Repeatability in lactation performance and the consequences for maternal reproductive success in gray seals

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Abstract. In mammals, the most significant maternal effect on offspring growth during lactation is the ability of females to efficiently transfer milk energy to their neonates. However, despite the importance of the transfer of milk energy to both maternal and offspring fitness, nothing is known about the extent to which variation among females may be attributed to differences in individual quality or environmental variation in natural populations. We measured repeatability over multiple lactation periods in components of lactation performance in free-ranging, multiparous gray seal (*Halichoerus grypus*) females to examine to what extent variation among females in pup weaning mass may be attributed to inherent differences in their physiological capacity to deliver milk energy. Levels of repeatability were high for milk composition ($r = 0.38$–0.50), daily milk output ($r = 0.46$), and the duration of lactation ($r = 0.57$), demonstrating that there are consistent differences among females in these characters across lactations and that the overall capacity of gray seal females to deliver milk energy to their pups is characteristic of individuals. The repeatability in pup weaning mass ($r = 0.48$) was consistent with the values for the components of total milk energy output and suggests that, over a large proportion of their reproductive life, individual gray seal females will consistently wean pups with greater or lesser probabilities of survival. Our results suggest that inherent differences among females in their physiological capacity to deliver milk energy may be an important component of variation in individual quality and, thus, lifetime reproductive success in mammals. High levels of repeatability also suggest that components of milk energy transfer may have a significant heritable genetic basis.

Key words: gray seal; Halichoerus grypus; *individual quality; lactation length; maternal effects; milk composition; milk energy output; repeatability; weaning mass.*

Introduction

Maternal effects occur when the phenotype of the mother affects the phenotype of her offspring in addition to the direct effects of the genes contributed by her. These effects are of interest to ecologists because of their potential to influence the evolutionary dynamics of populations (Räsänen and Kruuk 2007). Maternal effects are experienced by offspring as environmental effects and can represent the most important component of an individual's environment during development (Mousseau and Fox 1998). Therefore, variation among females in the quality of the environments they provide can have important consequences for variation in offspring phenotypes and, in turn, for variation in both maternal and offspring fitness. Identifying the sources of variation in maternal effects among individuals is necessary to understand the conditions under which maternal effects influence both population dynamics and the evolution of life history characters (Clutton-Brock 1988, Räsänen and Kruuk 2007).

In mammals, both offspring growth rate during lactation and the mass of offspring at weaning have a significant impact on their subsequent survival, growth, and reproductive success (e.g., Wauters et al. 1993, Festa-Bianchet et al. 2000, Hall et al. 2001). The most significant maternal effect on offspring growth during the lactation period is the ability of females to efficiently transfer milk energy to their neonates. Lactation is the most energetically demanding period in the life of a female mammal (Rogowitz 1996). Females offset these demands by increasing energy intake and/or mobilizing body energy stores and, as a result, it is frequently assumed that variation among females in milk production and, thus, in offspring growth is largely a function of variation in energy acquisition prior to and/or during the lactation period. However, studies on domesticated cattle, sheep, and goats suggest that individual females may vary in their physiological capacity to deliver milk energy to their offspring independent of variation in factors such as resource availability, levels of body energy stores, or body size. In these species both milk composition and daily milk output are characteristic of individual females as a consequence of significant levels of heritable genetic variation (e.g., Analla et al. 1996, Sanna et al. 1997, Van Tassell et al. 1999). In turn,

genetic differences in milk production explain a significant proportion of observed variation among females in the weaning mass of their offspring (e.g., Montaño-Bermudez and Nielsen 1990, Meyer et al. 1994). Although average milk composition and milk output during lactation have been characterized in many wild species (e.g., Oftedal and Iverson 1995), only a few studies have examined variation among individual females in one or both of these components (Stern et al. 1997, Mellish et al. 1999, Crocker et al. 2001, Gjøstein et al. 2004, Lang et al. 2005, Power et al. 2008), and no study has examined whether milk composition or the rate of milk production may vary across lactation periods within individual females in natural populations.

We studied variation in components of milk energy transfer across lactation periods within individual gray seal females (*Halichoerus grypus*). Lactation energetics in this species have been well studied (Iverson et al. 1993, Mellish et al. 1999), and the relationship between milk energy transfer, weaning mass, and the subsequent survival of offspring is known. Like other large-bodied phocid seals (Family Phocidae), gray seal females are capital breeders, supporting all of the energetic costs of lactation from the body energy reserves acquired prior to parturition (see Plate 1). Females give birth to a single pup each year beginning at age 4–5 years, and some will continue to reproduce to age 30 or more (Bowen et al. 2006). Pups consume only milk during the lactation period and all maternal care ends at weaning. At the end of a 16–18 day lactation period (Bowen et al. 1992b) females abruptly wean their pups and depart the colony. Pups then rely on the energy stores deposited during the suckling period to survive a 3–4 week post-weaning fast before going to sea to forage for the first time (Noren et al. 2008). Total milk energy intake, which is a product of milk energy content, daily milk output, and the duration of lactation, is the strongest predictor of pup weaning mass and condition in gray seals, accounting for 88% of observed variation among females (Mellish et al. 1999). In turn, weaning mass and condition affect post-weaning survival with larger, fatter pups having a greater probability of surviving to one year of age (Hall et al. 2001). Maternal energy stores at parturition play an important role in total milk energy transfer in this species; however, a substantial proportion of the variation in total milk energy output observed among females cannot be explained by variation in maternal postpartum mass (37%) or levels of body protein or fat stores (43% and 72%, respectively; Mellish et al. 1999), suggesting that individual females vary in their physiological capacity to deliver milk energy to their offspring.

We studied individual, multiparous gray seal females over multiple lactation periods to test the hypothesis that variation among females in offspring weaning mass is a consequence of inherent variation in their physiological capacity to deliver milk energy. To do this we measured the repeatability of milk composition, daily milk output, lactation length, and maternal postpartum mass. Repeatability measures the proportion of phenotypic variation in a trait that is attributable to either genetic or permanent environmental effects among individuals. Therefore, it quantifies the extent to which a trait is characteristic of individuals and can provide an upper bound to heritability (Falconer and Mackay 1996, Dohm 2002).

Materials and Methods

Field procedures

Our study was conducted on Sable Island (43°55′ N, 60°00′ W), located ~300 km east southeast of Halifax, Nova Scotia, Canada, during the 1999 through 2004 breeding seasons (December–January). The number of pups born on Sable Island has been increasing since the early 1960s, and during the years of the study pup production increased at a rate of ~7% per year with an estimated 41 500 pups born in 2004 (Bowen et al. 2003, 2007).

