Maternal age influences offspring behaviour and growth efficiency during provisioning in northern elephant seals

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Offspring growth rates impact fitness and can be influenced by maternal effects. Despite efforts to understand the influence of maternal traits (e.g. age, size, body condition) on reproductive effort, much less is known about how maternal traits and environment influence the behaviour of offspring and ultimately, how offspring behaviour may influence the efficiency of the translation of maternal investment into offspring growth. Offspring of capital breeders, such as the northern elephant seal, Mirounga angustirostris, are nursed exclusively from maternal body reserves and investment is limited by the resources acquired prior to parturition. Combined new and previously published milk energy intake and offspring storage data (N = 38) suggest impacts of maternal age on growth efficiency that are independent of rates of energy delivery. To determine the mechanisms underlying this effect of maternal age, behavioural data were collected from pups of 46 known-age females, from parturition to weaning, across 6 years and three different sites along the central California coast, representing 3954 seal-hours of observation. Pup behaviours were divided into five mutually exclusive categories that potentially impacted pup energetics. The offspring of older females spent more time resting, while offspring of younger females spent more time locomoting and distant from their mother. As pups developed, they spent more time suckling and locomoting and less time resting. Pup behaviour showed strong diel patterns, with activity decreasing over the day. The magnitude of these relationships varied between rookeries, suggesting influences of harem size, topography or environmental features on pup behaviour. Together these findings suggest direct impacts of maternal age and breeding experience on pup behaviour and growth efficiency.

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Early development is a significant contributor to the lifetime reproductive success and fitness of individuals (Clutton-Brock, 1991; Lindström, 1999). In mammals, maternal phenotype affects a mother’s capacity to invest in her offspring, influencing the growth rate and ultimately the size of the offspring (Clutton-Brock, 1991). Offspring size impacts future survival, age of first reproduction and fecundity, all of which are important in determining fitness (Beauplet, Barbraud, Chambellant, & Guinet, 2005; Dobson & Oli, 2001; Hofer & East, 1993; Hutchings, 1993; Lochmiller, Ditchkoff, & Sinclair, 2000; McAdam & Millar, 1999). While the rate and magnitude of parental investment directly impact offspring growth, growth rates reflect complex interactions between maternal effects, environmental conditions and offspring traits (McDonald, Goebel, Crocker, & Costa, 2012a). Many studies have investigated factors that influence growth rates, but relatively few have investigated the growth efficiency (proportion of allocated energy that goes to somatic growth and energy storage) and the mechanisms that influence efficiency.

Growth rates are influenced by both maternal effects and offspring traits in a wide range of species (Bernardo, 1996; McDonald Goebel, Crocker, & Costa, 2012b; Mousseau & Fox, 1998). Maternal effects occur when offspring phenotype is influenced by the maternal phenotype or maternal environment independent of offspring genotype. One mechanism underlying maternal effects is maternal behaviour (Adkins-Regan, Banerjee, Correa, & Schweitzer, 2013). Adaptive maternal effects may be environment specific (Champagne, Francis, Mar, & Meaney, 2003),
mediated by stress hormones (Adkins-Regan et al., 2013; Dantzer et al., 2013; Love, McGowan, & Sheriff, 2013; Sheriff & Love, 2013) and can be modulated by prior maternal experience (Champagne et al., 2008). Variation in maternal behaviour during provisioning, as influenced by maternal dominance or environmental interactions, has been identified as an important form of maternal effect (Altman & Alberts, 2005; Bowen, Ellis, Iverson, & Boness, 2001; Lloyd & Martin, 2004). Offspring traits also shape patterns of maternal investment. For example, in fur seals, pup traits such as size and age can influence maternal investment and energy allocation by mothers (McDonald et al., 2012a).

Growth rates are not only determined by energy investment by the mother, but are also influenced by how the offspring allocate energy. Maternal age and breeding experience may alter the expression of maternal care in ways that influence offspring behaviour and development (Altman & Alberts, 2005; Pittet, Cognard, Houdelier, Richard-Yris, & Lumineau, 2012). For example, in colonial breeders, size and prior breeding experience can influence harem position and the potential for harassment of offspring by conspecifics (McMahon & Bradshaw, 2004). This may result in increased allocation of ingested energy towards activity, rather than storage.

