$  offspring sex for lipid and protein concentrations mirror the effect shown here. Sons: open triangles, dotted line. Daughters: closed circles, solid line. (c) Model-averaged effect of scaled offspring birthdate. (d) Model-averaged effect of scaled maternal leg length (range: 488–562 mm). Details on multimodel inference are provided in Appendix S3.

**Table 7** Model selection summary for the effects of milk composition, sex of pouch young, birthdate and year on eastern grey kangaroo survival to weaning

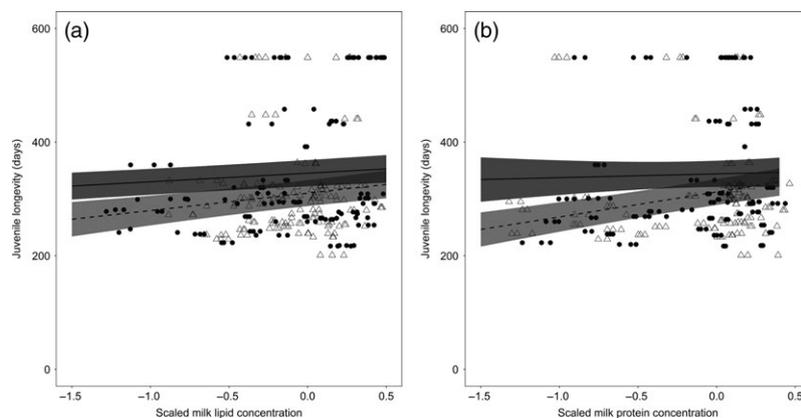
Model ID	LL	K	AIC <sub>c</sub>	$\Delta_i$	$w_i$
<b>Y {S <math>\times</math> lip + S <math>\times</math> prot}</b>	<b>-1221.91</b>	<b>12</b>	<b>2468.97</b>	<b>0.00</b>	<b>0.64</b>
<b>Y {S <math>\times</math> prot}</b>	<b>-1223.58</b>	<b>11</b>	<b>2470.14</b>	<b>1.17</b>	<b>0.36</b>
Y {all}	-1231.52	10	2483.84	14.87	0.00
Y {S $\times$ lip}	-1230.59	11	2484.14	15.17	0.00
Y {yr $\times$ bd}	-1344.58	6	2701.47	232.50	0.00
Y {a}	-1383.68	4	2775.51	306.54	0.00
Y {prot}	-1386.23	4	2780.61	311.64	0.00
Y {C}	-1402.46	4	2813.07	344.09	0.00
Y {lip}	-1419.23	4	2846.60	377.63	0.00
Y {S}	-1429.38	4	2866.90	397.93	0.00
Y {.}	-1438.05	3	2882.18	413.20	0.00

LL, log-likelihood; K, number of estimated parameters; AIC<sub>c</sub>, Akaike's Information Criterion corrected for small sample size;  $\Delta_i$ , difference between model AIC<sub>c</sub> and the lowest AIC<sub>c</sub> in the model set;  $w_i$ , Akaike model weight.

Models are ranked in descending order of support, and models with significant support ( $w_i \geq 0.05$ ) are in bold.

there might be higher costs to deplete protein stores than fat stores (Barboza & Parker, 2008). Our results provide a rare example of how forage production, and potentially temperature, affect maternal condition and milk composition in a wild terrestrial mammal. Eastern grey kangaroos exhibit a conservative reproductive strategy and are partly income breeders (Gélin, Coulson & Festa-Bianchet, 2016); lower milk partial energy in a low-forage year suggests that females attempt to maintain condition by transferring environmental costs to their young. Females, however, acted as capital breeders early in the season by using body stores to meet the high lactation costs, suggesting a resource-modulated lactation strategy.