Study females were a subset of those that were permanently marked between 1963 and 1989 with individually unique, hot-iron brands shortly after weaning and, thus, were of known age. In January of 1999, we selected 23 multiparous females with known parturition dates, covering the full age range (10–36 yr) of marked females in the population. Females and their pups were sampled on day 3 postpartum (early lactation) and again on day 12 postpartum (peak lactation). Day 3 postpartum was selected to allow the female–pup bond to develop and, thereby, minimize the risk of abandonment as a result of handling. Day 12 postpartum was selected as being representative of peak milk energy composition and output (Iverson et al. 1993). To the extent possible, all returning females initially sampled in 1999 were studied during successive lactation periods in 2000 through 2004. The presence of a study female in the colony was determined during daily surveys throughout the colony and/or during weekly whole-island censuses of all branded individuals as described in Bowen et al. (2006). In cases where returning females were not located on the day of parturition, birth date was estimated for recently born pups based on an assessment of pup age derived from pelage/morphology stage classes (Bowen et al. 2003).

Adult females were captured using a hinged pole net. Following capture, females and pups were weighed to the nearest 0.5 kg. Females were then mildly sedated with an intravenous injection of diazepam (25 mg; Sandoz Canada, Boucherville, Quebec, Canada) and given an intramuscular injection of oxytocin (1.5 mL at 20 IU/mL; Vétoquinol Canada, Lavaltrie, Quebec, Canada) to facilitate milk letdown. A milk sample (~60 mL) was then collected by suction. Milk samples were stored in 30-mL Nalgene bottles at −20°C until analysis for proximate composition. Prior to release on day 3 postpartum, pups were given an individually numbered hind-flipper tag (Rototag, Dalton Supplies Limited, Henley on Thames, UK) to permit post-

weaning identification. Pairs were sighted daily throughout lactation to ensure that the female and pup were still together and to obtain an accurate date of weaning. On the day of weaning, which is marked by the departure of the female, pups were weighed to the nearest 0.5 kg.

Severe weather conditions and the demands of concurrent sampling resulted in some early lactation samples being taken on day 4 postpartum ($n = 3$), and some late lactation samples being taken on day 10 ($n = 2$), day 11 ($n = 2$) or day 13 ($n = 6$) postpartum. On three occasions study females could not be sampled during early lactation and samples were obtained at day 12 postpartum only. Three females weaned prior to the second sampling. We were only able to obtain milk samples in late lactation during one season for female 450 and, therefore, she was excluded from analyses of milk composition, average milk energy, and daily milk output (see *Materials and methods: Data analysis*).

All sampling protocols were conducted in accordance with the requirements of the Canadian Council on Animal Care and were approved by Dalhousie University's Committee on Laboratory Animals and by the Department of Fisheries and Oceans Animal Care Committee.

Sample analysis

Milk samples were analyzed for fat (Roese-Gottlieb, AOAC 2000*a*), protein (macro-Kjeldahl, AOAC 2000*b*), and dry matter content (as described in Iverson et al. 1993). Milk samples were not analyzed for carbohydrate content as it has been previously demonstrated that this is a very minor component of phocid seal milks (Oftedal and Iverson 1995), as was confirmed by the low residuals from the sum of fat and protein compared to dry matter.

Data analysis

We estimated average milk energy and daily milk output between early and peak lactation for each female. In gray seals, milk fat content increases from parturition until reaching peak values at approximately day 8 postpartum and then remains relatively stable over the remainder of lactation (Iverson et al. 1993). Therefore, we calculated a weighted harmonic mean for a female's percentage milk fat by assuming a linear increase in milk fat content from initial sampling to day 8 postpartum, followed by a stable milk fat content through to the second sampling. The proportion of the measurement period spent in each phase was the weighting factor. Milk protein content remains relatively constant throughout lactation in gray seals (Iverson et al. 1993) and, therefore, we calculated an average of the early and peak lactation values. The average milk energy content (MJ/kg) was then calculated using the values of 39.3 MJ/kg and 23.6 MJ/kg for fat and protein, respectively (Blaxter 1989).

Given the high concentration of fat in gray seal milk and its greater energetic value relative to protein, variation in milk fat content both among and within females will have a significant impact on the observed repeatability for milk energy. Because it was not possible to obtain milk samples at the same time point of early lactation in successive lactations, the use of early milk fat values to calculate milk energy may increase the within-female variation in our estimates. However, because the rate of change in milk fat content over early lactation is known to differ among gray seal females (Mellish et al. 1999), the use of peak values alone would ignore a known source of variation among females and would overestimate milk energy and, potentially, repeatability for the sampling period. Therefore we used both early and peak milk fat contents in our analyses.

For each female, daily milk energy output (MEO, MJ/d) was estimated from her pup's mass gain (PMG, kg/d) between early and peak lactation, based on the regression equation for gray seals from Iverson et al. (Iverson et al. 1993; $r^2 = 0.998$): PMG = MEO $\times$ 0.031 − 0.156.

Daily milk output (kg/d) was then estimated by dividing the daily milk energy output by the average milk energy content of each female.