Northern elephant seals, Mirounga angustirostris, are capital breeders and phocid seals that provision their young from energy obtained during an 8-month, gestational foraging migration (Robinson et al., 2012). Maternal body reserves accrued during this trip are the primary determinants of reproductive effort in females (Crocker, Williams, Costa, & Le Boeuf, 2001). Lactation efficiency, the proportion of reproductive energy expenditure delivered to the offspring as milk energy, increases with age of the female (Crocker et al., 2001) and prior breeding experience positively influences weaning success (Sydeman, Huber, Emslie, Ribic, & Nur, 1991). Females are capable of increasing lactation efficiency by decreasing daily metabolic maintenance requirements (Fedak & Anderson, 1982). Older females’ more dominant positions in the harem leads to a lower rate of energy expenditure on agonistic and locomotor behaviours, allowing them to devote more energy towards lactation (McDonald & Crocker, 2006; Reiter, Panken, & Le Boeuf, 1981). The increase in lactation efficiency with age modifies the level of milk energy obtained from reproductive effort that is constrained by available body reserves. While this increase in milk energy delivered to pups of older females is important, it is not the sole factor in determining rates of pup growth. Growth efficiency, the proportion of milk energy stored and used towards growth by pups, depends on the rate of milk energy received relative to their daily metabolic requirements. In elephant seals, strong impacts of maternal body reserves on reproductive effort and milk production are modulated by differences in offspring metabolism (Crocke et al., 2001). Despite this variation, growth efficiency remains high, with pups storing an average of 84% of milk energy delivered by their mothers. Similar to lactation efficiency, growth efficiency can be increased by decreasing metabolic overhead and energy expenditure for activity. Thus, behaviours females exhibit to protect, supervise and maintain proximity to pups in the high-density and dynamic harem environments may influence offspring activity budgets and growth efficiency.

Harem site, density, topography and environmental conditions may also influence lactation efficiency and pup behaviour. Female location within a harem and environmental conditions influence phocid social systems (Ferguson, 2006). Some breeding sites require frequent movement of females and their offspring due to tidal or thermal exposure (Codde, Allen, Houser, & Crocker, 2016). Similarly, these environmental characteristics may affect male activity, a major source of harem disturbance (Norris, Houser, & Crocker, 2010). Colony and harem site-specific environmental factors may directly impact growth efficiency and modulate the ability of maternal experience to influence offspring behavioural phenotype.

We examined the influence of maternal age and milk delivery rate on growth efficiency using a combination of new and published pooled milk intake and energy storage data. To investigate the mechanisms that influence differences in growth efficiency, we investigated the effects of maternal age and pup development with age on pup behaviour. We compared pup behaviour at three different sites and in several different years to examine how local environmental conditions may influence pup behaviour and likely growth efficiency.

**METHODS**

**Study Site and Subjects**

This study was conducted during the northern elephant seal breeding season (December through March) across 6 years and three different mainland and island breeding sites along the central coast of California, U.S.A. Sites included Año Nuevo State Park, San Mateo County (ANO, 2009 and 2017), Point Reyes National Seashore, Marin County (PRNS, 2007) and Farallon Island National Wildlife Refuge, San Francisco County (SEFI, 2014–2016). These sites represent varying breeding habitats, each with different topography and tidal influences. The North Point harem sites at ANO are composed of sandy beaches with gradual slopes, making it ideal for breeding; however, mixed semidiurnal tidal patterns require diel movement of the entire harem, including females and their pups, and harems are highly exposed to storm surge. SEFI is a granite island off the coast of San Francisco that lacks sand and easy access points between the ocean and the harem. The difficulty of access requires seals to manoeuvre around and over large rocks to get to the breeding site but protects the harem from tidal movements. Furthermore, female movements have been observed in response to solar position (R. W. Berger, personal observation), indicating that the dark substrate and lack of sand potentially expose animals to thermal stress. The Drakes Beach harem at PRNS is relatively protected and lacks observable topographic and tidal challenges. However, the dark substrate and thermal environment cause females to move in response to solar radiation, with females moving to the water’s edge or even entering the water with their pups during thermal stress (Codde et al., 2016).

Forty-six known-age breeding adult female elephant seals, ages 3–16 years old, and their pups were observed from parturition date to weaning (25 ± 4 days) for a total of 3954 seal observation hours. No females were repeat subjects in the 2 years at ANO, while all 11 females at SEFI were followed in all 3 years of observations. Only one harem and site was observed in each year. Harem size varied between sites and years (Table 1). Upon arrival at the harem, known-age females were identified by numbered plastic flipper tags applied at weaning and were marked on the dorsolateral body.

**Table 1**

<table>
<thead>
<tr>
<th>Site</th>
<th>Observations</th>
<th>Seal-hours</th>
<th>Subjects</th>
<th>Harem size</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRNS</td>
<td>2458</td>
<td>615</td>
<td>9</td>
<td>164</td>
<td>2007</td>
</tr>
<tr>
<td>SEFI</td>
<td>6305</td>
<td>1576</td>
<td>11</td>
<td>73, 84, 83</td>
<td>2014, 2015, 2016</td>
</tr>
<tr>
<td>ANO</td>
<td>7052</td>
<td>1763</td>
<td>26</td>
<td>236, 148</td>
<td>2009, 2017</td>
</tr>
<tr>
<td>Total</td>
<td>15 815</td>
<td>3954</td>
<td>46</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

surface using hair dye (Clairol, Inc., Stamford, CT, U.S.A.) to allow for easier identification throughout the study (Le Boeuf & Peterson, 1969). A sample of 9–14 subjects was chosen at each observed harem based on observed parturition dates, a range of maternal ages and the ability to observe all study females at each sampling period. Pups were marked using hair bleach (Clairol, Inc.) shortly after parturition, to allow accurate mother–pup pair confirmation throughout lactation.