Kangaroos are polygynous (Fisher *et al.*, 2002), and successful sons could provide higher fitness returns compared to daughters (Trivers & Willard, 1973). Since milk protein is of paramount importance to pre-weaning growth (Landete-Castillejos *et al.*, 2001; Parker *et al.*, 2009), greater protein transfer by mothers could improve sons' reproductive potential by increasing their adult body size (Miller *et al.*, 2010). We found that mothers of sons produced milk with higher proportions of protein than mothers of daughters (Robert & Braun, 2012), but this effect was moderated by maternal body condition. Mothers in better relative condition favored protein allocation to sons



**Figure 3** Model-averaged effects of (a) scaled lipid concentration and (b) scaled protein concentration in milk of eastern grey kangaroos on offspring survival to weaning (i.e. 550 days). Sons: open triangles, dotted line. Daughters: closed circles, solid line. Details on multimodel inference are provided in Appendix S4.

while mothers in poorer condition favored protein allocation to daughters, revealing a condition-specific and sex-biased care strategy (Trivers & Willard, 1973). This result supports previous findings that heavier macropod mothers tend to have more sons (Sunnucks & Taylor, 1997; Le Gall-Payne *et al.*, 2015), probably because they can provide high-quality care (Robert, Schwanz & Mills, 2010). In marsupials, milk composition is autonomously controlled by the mammary gland and is unaffected by suckling patterns (Trott *et al.*, 2003). It is therefore unlikely that higher protein allowance for sons was a non-adaptive maternal response to higher suckling and nutrient demand (Landete-Castillejos *et al.*, 2005), because despite different compositions, partial energy density per unit volume of milk was similar for sons and daughters. We do not know if females produced different amounts of milk. Data on milk volume would allow a better understanding of sex-biased care, with potential sex differences in production and macronutrient dilution (Nommsen *et al.*, 1991; Hinde, 2009; Hinde *et al.*, 2014).

Surprisingly, structural size affected milk composition independently of maternal condition. Larger females produced high-protein milk compared to smaller females. Kangaroos have indeterminate skeletal growth, so larger individuals tend to be older (Jarman, 1989). Age could therefore partly explain the effect of size, as experienced females may select better food patches. Alternatively, larger females may be able to allocate more resources to lactation (Pomeroy, Fedak & Anderson, 1999) because smaller females face a steeper trade-off between allocating protein stores to structural growth or to milk production (Stearns, 1989).

In 2015, when forage production and maternal condition were poor, offspring survival and population reproductive rate were lower compared to 2014. Model selection revealed that offspring longevity was affected by maternal condition, birthdate and milk composition. Higher lipid concentrations increased longevity for both sexes, but low protein concentrations had a greater negative effect on the longevity of sons. Higher milk protein during mid lactation was also associated with higher offspring mass at 14 months in captive tamar wallabies (Robert &

Braun, 2012). In kangaroos, sons require more protein than daughters, as they grow faster during the pouch stage (Poole *et al.*, 1982), and selection favors rapid structural growth in male juveniles of sexually dimorphic species (Arnould *et al.*, 2001). Marsupial milk composition follows a temporal protein delivery sequence, partly acting as signal for tissue development (Sharp *et al.*, in press). Apart from retarding structural growth, deficiency in specific compounds could also cause abnormal development (Sharp *et al.*, in press) and weaken resistance to pathogens (Daly *et al.*, 2007; Joss *et al.*, 2009), jeopardizing survival. Our results are conservative, because it is possible that we overestimated the age of high-provisioned young. Our analyses could not compare milk attributes to early survival, which was likely affected by poor environmental conditions.

We found that early-born young survived longer. A similar result in mountain goats (*Oreamnos americanus*) was attributed to early-born kids having a longer period of access to high forage quality in a temperate environment (Côté & Festa-Bianchet, 2001). We remain cautious about the negative effect of birthdate on longevity, as seasonal conditions likely fluctuate between years in our study area.

In conclusion, we found strong support for sex-biased milk macronutrient allocation in wild kangaroos. Asynchronous reproduction revealed effects of environmental conditions on a direct component of maternal care. Our study is a rare example of concomitant deterioration in milk quality and forage production in a large wild terrestrial mammal.

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## Supporting Information

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

**Appendix S1.** Candidate model lists.

**Appendix S2.** Model selection and multimodel inference summaries for protein and lipid concentrations.

**Appendix S3.** Detailed multimodel inference summaries for lipid-to-protein ratio (Tables S7-NDASH-S14).

**Appendix S4.** Detailed multimodel inference summaries for survival to weaning (Tables S15-NDASH-S22).

**Appendix S5.** Distribution of the macronutrient concentrations.

[Correction added on 31 March 2017, after first online publication: The Supporting Information was previously omitted and this has been corrected in this current version.]