Variance components derived from linear mixed-effects (LME) models with female identity as a random effect were used to calculate repeatability (intraclass-correlation coefficient) following West et al. (2007). Repeatability was estimated for milk fat and protein contents at peak lactation only as both components are stable during this period. Percentage values were arcsine transformed prior to analysis. Female age, both as linear and quadratic function, was entered as a covariate in all models. Although maternal postpartum mass is known to influence the duration of lactation in gray seals, it is not a factor in milk composition or daily milk output (Mellish et al. 1999); therefore day 3 postpartum female mass (as a proxy for maternal postpartum mass; Bowen et al. 2006) was entered as a covariate in the LME analyses for lactation length and weaning mass only. Mass loss per day is relatively constant over lactation in gray seals (Mellish et al. 1999); therefore, where female mass was obtained on day 4 postpartum, mass was adjusted to day 3 postpartum prior to analysis using the mass loss per day for each individual female for that lactation period. Male pups are, on average, larger than female pups throughout lactation; therefore, pup sex was entered as a covariate into the LME for daily milk output and weaning mass. Pup sex was not entered into the LME for lactation length because previous analyses suggest that the difference in lactation length between male and female pups (<0.5 d; Bowen et al. 2006) is unlikely to be biologically significant. LME analyses were conducted in S-Plus version 7.0 (S-Plus 2005). Only females with ≥ 2 observations were included in the analyses. Competing models were evaluated on the basis of the lowest second order Akaike information criteria (AIC$_c$), smallest ΔAIC$_c$, and highest AIC weights (w; Burnham and Anderson 2002). Repeatability (r) was
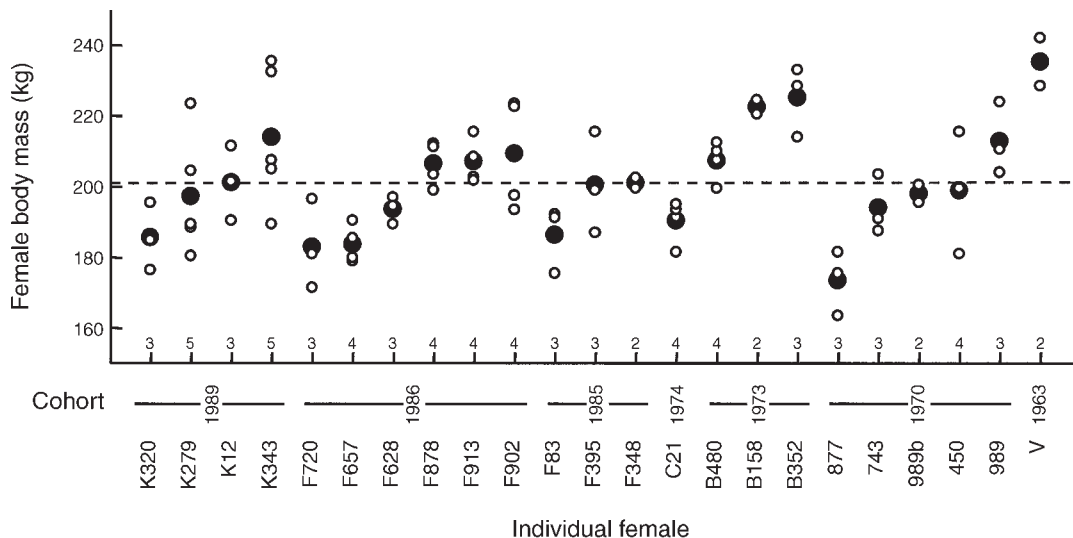
Fig. 1. Average (solid circles) and absolute (open circles) day 3 postpartum body mass of individual gray seal females (*Halichoerus grypus*) on Sable Island, Nova Scotia, Canada, over multiple lactation periods. The dashed line is the estimated marginal mean obtained from the linear mixed-effects model with female as a random effect only. Numbers above the axis indicate the number of observations per individual.

calculated from the within- (σ^2) and among- (σ_a^2) variance components following West et al. (2007): $r = \sigma_a^2/(\sigma^2 + \sigma_a^2)$.

Coefficients of variation (CV) were calculated using the among-female standard deviation and the overall fitted average obtained from the LME models with female identity as a random effect only. Correlation coefficients (ρ) were obtained using PROC MIXED in SAS (SAS Institute 2003) following the procedure of

Hamlett et al. (2004) for the estimation of correlation coefficients in the presence of repeated measures. Standard errors are reported throughout.

RESULTS

There was a 57-kg range in the average day 3 postpartum mass among the females (CV 6.4%, Fig. 1). Day 3 postpartum mass varied with age, with support for age as both linear and nonlinear effects; however, the Akaike

TABLE 1. Repeatability estimates (r) for components of lactation performance in gray seals (*Halichoerus grypus*) on Sable Island, Nova Scotia, Canada, based on the variance components from linear mixed-effects models.

Lactation component	Model	n	df	AIC_c	Δ	w	σ_a^2	σ^2	r
Female mass† (kg)	female (random effect only)	23, 76	1	621.7	6.1	0.03	165.1	124.2	0.57
	female age		2	**616.9**	**1.3**	**0.34**	180.8	108.0	0.63
	female age + female age^2		3	**615.6**	**0.0**	**0.63**	271.5	82.9	0.77
Milk fat, peak lactation (%)	female (random effect only)	22, 73	1	**267.9**	**0.0**	**0.94**	1.377	1.371	0.50
Milk protein, peak lactation (%)	female (random effect only)	22, 73	1	**173.5**	**0.0**	**0.98**	0.252	0.409	0.38
Milk energy (MJ/kg)	female (random effect only)	22, 70	1	**192.0**	**0.0**	**0.95**	0.288	0.618	0.32
Milk output (kg/d)	female (random effect only)	22, 70	1	**132.4**	**0.0**	**0.92**	0.196	0.231	0.46
Lactation length (d)	female (random effect only)	23, 74	1	319.2	6.2	0.04	2.253	2.731	0.42
	female mass		2	**313.0**	**0.0**	**0.84**	2.688	2.022	0.57
Total milk energy output (MJ)	female (random effect only)	23, 72	1	1013.3	23.7	0.00	75 419.3	45 640.9	0.62
	female mass + female age + female age^2		4	**989.5**	**0.0**	**0.92**	46 831.9	38 646.8	0.55
Weaning mass (kg)	female (random effect only)	23, 74	1	497.6	13.2	0.00	50.4	25.5	0.66
	pup sex + female mass + female age + female age^2		5	**484.4**	**0.0**	**0.77**	19.7	21.8	0.48

Notes: Favored models (in bold) were selected on the basis of lowest second-order Akaike's information criteria (AIC_c), lowest ΔAIC_c, and highest Akaike weight (w). For the complete set of models run see the Appendix. For n, the first value is the number of females; the second value is the number of observations included in the models. Variance components are: σ_a^2, among-groups variance; σ^2, within-group variance.
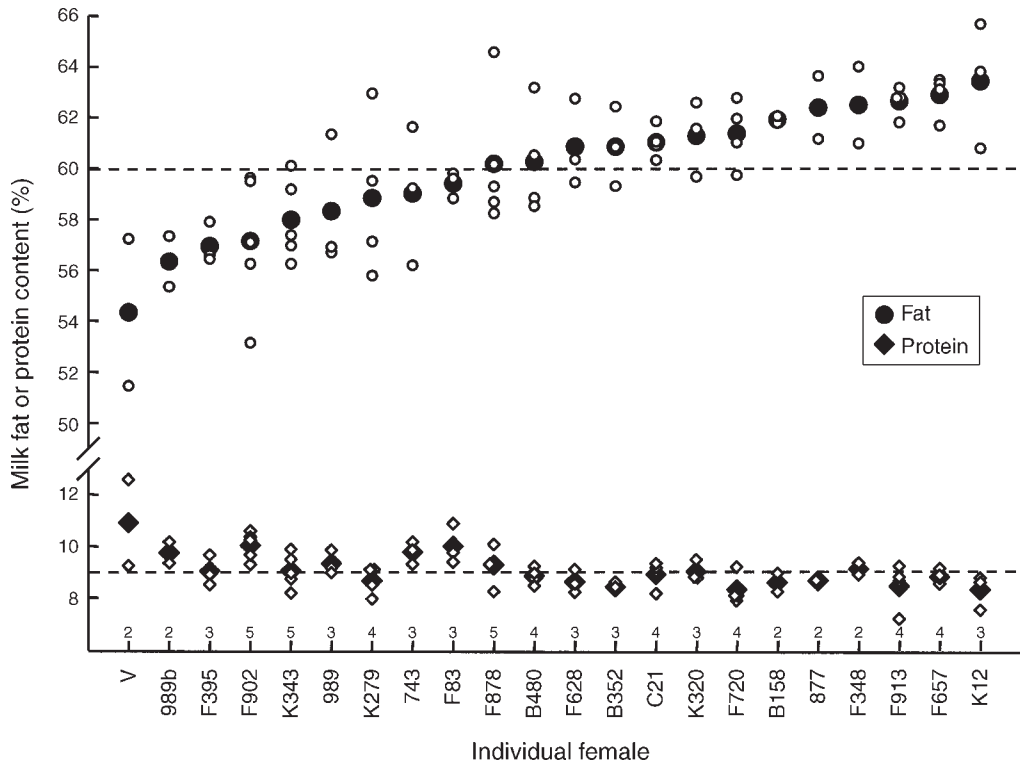
† Day 3 postpartum.