**Offspring Behaviour**

Pup behaviour was recorded using instantaneous scan sampling. Behaviour was noted every 15 min for 4–5 h per day during daylight hours, 4–7 days per week at each site and year (Table 1). For each scan, behaviour of the pup was categorized as suckling, resting, vocalizing, locomoting or ‘distant’. The behaviours were defined as (1) suckling: pup in suckling position with its mother, (2) resting: sleeping or slight movement, (3) vocalizing: pup making audible vocal calls and (4) locomoting: full body length movements of the pup. The fifth behaviour, ‘distant’, was recorded when pups were at least two pup body lengths away from their mother (Baldi, Campagna, Pedraza, & Le Boeuf, 1996; Boness, Craig, Honigman, & Austin, 1998). Each of these categories were mutually exclusive and only one behaviour was recorded for each pup per 15 min scan. If two behaviours appeared to be present for one observational scan, the behaviour that was the most energetically demanding was recorded. For example, if a pup was vocalizing while locomoting, locomoting was recorded, as it is considered the more energetically costly behaviour. In the rare event that a mother–pup pair was not visible or could not be identified, no data were recorded for that pair for that scan.

**Milk Intake and Growth Efficiency**

Milk intake and energy storage were measured in 38 pups with known-age mothers. Data from 16 pups were included in a previously published study (Crocker et al., 2001), 22 additional measurements were taken using identical methods during other studies that required female immobilization and pup sampling during 1995–2009. Mothers were chemically immobilized at 2–5 days postpartum using ~1.0 mg/kg of telnetamine/zolazepam (Telazol, Fort Dodge, IN, U.S.A.). Milk samples were taken after injection of oxytocin into the mammary gland for proximate analysis using previously described methods (Crocker et al., 2001). Pups were weighed using a canvas restraint bag attached to a scale suspended from a tripod. This procedure was repeated on day 22–24 postpartum.

Milk energy intake and energy storage by pups were assessed using water isotope dilution (Ortiz, Costa, & Le Boeuf, 1978). Detailed methods and calculations are provided in the Appendix. Briefly, during the initial procedure the pup was injected with 64 MBq tritiated water (HTO) in 5 ml of sterile saline and allowed to equilibrate, at which time an equilibrium blood sample was collected. The second procedure at 22–24 days postpartum followed identical methods except that a pre-injection blood sample was taken in order to determine any residual isotope activity. These injections were used to determine changes in total body water (TBW) between measurements. Using tritiated water turnover rates, the average proportion of water in each female’s milk and an estimate of metabolic water production, we calculated milk intake. (Kretzmann, Costa, & Le Boeuf, 1993; Oppedal & Iverson, 1987). The change in specific isotopic activity between the first treatment and post-equilibration sample and the second pre-injection sample, combined with TBW and mass measurements was used to calculate water influx (Nagy & Costa, 1980). An estimate of metabolic water production based on average body mass was subtracted from water influx to estimate milk water intake (Crocker et al., 2001). Milk composition was used to estimate milk energy intake and compared to changes in pup mass and body composition derived from the water isotope dilution space (Crocker et al., 2001). Body composition was estimated using total body water values (see Appendix for details). Pup energy storage during growth was calculated from the change in fat and fat-free mass components between the beginning and end of lactation (see Appendix). Growth efficiency was calculated as energy stored divided by milk energy delivered.

One potential confound in the measurement of growth efficiency using these methods is the assumption of a standard metabolic rate for each pup in estimating metabolic water production that is subtracted from water influx to estimate milk delivery (Crocker et al., 2001; Kretzmann et al., 1993). Although metabolic water production is low compared to milk water ingestion, variation in pup metabolism may induce small errors in estimates of milk energy delivery. If a pup’s metabolic rate is higher than assumed, metabolic water production will be underestimated, leading to an overestimation of milk intake, and as a consequence, growth efficiency will be biased low. In contrast, if a pup’s metabolic rate is lower than assumed, metabolic water production will be overestimated, leading to an underestimation of milk intake and pup growth efficiency being biased high. Thus, use of an assumed metabolic rate relative to body size to estimate metabolic water production for milk intake measurements may slightly exaggerate existing differences in growth efficiency.

**Ethical Note**

All procedures were approved by the Sonoma State University Institutional Animal Care and Use Committee (IACUC protocol numbers 0734, 1757) and performed under National Marine Fisheries Service (NMFS) marine mammal permits 87-1743, 14636 and 19108. Pups were kept with their mothers and protected during sampling procedures. Mothers recovered rapidly from immobilization and quickly resumed normal maternal behaviour. No mothers and pups were separated or harmed as a result of the research.