Fig. 2. Average (solid symbols) and absolute (open symbols) milk fat and milk protein content at peak lactation for individual gray seal females over multiple lactation periods ranked from lowest to highest average milk fat content. Dashed lines are the estimated marginal means obtained from linear mixed-effects models with female as a random effect only. Numbers above the axis indicate the number of observations per individual.

weight for age as a nonlinear effect was much larger (Table 1). Estimated marginal means (averages from the LME model) indicated that the day 3 postpartum mass of females increased between the ages of 10 and 13 years (Fig. 1). Repeatability for day 3 postpartum mass was high for both models with repeatability highest for the model with age as a nonlinear function (Table 1).

Milk fat content at peak lactation ranged from an average of 54.3% to 63.4% with an overall average of 60.0% ± 0.49% (CV = 3.3%, Fig. 2). Milk protein content at peak lactation ranged from 8.3% to 10.9% with an overall average of 9.0% ± 0.13% (CV = 5.6%, Fig. 2). There was no evidence of variation with female age in either milk component (Table 1; for results from all models run see the Appendix). Repeatability for milk fat content at peak lactation was greater than that for milk protein content (Table 1) indicating a greater consistency within females for milk fat content. Variation in average milk energy content among females was low (CV = 2.2%) ranging from 23.1 MJ/kg to 25.2 MJ/kg (overall average, 24.2 ± 0.15 MJ/kg; Fig. 3e). There was no evidence of variation in average milk energy content with female age (Table 1). Repeatability for average milk energy content was lower than that for either peak milk fat or protein content (Table 1).

Daily milk output varied substantially among females (CV = 13.5%) with a twofold range in the average values

(2.1–4.1 kg/d, overall average, 3.2 ± 0.11 kg/d; Fig. 3d). There was no evidence for variation with maternal age or pup sex. Repeatability for daily milk output was high (Table 1).

Lactation length also varied substantially among females (CV = 8.6%), ranging from an average of 12.3 d to 21.0 d (overall average, 17.3 ± 0.37 d; Fig. 3c). Lactation length did not vary with female age but did vary with female mass (Table 1). However, including day 3 postpartum mass as a covariate had a greater effect on the within-group variance than the among-group variance, resulting in a higher repeatability for lactation length when day 3 postpartum mass was included in the model (Table 1). Nevertheless, lactation length was not strongly correlated with day 3 postpartum mass ($\rho = 0.311$; $n = 23$ females, 74 observations) suggesting that variation in female postpartum mass explained only a small proportion of the overall variation in lactation length among females.

Weaning mass exhibited a more than twofold variation among females (CV = 14.7%), ranging from an average of 29.8 kg to 63.0 kg (overall average, 48.4 ± 1.60 kg; Fig. 3a). Weaning mass varied with pup sex, female mass, and female age as a nonlinear effect (Table 1). The effect for female mass was consistent with the influence of mass on lactation length (Table 1). However, in contrast to the results for day 3 postpartum
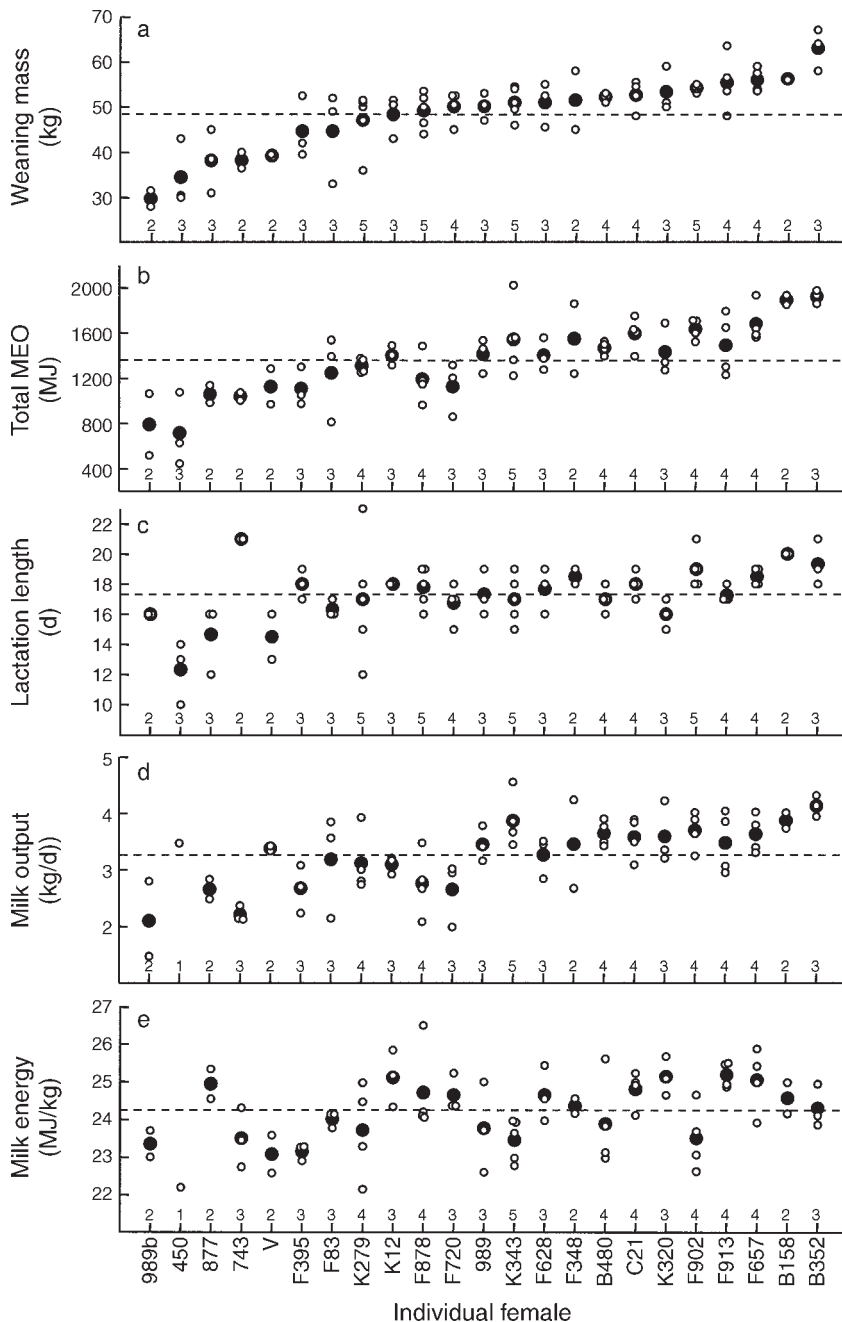
FIG. 3. Average (solid circles) and absolute (open circles) values for components of lactation performance of individual gray seal females over multiple lactation periods ranked from lowest to highest average weaning mass. Dashed lines are the estimated marginal means obtained from linear mixed-effects models with female as a random effect only. Numbers above the axis indicate the number of observations per individual. MEO is milk energy output.

mass, where the age effect was attributed to the youngest females, the estimated marginal means for weaning mass by age indicated that the six oldest females in the study had the five smallest average weaning masses (Fig. 3a). Estimated total milk energy output (calculated as the product of average milk energy, daily milk output, and lactation length) varied nonlinearly with female postpartum mass and age (Table 1) with five of the six oldest

females having relatively low outputs (Fig. 3b). Repeatability for weaning mass was still high after accounting for variation in pup sex, female mass, and female age as a nonlinear function (Table 1). Weaning mass was positively correlated with milk energy, daily milk output, and lactation length (Table 2). Milk energy and daily milk output and milk energy and lactation length were only weakly correlated; there was no

TABLE 2. Estimated correlation coefficients (ρ) among repeated measures of components of lactation performance in gray seals.

Lactation component	Lactation length (d)		Milk output (kg/d)		Milk energy (MJ/kg)	
	ρ	n	ρ	n	ρ	n
Weaning mass (kg)	0.498	23, 77	0.801	22, 69	0.325	22, 69
Lactation length (d)			<−0.001	22, 69	−0.164	22, 69
Milk output (kg/d)					0.128	22, 70

Note: For n, the first value is the number of females; the second value is the number of observations included in the analyses.

relationship between lactation length and daily milk output (Table 2).

DISCUSSION

To our knowledge, this study represents the first report of repeatability for components of milk energy transfer in a free-ranging mammal. The variation among females observed for individual components (Figs. 1–3) was consistent with the variation previously reported for gray seals (Iverson et al. 1993, Mellish et al. 1999, Bowen et al. 2006), indicating that the females studied were representative of the population. Our results indicate high levels of repeatability for milk energy, the rate of milk production, and the duration of lactation demonstrating that the physiological capacity to deliver milk energy to offspring is characteristic of individual females. The high repeatability in offspring weaning mass indicates that these female effects also have important consequences for offspring and, presumably, maternal fitness.

Milk composition and milk energy

Despite substantial variation in the proximate composition of milk among species, the basic mechanisms of synthesis and secretion of milk components are consistent across all mammals studied (Mepham 1983). Nevertheless, the factors which mediate the relative amounts of products that are produced by the mammary gland remain poorly understood. Nutrient partitioning by the mammary gland appears to be tightly controlled, and milk composition shows little plasticity in response to variation in diet, levels of energy intake, body energy stores, or the suckling demands of offspring (Roberts et al. 1985, Iverson et al. 1991, Mellish et al. 1999, Trott et al. 2003). The high repeatability for milk fat and protein contents in gray seals (Table 1) suggests that nutrient partitioning by the mammary gland is tightly controlled within individuals and that milk composition (and, thus, milk energy) is characteristic of individual females. Our results also suggest that the substantial individual variation observed in milk composition in other free-ranging species (Stern et al. 1997, Lang et al. 2005, Power et al. 2008) may represent consistent differences among females. The greater repeatability (Table 1) and lower overall variability in milk fat compared to milk protein at peak lactation suggests that milk fat content may be more tightly regulated than milk protein content

in gray seals, possibly reflecting the greater importance of fat to the rapid transfer of milk energy in phocid seals and other species with lipid-rich milks.

The repeatability of both milk fat and protein contents at peak lactation were within the range of the estimated repeatability for these components in domesticated species (milk fat, $r = 0.33$–0.61; milk protein, $r = 0.33$–0.69) in which significant levels of heritability have been found (milk fat, $h^2 = 0.14$–0.59; milk protein, $h^2 = 0.22$–0.55; Analla et al. 1996, Cappio-Borlino et al. 1997, Sanna et al. 1997, Roman et al. 2000) suggesting that, like domestic species, variation among gray seal females in the proximate composition of their milk (and, thus, milk energy) may have a significant heritable genetic basis.

Daily milk output

Studies in both domestic and laboratory species indicate that variation in daily milk output is a function of variation in the number of secretory cells in the mammary gland (mammary gland size) and, to a lesser extent, the activity of those cells (Knight 2000). The total mass of mammary glands taken from pregnant gray seals ($n = 6$; W. D. Bowen, *unpublished data*) and lactating harbor seals ($n = 13$; Bowen et al. 1992a) suggest that mammary gland size does vary substantially among phocid females (CV = 34% and 14%, respectively) and that this variation is independent of variation in body size. However, it remains to be determined whether variation in mammary gland size alone is sufficient to explain the observed variation in the daily milk output of phocid females (e.g., Oftedal et al. 1993, Hindell and Slip 1997, Wheatley et al. 2008; see *Results*), or whether females also differ in the secretory activity of their mammary epithelial cells. Nevertheless, the observed repeatability for daily milk output (Table 1) suggests that mammary gland size and activity are consistent within multiparous females across lactations. Given the consistency in mammary gland structure and function among species (Cowie et al. 1980, Akers 2002), our results suggest that the substantial individual variation observed in the rate of milk production in other free-ranging mammals (Stern et al. 1997, Crocker et al. 2001, Gjøstein et al. 2004) may also represent consistent individual difference among females. As with milk composition, the repeatability for daily milk output was within the range

estimated for domesticated species ($r = 0.44–0.50$), in which significant levels of heritability have been found ($h^2 = 0.30$; Sanna et al. 1997, Van Tassell et al. 1999) suggesting that variation among females in daily milk output may also have a significant heritable genetic basis in gray seals.