**Statistical Analysis**

Data were analysed using SAS 9.4 software (SAS Institute, Cary, NC, U.S.A.). Simple polynomial regression was used to assess the impact of maternal age on pup growth efficiency with a quadratic curve yielding the best fit to the data. The rate of milk energy intake potentially positively influences offspring growth efficiency and has been shown in elephant seals to increase with maternal age (Crocker et al., 2001), so we included milk intake in the model to better differentiate potential age impacts of maternal behaviour. A general linear model (GLM) was used to assess the concurrent effects of milk energy delivery rate and maternal age on growth efficiency. We evaluated potential multicollinearity using variance inflation factors (VIF) and compared the relative importance of these factors using standardized regression coefficients (StdB). Generalized linear mixed models (GLMM) with a multinomial response distribution and a logit linking function were used to assess the impact of maternal age, time of day (TOD), days postpartum (DPP) and site on pup behaviour. These models included a random effect term for individual female. Since behaviour varied between sites, an interaction term between site and each of the other fixed effects was also included in the model. At sites where data were collected for multiple years (SEFI and AN0), year was included as a fixed effect in site-specific models. If year was found...
to have a significant effect on pup behaviour, a follow-up analysis was run including interaction terms of year with the other fixed effects. Influence of significant effects evident in multinomial response models on specific pup behaviours were analysed using post hoc tests by modelling binary distributions of the behaviours of interest (e.g. suckling versus not suckling). The magnitude of impacts on binary responses were visualized using logistic plots of the generalized linear mixed model’s parameter estimates. When significant site interactions were present, these plots were made individually for each site.

RESULTS

Milk Intake and Growth Efficiency

Pups stored a mean (±SD) of 83.3 ± 5.5% of milk energy delivered. Growth efficiency increased with maternal age until ~10 years ($r^2 = 0.80, F_{2,35} = 71.6, P < 0.0001$; Fig. 1). Maternal age was the major driver of variation in pup growth efficiency ($F_{1,35} = 27.3, P < 0.0001$, StdB = 0.63, VIF = 1.47). Milk energy delivery rate influenced growth efficiency ($F_{1,35} = 4.4, P = 0.04$, StdB = 0.25, VIF = 1.47).

Offspring Behaviour

We collected a total of 3954 seal observation hours from 46 female–pup pairs during 6 years across the three breeding sites (1 year at PRNS, 2 years at ANO, 3 years at SEFI). Data were not collected at two sites concurrently during the same year. Pup behaviour was dominated by low-activity behaviours across all sites and years. Resting represented 72% of observations, followed by suckling, which represented 18% of observations (Fig. 2). Energy-demanding behaviours that potentially reduce growth efficiency were less common. Vocalizing occurred in 5% of behavioural samples and locomoting or being distant from the mother each occurred in 3% of the behavioural samples. Behavioural time budgets varied between the sites ($F_{1,15 758} = 3.87, P = 0.02$). Time spent suckling and vocalizing were relatively consistent between sites. However, pups spent more time locomoting or distant from their mothers and less time resting at PRNS (Fig. 2).

Effects of maternal age

Maternal age influenced offspring behaviour across all breeding sites and years ($F_{1,15 758} = 4.56, P = 0.03$). The effect of maternal age differed marginally between the colonies ($F_{1,15 758} = 2.67, P = 0.06$).

Post hoc examinations of binary variables revealed positive effects of increasing maternal age on time spent resting (Table 2, Fig. 3a), while time spent locomoting (Table 2, Fig. 3b) and distant from the mother (Table 2, Fig. 3c) decreased with maternal age. Comparisons of logistic plots of the significant binary behavioural responses between sites suggested the strongest differences in maternal effects were for proportion of time spent distant from females (Fig. 4). Maternal age had strong negative impacts on the probability of pup separation at ANO and PRNS that were absent at SEFI.

Effects of development

Pup activity budgets changed with development across the suckling period ($F_{1,15 758} = 31.17, P < 0.0001$), and pup age was the strongest driver of behavioural changes among the explanatory factors. The magnitude of this relationship varied between sites ($F_{1,15 758} = 16.33, P < 0.0001$). The time pups spent suckling increased with days postpartum up to weaning across all sites (11–28%; Table 2, Fig. 5a). However, this change was much smaller in magnitude at the PRNS site. Pups became more active over the suckling period, spending less time resting and more time locomoting as they developed (Table 2, Fig. 5b and c). There was no consistent effect on vocal behaviour as the pattern varied markedly between sites. Pups vocalized more as they developed at PRNS, showed little changes with development at ANO and vocalized more as neonates at SEFI (Table 2, Fig. 6).