Lactation length

Because large-bodied fasting phocid seals are capital breeders, the absolute level of body energy stores available at parturition will place a limit on the duration of lactation. Although the specific cue(s) for weaning in phocid seals are unknown, females must, and do (Mellish and Iverson 2001), terminate lactation before body energy stores become critically depleted. However, variation among gray seal females in body mass at parturition (as a measure of absolute energy stores) explains only some of the observed variation in lactation length (28%; Bowen et al. 2006; see *Results*). This is a consequence of two factors. First, females differ substantially in their rate of daily energy expenditure, and this rate is independent of female mass or body composition at parturition (Mellish et al. 1999). Second, there is considerable variation among females in the proportion of energy stores expended over the course of lactation (44–85%, Mellish et al. 1999; 32–65%, S. L. C. Lang, S. J. Iverson, and W. D. Bowen, *unpublished data*), which is also independent of female mass or body composition at parturition. Therefore, variation in lactation length, both among and within gray seal females, will depend not only on the variation in the absolute level of body energy stores at parturition, but also on variation in daily energy expenditure and in the proportion of body energy stores expended prior to weaning.

Given the high level of repeatability in day 3 postpartum mass, the observed repeatability in lactation length (Table 1) suggests that both daily energy expenditure and the proportion of energy stores expended over the course of lactation were consistent within females across lactations. This has two implications for the regulation of lactation length in gray seals and, presumably, other large-bodied phocids, which fast throughout lactation. First, it suggests that the proportion of body energy stores used by females may act as a consistent cue for entrance into oestrus and the subsequent termination of lactation. Changes in body energy balance do play a role in the regulation of oestrus in mammals (Wade and Schneider 1992). Adipose tissue is a highly active endocrine organ that secretes a number of bioactive peptides (adipokines; Guerre-Millo 2004) some of which are secreted in proportion to the levels of body fat and are known to interact with hormones related to the regulation of ovulation (Chanda et al. 2003). In large-bodied phocid seals the mobilization of body fat accounts for ~90% of maternal energy loss over the course of lactation (Mellish et al. 1999, Crocker et al. 2001). Therefore, changes in the levels of body energy stores, particularly fat, have the potential to act as a metabolic signal for triggering oestrus and weaning (see Mellish and Iverson 2005). If, as our data suggest, the proportion of body energy stores used serves as a consistent cue for weaning, this would place a limit on the duration of lactation within females and, as a result, their ability to transfer available energy to their pups.

Our results also suggest that while variation in parturition mass explains only some of the observed variation in lactation length among females, it will have a substantial effect on variation in lactation length within females (Table 1). Like many other mammals, gray seal females begin to reproduce before reaching full adult body size with postpartum body mass increasing into the early to middle teens and then remaining relatively constant thereafter (Bowen et al. 2006). Therefore, lactation length is expected to increase with age in younger females as a result of age-associated changes in body mass (e.g., females K279 and K343; Figs. 1 and 3c). Changes in lactation length within females may also occur in response to changes in environmental conditions, which affect the abundance or distribution of prey and, thus, the ability of females to acquire body energy stores prior to parturition (e.g., Antonelis et al. 2003, Crocker et al. 2006, Proffitt et al. 2007). During our study, pup production on Sable Island continued to increase, suggesting that environmental conditions were generally favorable (Bowen et al. 2007). Longer term studies over more variable environmental conditions may show a greater variation in parturition mass within females and, therefore, a lower level of repeatability in both body mass and lactation length than we observed in the present study. Nevertheless, the evidence for a consistent cue for weaning suggests that individual females will be unable to buffer poor environmental conditions by extending their lactation length.

To what extent the observed repeatability in lactation length may represent heritable genetic variance is uncertain. Because lactation length in gray seals depends not only on variation in the absolute level of body energy stores available at parturition, but also on variation in daily energy expenditure and in the proportion of body energy stores expended prior to weaning, the extent to which lactation length may be heritable will depend on the degree to which additive genetic variance explains variation in each of these traits. Heritable genetic variation for adult body mass has been found in bighorn sheep (*Ovis canadensis*; e.g., Coltman et al. 2005); however, these results represent the only available data for free-ranging large mammals. As with body mass, quantitative genetic studies of metabolism in wild mammals are rare. However, significant levels of heritable genetic variation have been found for mass-specific resting metabolic rates in free-living weasels (*Mustela nivalis*; Szafrańska et al. 2007) and mass-specific basal metabolic rates in captive-reared bank voles (*Clethrionomys glareolus*; Sadowska et al. 2005), suggesting that heritable genetic

PLATE 1. Grey seal female nursing her pup at approximately 5 days postpartum on Sable Island, Nova Scotia, Canada. Photo credit: S. L. C. Lang.

variation could contribute to differences among gray seal females in the portion of daily energy expenditure that is devoted to maintenance metabolism.

Consequences for pup weaning mass

In large-bodied phocid seals total milk energy output is the most significant predictor of pup weaning mass (Mellish et al. 1999, Crocker et al. 2001). Our results demonstrate that milk energy, daily milk output, and lactation length are all characteristic of individual multiparous gray seal females. Because total milk energy output is a product of these, the overall capacity of females to deliver milk energy to their pups will also be characteristic of individuals (Table 1). The lack of correlation among milk energy, daily milk output, and lactation length (Table 2) indicates that these traits are independent of one another. This has two important implications for variation among females in total milk energy output. The first is that there is no trade-off among these traits within individuals; females with high levels of daily milk output or high milk energy do not have shorter lactation lengths or vice versa (Fig. 3). Therefore, while a female's milk energy and daily milk output will influence the time it takes for her to reach the threshold cue for weaning (see *Discussion: Lactation length*), there is no relationship between the factors which determine a female's threshold cue for weaning and those which determine a female's daily milk output or milk composition. The second implication is that quality in one trait will not be indicative of quality in another; females with above-average daily milk outputs

do not necessarily have above-average levels of milk energy or above-average lactation lengths (Fig. 3). Thus, a gray seal female's overall capacity to deliver milk energy to her pup cannot be inferred from the value for a single component of total milk energy output.

Individual differences in the survival of offspring among females is one of the most important components of variation in lifetime reproductive success (Clutton-Brock 1988). The observed repeatability in pup weaning mass, suggests that the lactation performance of individual gray seal females will be consistent through a large proportion of their reproductive life. From the point at which full adult body size is reached in their early teens until their late 20s or early 30s, individual females will consistently wean pups with greater or lesser probabilities of survival to one year of age (Hall et al. 2001). Thus, individual gray seal females will vary substantially in their contribution to the next generation.

In large mammals, which offset the costs of lactation either partly or entirely through the mobilization of body energy stores, studies examining variation in lifetime reproductive success (measured as offspring growth or survival to weaning) and its phenotypic determinants have typically focused on variation in maternal body mass or size as an indicator of variation in individual quality (e.g., Festa-Bianchet et al. 1998, Gaillard et al. 2000). However, our results suggest that the levels of expenditure by females during lactation may not be determined by body mass or size alone. Although factors influencing lactation length are likely to differ for species with longer lactation lengths and a

gradual weaning process, the consistency observed among species in the mechanisms of milk synthesis and secretion and in mammary gland structure (Cowie et al. 1980, Mepham 1983, Akers 2002) suggests that differences in both milk energy and the rate of milk production among females may be an important component of variation in individual quality and, thus, lifetime reproductive success.