Diel patterns

Consistent diel effects on behaviour across all sites were not evident ($P = 0.33$). However, strong differences in diel patterns between sites were present ($F_{1,15 758} = 18.67, P < 0.0001$). Diel patterns of resting, vocalizing and separation varied between sites (Table 2). Pups were more vocal early in the day at ANO and PRNS, but this pattern was not evident at SEFI (Fig. 7). Similarly, the strong increase in time spent resting across the day at PRNS was weaker at ANO and absent at SEFI (Fig. 7). The strongest variation in diel patterns between sites was for the probability of mother–pup separation. Pups at PRNS were twice as likely to be separated early in the morning compared to late in the day, pups at ANO were 10 times more likely to be separated late in the day and there was no diel pattern evident at SEFI (Fig. 7).

Annual differences

Despite annual differences in weather, no annual differences in pup behaviours were evident across the 3 years of observations at

![Figure 1.](image) Changes in growth efficiency measured as energy stored/milk energy input with maternal age. Pup growth efficiency = $54.5 + 6.7 \times Age - 0.3 \times Age^2$; $r^2 = 0.70, P < 0.001$.

![Figure 2.](image) Percentage of observations of each behaviour exhibited at each study site. Black bars = Año Nuevo State Park; grey bars = Point Reyes National Seashore; hatched bars = Southeast Farallon Island.

Post hoc analyses of binary variables revealed positive effects of increasing maternal age on time spent resting (Table 2, Fig. 3a), while time spent locomoting (Table 2, Fig. 3b) and distant from the mother (Table 2, Fig. 3c) decreased with maternal age. Comparisons of logistic plots of the significant binary behavioural responses between sites suggested the strongest differences in maternal effects were for proportion of time spent distant from females (Fig. 4). Maternal age had strong negative impacts on the probability of pup separation at ANO and PRNS that were absent at SEFI.
Table 2
Model results from a generalized linear mixed model examining effects of explanatory variables on binary behavioural states

<table>
<thead>
<tr>
<th></th>
<th>Suckling</th>
<th></th>
<th>Resting</th>
<th></th>
<th>Vocalizing</th>
<th></th>
<th>Locomoting</th>
<th></th>
<th>Distant</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>DPP</td>
<td>74.49</td>
<td>&lt;0.0001</td>
<td>100.24</td>
<td>&lt;0.0001</td>
<td>2.41</td>
<td>0.12</td>
<td>7.97</td>
<td>0.005</td>
<td>3.31</td>
<td>0.07</td>
</tr>
<tr>
<td>TOD</td>
<td>3.21</td>
<td>0.07</td>
<td>2.43</td>
<td>0.12</td>
<td>22.00</td>
<td>&lt;0.0001</td>
<td>8.47</td>
<td>0.004</td>
<td>5.31</td>
<td>0.02</td>
</tr>
<tr>
<td>Age</td>
<td>0.11</td>
<td>0.74</td>
<td>5.71</td>
<td>0.02</td>
<td>1.21</td>
<td>0.27</td>
<td>7.68</td>
<td>0.006</td>
<td>16.41</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Site</td>
<td>0.62</td>
<td>0.54</td>
<td>5.06</td>
<td>0.006</td>
<td>9.40</td>
<td>&lt;0.0001</td>
<td>0.72</td>
<td>0.49</td>
<td>8.58</td>
<td>0.0002</td>
</tr>
<tr>
<td>DPP*site</td>
<td>7.73</td>
<td>0.0004 0.74</td>
<td>0.48</td>
<td>18.67</td>
<td>&lt;0.0001</td>
<td>3.47</td>
<td>0.03</td>
<td></td>
<td>1.72</td>
<td>0.18</td>
</tr>
<tr>
<td>Age*site</td>
<td>1.09</td>
<td>0.34</td>
<td>1.26</td>
<td>0.19</td>
<td>1.64</td>
<td>0.19</td>
<td>1.73</td>
<td>0.18</td>
<td>8.77</td>
<td>0.0002</td>
</tr>
<tr>
<td>TOD*site</td>
<td>1.60</td>
<td>0.20</td>
<td>8.54</td>
<td>0.0002</td>
<td>21.7</td>
<td>&lt;0.0001</td>
<td>1.61</td>
<td>0.20</td>
<td>10.86</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

DPP – days postpartum; TOD – time of day; Age – maternal age. The model included a random effect of maternal identity (ID). Statistically significant results are in bold. ‘Distant’ signifies pup being greater than two pup lengths away from the mother. Denominator df = 15,762 for all models.

Figure 3. Probability of pups being observed (a) resting, (b) locomoting or (c) distant from their mothers with increasing maternal age. Logistic plots use the regression parameters from the generalized linear mixed model.