While repeatability cannot be directly equated with heritability (Falconer and Mackay 1996, Dohm 2002), our results represent the first evidence that variation among females in their physiological capacity to deliver milk energy to their offspring may have a significant heritable genetic basis in a free-ranging mammal. This suggests that not only is there a potential for levels of maternal expenditure in capital breeding mammals to respond to selection but that traits directly related to the transfer of milk energy may be important components of maternal genetic effects (Wolf et al. 1998) on offspring growth.

Literature Cited

Akers, R. M. 2002. Lactation and the mammary gland. Iowa State Press, Ames, Iowa, USA.

Analla, M., I. Jiménez-Gamero, A. Muñoz-Serrano, J. M. Serradilla, and A. Falagán. 1996. Estimation of genetic parameters for milk yield and fat and protein contents of milk from Murciano-Granadina goats. Journal of Dairy Science 79:1895–1898.

Antonelis, G. A., J. D. Baker, and J. J. Polovina. 2003. Improved body condition of weaned Hawaiian monk seal pups associated with El Niño events: potential benefits to an endangered species. Marine Mammal Science 19:590–598.

AOAC. 2000a. AOAC official method 905.02 for fat in milk, Roese-Gottlieb method. Page 18, chapter 33 in W. Horwitz, editor. Official methods of analysis. AOAC International, Gaithersburg, Maryland, USA.

AOAC. 2000b. AOAC official method 991.20 for nitrogen (total) in milk. Kjeldahl methods. Page 10, chapter 33 in W. Horwitz, editor. Official methods of analysis. AOAC International, Gaithersburg, Maryland, USA.

Blaxter, K. 1989. Energy metabolism in animals and man. Cambridge University Press, Cambridge, UK.

Bowen, W. D., S. J. Iverson, J. I. McMillan, and D. J. Boness. 2006. Reproductive performance in grey seals: age-related improvement and senescence in a capital breeder. Journal of Animal Ecology 75:1340–1351.

Bowen, W. D., J. I. McMillan, and W. Blanchard. 2007. Reduced population growth of gray seals at Sable Island: evidence from pup production and age of primiparity. Marine Mammal Science 23:48–64.

Bowen, W. D., J. McMillan, and R. Mohn. 2003. Sustained exponential population growth of grey seals at Sable Island, Nova Scotia. ICES Journal of Marine Science 60:1265–1274.

Bowen, W. D., O. T. Oftedal, and D. J. Boness. 1992a. Mass and energy transfer during lactation in a small phocid, the harbor seal (Phoca vitulina). Physiological Zoology 65:844–866.

Bowen, W. D., W. T. Stobo, and S. J. Smith. 1992b. Mass changes of grey seal (Halichoerus grypus) pups on Sable Island: differential maternal investment reconsidered. Journal of Zoology London 227:607–622.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Second edition. Springer, New York, New York, USA.

Cappio-Borlino, A., B. Portolano, M. Todaro, N. P. P. Macciotta, P. Giaccone, and G. Pulina. 1997. Lactation curves of Valle del Belice dairy ewes for yields of milk, fat and protein estimated with test day models. Journal of Dairy Science 80:3023–3029.

Chanda, D., Abhilasha, and A. Krishna. 2003. Seasonal adiposity and delayed ovulation in a vespertilionid bat, Scotophilus heathi: role of tumor necrosis factor-α. Physiological and Biochemical Zoology 76:271–280.

Clutton-Brock, T. H., editor. 1988. Reproductive success: studies of individual variation in contrasting breeding systems. University of Chicago Press, Chicago, Illinois, USA.

Coltman, D. W., P. O'Donoghue, J. T. Hogg, and M. Festa-Bianchet. 2005. Selection and genetic (co)variance in bighorn sheep. Evolution 59:1372–1382.

Cowie, A. T., I. A. Forsyth, and I. C. Hart. 1980. Hormonal control of lactation. Springer-Verlag, New York, New York, USA.

Crocker, D. E., D. P. Costa, B. J. Le Boeuf, P. M. Webb, and D. S. Houser. 2006. Impact of El Niño on the foraging behaviour of female northern elephant seals. Marine Ecology Progress Series 309:1–10.

Crocker, D. E., J. D. Williams, D. P. Costa, and B. J. Le Boeuf. 2001. Maternal traits and reproductive effort in northern elephant seals. Ecology 82:3541–3555.

Dohm, M. R. 2002. Repeatability estimates do not always set an upper limit to heritability. Functional Ecology 16:273–280.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. Fourth edition. Prentice Hall, London, UK.

Festa-Bianchet, M., J. M. Gaillard, and J. T. Jorgenson. 1998. Mass- and density-dependent reproductive success and reproductive costs in a capital breeder. American Naturalist 152:367–379.

Festa-Bianchet, M., J. T. Jorgenson, and D. Reale. 2000. Early development, adult mass and reproductive success in bighorn sheep. Behavioral Ecology 11:633–639.

Gaillard, J. M., M. Festa-Bianchet, D. Delorme, and J. Jorgenson. 2000. Body mass and individual fitness in female ungulates: bigger is not always better. Proceedings of the Royal Society of London B 267:471–477.

Gjøstein, H., O. Holand, and R. B. Weladji. 2004. Milk production and composition in reindeer (Rangifer tarandus): effect of lactational stage. Comparative Biochemistry and Physiology A 137:649–656.

Guerre-Millo, M. 2004. Adipose tissue and adipokines: for better or worse. Diabetes and Metabolism 30:13–19.

Hall, A. J., B. J. McConnell, and R. J. Barker. 2001. Factors affecting first-year survival in grey seals and their implications for life history strategy. Journal of Animal Ecology 70:138–149.

Hamlett, A., L. Ryan, and R. Wolfinger. 2004. On the use of PROC MIXED to estimate correlation in the presence of repeated measures. SAS Users Group International, Pro-

ceedings of the Statistics and Data Analysis Section Paper 198-29:1–7.

Hindell, M. A., and D. J. Slip. 1997. The importance of being fat: maternal expenditure in the southern elephant seal *Mirounga leonina*. Pages 72–77 *in* M. A. Hindell and C. Kemper, editors. Marine mammal research in the Southern Hemisphere volume 1: status, ecology and medicine. Surrey Beatty and Sons, Chipping Norton, UK.

Iverson, S. J., W. D. Bowen, D. J. Boness, and O. T. Oftedal. 1993. The effect of maternal size and milk energy output on pup growth in grey seals (*Halichoerus grypus*). Physiological Zoology 66:61–88.

Iverson, S. J., O. T. Oftedal, and D. J. Boness. 1991. The effect of El Niño on pup development in the California sea lion (*Zalophus californianus*). II. Milk intake. Pages 180–184 *in* F. Trillmich and K. A. Ono, editors. Pinnipeds and El Niño: responses to environmental stress. Springer-Verlag, Berlin, Germany.

Knight, C. H. 2000. The importance of cell division in udder development and lactation. Livestock Production Science 66: 169–176.

Lang, S. L. C., S. J. Iverson, and W. D. Bowen. 2005. Individual variation in milk composition over lactation in harbour seals (*Phoca vitulina*) and the potential consequences of intermittent attendance. Canadian Journal of Zoology 83: 1525–1531.

Mellish, J. E., and S. J. Iverson. 2001. Blood metabolites as indicators of nutrient utilization in fasting, lactating phocid seals: does depletion of nutrient reserves terminate lactation? Canadian Journal of Zoology 79:303–311.

Mellish, J. E., and S. J. Iverson. 2005. Postpartum dynamics of reproductive hormones in gray and hooded seals. Marine Mammal Science 21:162–168.

Mellish, J. E., S. J. Iverson, and W. D. Bowen. 1999. Variation in milk production and lactation performance in grey seals and consequences for pup growth and weaning characteristics. Physiological and Biochemical Zoology 72:677–690.

Mepham, T. B. 1983. Biochemistry of lactation. Elsevier Science, Amsterdam, Netherlands.

Meyer, K., M. J. Carrick, and B. J. P. Donnelly. 1994. Genetic parameters for milk production of Australian beef cows and weaning weight of their calves. Journal of Animal Science 72: 1155–1165.

Montaño-Bermudez, M., and M. K. Nielsen. 1990. Biological efficiency to weaning and to slaughter of crossbred beef cattle with different genetic potential for milk. Journal of Animal Science 68:2297–2309.

Mousseau, T. A., and C. W. Fox, editors. 1998. Maternal effects as adaptations. Oxford University Press, Oxford, UK.

Noren, S. R., D. Boness, S. J. Iverson, J. McMillan, and W. D. Bowen. 2008. Body condition at weaning affects the duration of the postweaning fast in gray seal pups (*Halichoerus grypus*). Physiological and Biochemical Zoology 81:269–277.

Oftedal, O. T., W. D. Bowen, and D. J. Boness. 1993. Energy transfer by lactating hooded seals and nutrient deposition in their pups during the four days from birth to weaning. Physiological Zoology 66:412–436.

Oftedal, O. T., and S. J. Iverson. 1995. Phylogenetic variation in the gross composition of mammalian milks. Pages 790–827 *in* R. G. Jensen and M. Thompson, editors. The handbook of milk composition. Academic Press, San Diego, California, USA.

Power, M. L., C. E. Verona, C. Ruiz-Miranda, and O. T. Oftedal. 2008. The composition of milk from free-living common marmosets (*Callithrix jacchus*) in Brazil. American Journal of Primatology 70:78–83.

Proffitt, K. M., R. A. Garrott, J. J. Rotella, and K. E. Wheatley. 2007. Environmental and senescent related variations in Weddell seal body mass: implications for age-specific reproductive performance. Oikos 116:1683–1690.

Räsänen, K., and L. E. B. Kruuk. 2007. Maternal effects and evolution at ecological time-scales. Functional Ecology 21: 408–421.

Roberts, S. B., T. J. Cole, and W. A. Coward. 1985. Lactational performance in relation to energy intake in the baboon. American Journal of Clinical Nutrition 41:1270–1276.

Rogowitz, G. L. 1996. Trade-offs in energy allocation during lactation. American Zoologist 36:197–204.

Roman, R. M., C. J. Wilcox, and F. G. Martin. 2000. Estimates of repeatability and heritability of productive and reproductive traits in a herd of Jersey cattle. Genetics and Molecular Biology 23:113–119.

Sadowska, E. T., M. K. Labocha, K. Baliga, A. Stanisz, A. K. Wróblewska, W. Jagusiak, and P. Koteja. 2005. Genetic correlations between basal and maximum metabolic rates in a wild rodent: consequences for evolution of endothermy. Evolution 59:672–681.

Sanna, S. R., A. Carta, and S. Casu. 1997. (Co)variance component estimates for milk composition traits in Sarda dairy sheep using a bivariate animal model. Small Ruminant Research 25:77–82.

SAS Institute. 2003. SAS version 9.1.3. SAS Institute, Cary, North Carolina, USA.

S-Plus. 2005. S-Plus version 7.0. Insightful Corporation, Seattle, Washington, USA.

Stern, A. A., T. H. Kunz, E. H. Studier, and O. T. Oftedal. 1997. Milk composition and lactational output in the greater spear-nosed bat, *Phyllostomus hastatus*. Journal of Comparative Physiology B 167:389–398.

Szafrańska, P. A., K. Zub, and M. Konarzewski. 2007. Long-term repeatability of body mass and resting metabolic rate in free-living weasels, *Mustela nivalis*. Functional Ecology 21: 731–737.

Trott, J. F., K. J. Simpson, R. L. C. Moyle, C. M. Hearn, G. Shaw, K. R. Nicholas, and M. B. Renfree. 2003. Maternal regulation of milk composition, milk production, and pouch young development during lactation in the tammar wallaby (*Macropus eugenii*). Biology of Reproduction 68:929–936.

Van Tassell, C. P., G. R. Wiggans, and H. D. Norman. 1999. Method R estimates of heritability for milk, fat and protein yields of United States dairy cattle. Journal of Dairy Science 82:2231–2237.

Wade, G. N., and J. E. Schneider. 1992. Metabolic fuels and reproduction in female mammals. Neuroscience and Behavioural Reviews 16:235–272.

Wauters, L., L. Bijnens, and A. A. Dhondt. 1993. Body mass at weaning and juvenile recruitment in the red squirrel. Journal of Animal Ecology 62:280–286.

West, B. T., K. B. Welch, and A. T. Gałecki. 2007. Linear mixed models: a practical guide using statistical software. CRC Press, Boca Raton, Florida, USA.

Wheatley, K. E., C. J. A. Bradshaw, R. G. Harcourt, and M. A. Hindell. 2008. Feast or famine: evidence for mixed capital-income breeding strategies in Weddell seals. Oecologia 155: 11–20.

Wolf, J. B., E. D. Brodie, J. M. Cheverud, A. J. Moore, and M. J. Wade. 1998. Evolutionary consequences of indirect genetic effects. Trends in Ecology and Evolution 13:64–69.

APPENDIX

Results for all linear mixed-effects models run for the components of lactation performance in gray seals (*Ecological Archives* E090-177-A1).