Figure 4. Probability of pups being observed distant from their mothers with increasing maternal age at each site (ANO: Año Nuevo State Park; PRNS: Point Reyes National Seashore; SEFI: Farallon Island National Wildlife Refuge). Logistic plots use the regression parameters from the generalized linear mixed model.
SEFI ($P = 0.15$). In contrast, strong annual differences were evident between the 2 study years at ANO. Effects of maternal age ($F_{1,7019} = 6.04, P = 0.01$) and time of day ($F_{1,7019} = 7.16, P = 0.0008$) varied between years while developmental patterns did not ($P = 0.75$). Effects of maternal age on resting behaviour and separation were much stronger in 2009 compared to 2017, with occurrence of resting behaviour increasing and separation incidence decreasing with increasing maternal age. Average precipitation and storms occurred in 2009, whereas high rainfall and storm surge occurred in 2017. ANO pups were more likely to be separated from their mothers late in the day in 2017 than in 2009.

**DISCUSSION**

Growth efficiency was high in elephant seal pups when compared to offspring growth efficiency of other mammalian species (McDonald et al., 2012a). These high rates of growth efficiency are likely enabled by the behaviour of pups prior to weaning, which was dominated by resting and suckling and relatively rare use of locomotor behaviours. As expected, this efficiency was influenced by the rate of milk energy delivery. However, comparison of standardized partial regression coefficients suggest that maternal age was 2.5 times more important than milk energy delivered in its effects on growth efficiency, with strong effects evident up to age 10, after which growth efficiency stabilized or declined slightly. This effect was likely mediated by maternal effects on offspring behaviour. Pups of older females spent more time resting, and locomotor behaviour and pup separation were reduced.

Elephant seals breed relatively consistently from the primiparity at age 3 or 4 years to as old as 18 years (Reiter & Le Boeuf, 1991). While previous studies have suggested that natality rates are high...
and invariant (Le Boeuf & Reiter, 1988), more recent studies that tracked females with satellite telemetry suggested that females that forgo breeding return to shore outside of the breeding season and that natality rates may be slightly lower (84%, Robinson et al., 2012), especially after El Niño events (Crocker, Costa, Le Boeuf, Webb, & Houser, 2006; Huber, 1987). In either case, age is a reasonable proxy for prior breeding experience. Due to indeterminate growth displayed by female elephant seals (Deutsch, Crocker, Costa, & Le Boeuf, 1994), older females have body size advantages that may confer collateral benefits in agonistic interactions with conspecifics in the breeding harem (McDonald & Crocker, 2006). Older females display more dominance and hold more central positions in the harem (Le Boeuf & Reiter, 1988; Reiter et al., 1981; Sydeman et al., 1991). This central positioning allows females to minimize harassment by subordinate males, potentially resulting in less movement or lower probability of separation from their pups. The importance of harem location to minimizing disturbance as opposed to direct manipulation of pup behaviour through maternal interactions is supported by a previous study on maternal behaviour that found older females spent more time resting and less time interacting with their pups (McDonald & Crocker, 2006).

Despite strong effects of maternal age on pup activity, no effects on time spent suckling were evident. A previous investigation reported strong increases in milk energy and milk volume delivery rates with maternal mass and age (Crocker et al., 2006). In concert with similar nursing times, these findings suggest higher milk flow rates relative to time spent suckling with increased female age. Some previous studies in pinnipeds have used time spent nursing as a proxy for milk energy intake. As found in horses (Cameron, 1998; Cameron, Stafford, Linklater, & Veltman, 1999), our results suggest that caution should be taken using this approach and that varying rates of milk delivery may occur during similar suckling durations.

Several aspects of pup behaviour changed with development across the suckling period. The proportion of time suckling more than doubled between parturition and weaning. Similar increases in suckling frequency and duration were reported in early studies on northern elephant seals at Ano Nuevo Island (Le Boeuf, Whiting, & Gantt, 1973). Our data suggest that pups suckle nearly 10 times more frequently than reported in this previous study, with shorter intervals between suckling bouts (1.5 h versus 7.7 h). This difference may reflect the potential for disturbance at higher harem densities on Ano Nuevo Island compared to the current study harems. Similar decreases in suckling interval with pup age have also been reported in southern elephant seals, Mirounga leonina, Weddell seals, Leptonychotes weddellii, and harbour seals, Phoca vitulina (Bryden, 1968; Newby, 1973; Tedman & Bryden, 1979), while no changes in suckling frequency were reported in harp seals, Phoca groenlandica, or grey seals, Halichoerus grypus (Kovacs & Lavigne, 1986). As with previous studies, these estimates assume that night-time behaviour was similar to observation periods during the day. Together with well-established increases over lactation in milk fat content (Crocker et al., 2001; Kretzmann et al., 1993), the increase of suckling frequency with pup age suggests potential increases in milk energy delivery late in lactation. This supports the idea that increased energetic costs of lactation in concert with declining adipose reserves forces females to increase protein catabolism late in lactation, ultimately constraining the magnitude and duration of reproductive effort (Crocker, Webb, Costa, & Le Boeuf, 1998).

Figure 7. Diel patterns of pup behaviour at each site (ANO: Ano Nuevo State Park; PRNS: Point Reyes National Seashore; SEFI: Farallon Island National Wildlife Refuge). Logistic plots use the regression parameters from the generalized linear mixed model.
The other important developmental change that occurred was an increase in pup activity. As pups age, they become more mobile, represented by a decrease in time spent resting and an increase in locomotor behaviour. Interestingly, despite this increase in activity, there was no increase in the probability of separation over development, suggesting that functional mother–pup recognition had developed by late in lactation.

Strong diel patterns in behaviour were evident for several behavioural categories. In general, pups were more active early in the day, exhibiting increased vocalization and locomotion, and they became less active as the day progressed (Table 2). This pattern may reflect thermal stress on dark lanugo pups (Codde et al., 2016) or carryover effects from increased activity at dawn. A crepuscular change in activity was described previously for weaned pups (Reiter et al., 1981). Despite this, there was a weak increase in the probability of separation later in the day across all breeding sites.

Our data suggest that the environmental characteristics of each site played an important role in modulating effects of all three explanatory factors for pup behaviour, including maternal effects. The SEFI rookery is located on an elevated granite terrace away from the water’s edge, requiring seals to climb up and over large rocks to access the harem. Historically, within the last century, this harem was covered in sand with sloping access points to the water, providing a mechanism for seals to cool themselves. However, due to years of extreme winter storms, the breeding site is now devoid of sand and is composed mostly of compact dirt and rocks. This to years of extreme winter storms, the breeding site is now devoid of sand and is composed mostly of compact dirt and rocks. This eliminates the seals’ ability to dig down into wet sand or flip sand onto their backs to cool themselves in the heat of the day. Instead, during the peak heat of the day, female seals move towards and into a puddle that forms on the edge of the harem from winter rain storms. The extreme movement exhibited by these females leads to higher occurrence of pup movement and loss of the impacts of storms. The extreme movement exhibited by these females leads to higher occurrence of pup movement and loss of the impacts of maternal age on behaviour. Probability of pups being distant from mothers at SEFI stayed relatively low across all ages of females. This may be due to the small structure and topography of the SEFI harem, which may in itself decrease pups’ ability to wander.

Seals at the Drake’s Beach site at PRNS display a diurnal movement pattern in response to increased solar radiation. The PRNS breeding site consists of sandy, gradual sloping beaches devoid of tidal influences. However, during peaks in cyclical solar radiation, females—pup pairs traverse the beach to the water’s edge and, in some instances, enter the water (Codde et al., 2016). This behaviour increases times of locomoting for pups and may increase chances of pups becoming distant from females. The extra movement that pups engaged in with their mothers during the day may have contributed to the larger increase in resting behaviour, displayed at the end of the day by PRNS seals, that was not seen at ANO or SEFI.

Unlike SEFI and PRNS, pups at ANO were influenced by a semi diurnal tidal pattern resulting in diel movement of the entire harem. Harem density increases dramatically at high tide followed by females and pups redistributing on the beach at low tide. These movements were associated with a dramatic increase in mother—pup separation as the day progressed through the tidal cycle. Effects of maternal age on separation were strongest at ANO, with younger females being much more likely to become separated from their pups. The strong annual differences at ANO but not SEFI suggest the importance of topography and weather in modulating maternal and diel effects. According to the National Oceanographic and Atmospheric Administration (NOAA) National Centers for Environmental Information (2017), the 2017 California winter storm season was one of the strongest in decades, ranking second on record for rainfall. The ANO harem was presented with anomalous challenges in the form of increased winds, and extreme storm surges. These differences reduced the apparent impacts of maternal age on pup behaviour and created a strong diel pattern in separation that was not present in 2009. In contrast, no annual differences in drivers of pup behaviour were evident at SEFI across 3 years of widely differing weather. Together, these patterns suggest site-specific environmental factors, including topography, harem size, thermal stress and exposure to tides and storm surge may modify the ability of maternal effects to influence pup behaviour and create diel patterns of behaviour that are site specific. Similarly, the high growth efficiency and proportions of time spent resting and suckling by pups may reflect the more optimal conditions at our smaller study harem when compared to the larger, denser breeding groups on the primary island colonies.

Conclusion

Maternal age had strong positive impacts on growth efficiency of suckling pups. Maternal experience and size create maternal effects that reduce energetically expensive locomotor behaviours, increase the time pups spend resting and reduce the probability of mother–pup separation. In addition, strong diel patterns and changes with development were evident in pup behaviour. Many of these effects differed between breeding sites, suggesting site-specific influences of the environment that modulate maternal effects and the other drivers of pup behaviour. Northern elephant seals are strongly philopatric (Reiter et al., 1981), suggesting that some aspects of maternal effects may be altered by site-specific environmental effects during prior breeding experiences. Our data support the evidence for increased weaning success and pup growth with maternal age documented in previous studies (e.g. Reiter et al., 1981; Sydeman et al., 1991) and suggest that maternal effects may directly modify the magnitude of parental investment measured at the level of offspring resources at weaning by altering offspring behavioural phenotype.

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References


to have no measurable tritiated water (HTO) in their plasma prior to the isotope injection. Pups were removed and injected with 64 MBq HTO in 5 ml of sterile saline. The syringe was gravimetrically calibrated in order to determine exact injection volume. For the previously published data (Crocker et al., 2001), the pup was injected intraperitoneally and allowed to equilibrate for 3 h. In all subsequent measurements, the water isotope was injected intravenously and allowed to equilibrate for 90 min (Kelso, Champagne, Tift, Houser, & Crocker, 2012). After the equilibration period, two blood samples were collected 10 min apart to confirm equilibration of the isotope. A second procedure at 22–24 days postpartum followed identical methods except that a pre-injection blood sample was taken in order to determine any residual isotope activity and the pup was then injected with 37 MBq HTO in 3 ml of sterile saline. During both the first and second treatment the pup was weighed using a canvas restraint bag attached to a hand winch and scale (MSI tension dynamometer, Seattle, WA, U.S.A.; ±0.1 kg) suspended from an aluminum tripod.

Blood samples were collected in serum vacutainers and centrifuged, and the serum was frozen until the time of analysis. Samples were thawed and the water was extracted using distillation (Ortiz et al., 1978). Samples were analysed for specific activity in triplicate. Two hundred μl of sample water was assayed in 9 ml of Betaphase scintillation cocktail (Westchem, San Diego, CA) using a Beckmann model LS 6500 liquid scintillation counter (Beckmann, Orange County, CA). To calculate total body water (TBW), the total amount of radioactivity injected was divided by the measured density was calculated from a value for each milk component. This allowed identical methods except that a pre-injection blood sample was collected 10 min before and 5 min after the isotope injection. A second procedure at 22–24 days postpartum allowed identical methods except that a pre-injection blood sample was collected 10 min before and 5 min after the isotope injection. A second procedure at 22–24 days postpartum was taken in order to determine any residual isotope activity and the pup was then injected with 37 MBq HTO in 3 ml of sterile saline. During both the first and second treatment the pup was weighed using a canvas restraint bag attached to a hand winch and scale (MSI tension dynamometer, Seattle, WA, U.S.A.; ±0.1 kg) suspended from an aluminum tripod.

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\[ r_{et} + 2000 \left( TBW_2 - TBW_1 \right) / \left( t_2 - t_1 \left( M_1 + M_2 \right) \right) \]

where \( (t_2 - t_1) \) is the time elapsed between treatments, \( TBW_1 \) and \( TBW_2 \) are the two dilution measurements and \( M_1 \) and \( M_2 \) are the two mass measurements (Nagy & Costa, 1980). The milk intake rate \( (M_i) \) was calculated as \( (m_n - m_o) / W \), where \( m_o \) is the rate of oxidatively formed water and \( W \) is the calculated average proportion of water in each female’s milk. Pup metabolism was estimated using the pup’s average mass and the regression equation from Crocker et al. (2001) and \( m_o \) was calculated using the conversion factor of 0.02629 g H₂O/kJ (Schmidt-Nielsen, 1975). Each pup’s total milk intake \( (M_i) \) was calculated as: \( M_i \times \text{lactation duration} \) (d). To use this method, we assume that milk water and oxidative water production are the only sources of water input.

An average energetic density was calculated for each female’s milk using the two end point milk compositions and a curve derived using milk composition data from this study and all previous studies on northern elephant seals (Crocker et al., 2001; Kretzmann et al., 1993; Riedman & Ortiz, 1979). Average energy density was calculated from a value for each milk component. This value was obtained for lipid and water content by integrating over the largest portion of the curve for each component that could connect the two end points. As the change in milk protein content over lactation was monotonic, the two end points were averaged. This method yielded independently derived mean compositions for each of the milk components, which summed to within 95–100%. Protein and lipid components were multiplied by their energy equivalents to obtain a mean energy density for each female’s milk. Milk energy delivered was calculated by multiplying the total milk intake \( (M_i) \) for each pup by the estimated energy density of each mother’s milk.

Body composition was estimated using TBW values assuming that no free water is present in lipid and that fat-free mass has a hydration state of 73.3% free water (Iverson, Bowen, Boness, & Oftedal, 1993; Worthy, Morris, Costa, & Le Boeuf, 1992). Lipid mass was calculated as follows: \( \text{body mass} - 1.37 \times \text{TBW} \). Pup energy storage during growth was calculated from the change in fat and fat-free mass components between the measurements. Energy stored as fat was estimated as 39.33 MJ/kg of fat stored. Energy stored as lean mass was estimated by assuming lean tissue was 73% water and 27% protein and that protein stored had an energy content of 23.5 MJ/kg (Crocker et al., 2001). Growth efficiency was calculated as energy stored divided by milk energy delivered.