Development of fasting abilities in subantarctic fur seal pups: balancing the demands of growth under extreme nutritional restrictions

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Summary

1. Surviving prolonged food deprivation requires various metabolic adaptations such as energy and protein sparing, which can be highly conflicting with energy-demanding stages of an animal's life history such as growth.

2. Due to the maternal attendance pattern, subantarctic fur seal (*Arctocephalus tropicalis* Gray) pups must repeatedly endure exceptionally long fasts of increasing duration throughout the 10-month lactation period. Little is known of (i) how these infants adapt to such extreme energetic constraints while sustaining growth and development; and (ii) the ecological implications of repeated prolonged fasting in early life in terms of offspring survival, maternal care and growth strategy in this species, as well as the evolutionary consequences of such life history trait.

3. Physiological responses to prolonged fasting and how they change with development throughout the pre-weaning period were investigated. Results show that beginning with their first fast, subantarctic fur seal pups are able to mobilize lipid reserves preferentially while conserving protein stores in response to nutritional deprivation. As pup age, profound changes in energy expenditure allow the implementation of an efficient strategy of fat storage and lean body mass preservation, which proves highly adaptive in the face of the low maternal provisioning rates experienced.

4. Despite increasing fasting durations, pup mortality decreased markedly throughout the maternal dependence period. Consistent with predictions, field measurements indicate that fasting endurance, although limited in early life, increases up to durations of nearly 3 months with age. Results suggest that the maternal provisioning strategy could be constrained by these ontogenetic changes in pup fasting abilities.

5. Furthermore, extreme energetic constraints and local density-dependent effects appear to exert a strong selective pressure upon the adoption of a convergent growth strategy between the sexes aiming to maximize fat storage and pre-weaning survival.

6. The issues of resulting trade-offs between pre- and post-weaning survival and the evolutionary consequences of extreme fasting abilities are also addressed.

Key-words: body fat, energy conservation, fuel partitioning, growth strategy, maternal care, offspring survival, pinnipeds, protein sparing

Introduction

To survive and reproduce or grow, animals must adaptively allocate resources among competing physiological systems in a fashion complementary to current or impending environmental conditions (Nelson & Demas 1996; Ricklefs & Wikelski 2002). If environments remained constant, animals

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would have little need to store energy reserves. However, animals living in fluctuating environments may be confronted with situations where food resources become inadequate to cover nutritional requirements and, thus, need to store energy reserves in the form of body fat to bridge the period of poor food availability and minimize the risk of succumbing to starvation (Owen-Smith 2004; Wang, Hung & Randall 2006).

Correspondingly, a wide range of bird and mammal species (e.g. seals, bears, baleen whales, penguins, hibernators) have evolved the ability to undergo phases of complete abstinence from food and water throughout periods of poor food availability and/or extreme climatic conditions as a natural part of their life history, generally associated with migration, hibernation, reproduction and moulting periods (Mrosovsky & Sherry 1980; Castellini & Rea 1992). Thereby, they have acquired the capability to avoid strong environmental constraints, such as those linked to highly seasonal milieus, and colonize *a priori* unfavourable ecological niches. To do so, these fasting-adapted species adopt a common strategy of extensive fat storage in preparation for periods of food deprivation, energy conservation, preferential mobilization of body fat reserves and protein sparing in absence of feeding (Castellini & Rea 1992, Cherel & Groscolas 1999). The amount of body fat stored determines both the ability to sustain the state of metabolic economy while fasting (the phase II fasting), which in turn determines their resistance to extended fasting (Le Maho, Robin & Cherel 1988; Robin *et al.* 1988; Cherel *et al.* 1992; Noren, Rea & Loughlin 2009), and the level of body protein sparing attained (Goodman *et al.* 1980; Cherel & Groscolas 1999; Noren & Mangel 2004; Rea, Rosen & Trites 2007), which is critical for survival (Le Maho, Robin & Cherel 1988; Caloin 2004).

In the young of non-fasting adapted species, allocation of energy to storage is made at the expense of growth and development (Owen-Smith 2004). As early developmental traits affect future performances and survival, as shown in various long-lived bird (Olsson 1997; Naef-Daenzer, Widmer & Nuber 2001; Blas *et al.* 2007) and mammal species (Festa-Bianchet *et al.* 1997; McMahon, Burton & Bester 2000; Hall, McConnell & Barker 2001), nutritional conditions in early life are likely to have important ecological implications at both the individual and population scales (Lindström 1999). In addition, due to the competing demands of growth and development, infants are generally less able to survive significant periods of food restriction than their older counterparts. Hence, whereas long-term fasts are a natural component of adult life in many vertebrate species, extended fasting is rare in infants. One group of species where this occurs as an integral part of their life history, however, is otariid seals (fur seals and sea lions) (Bonner 1984). Throughout lactation (4 months to 3 years, depending on species), adult female otariid seals adopt a central place foraging strategy, alternating between short nursing periods ashore (1–4 days) and long foraging trips to sea during which their pup remains on land. The duration of maternal foraging trips (2–8 days in most species) reflects the distance females must travel in search of prey and determines the length of the natural fasts pups must repeatedly endure while their mother is absent at sea throughout the maternal dependence period (Gentry & Kooyman 1986; Costa 1991). Thus, from an early age (1–2 weeks), otariid infants are already able to withstand recurrent periods of food deprivation (Arnould, Green & Rawlins 2001) that would prove lethal in most mammalian species (Swiatek *et al.* 1968; Mellor & Cockburn 1986; Owen 1989; Kellogg & Lukefahr 2005).

At Amsterdam Island (southern Indian Ocean), subantarctic fur seals (*Arctocephalus tropicalis* Gray) represent the most extreme example of the otariid life history pattern. Lactating females undertake the longest maternal foraging trips of any otariid seal due to the great distances they must travel to feed on myctophid fish in the subtropical front (up to 1800 km away from breeding colony) (Georges & Guinet 2000; Beauplet *et al.* 2004). Pups of this species (Fig. 1) therefore are forced to undergo extreme fasting bouts repeatedly from birth to weaning. Furthermore, because food availability within the subtropical front dramatically decreases throughout autumn and winter months, these fasts increase in duration throughout the 10-month lactation period: from an average of 14 days in summer (at 0–3 months of age) to > 30 days in winter (at 7–9 months of age), with records regularly exceeding 2 months (Georges & Guinet 2000; Beauplet *et al.* 2004; Verrier *et al.* 2009). In contrast, maternal absences of moderate length (≤8 days) cause significant mortality by starvation in the closely related Antarctic fur seal (*A. gazella*) pup (McCafferty *et al.* 1998) and, in years where environmental perturbations such as El Niño events affect food availability for mother provisioning pups, up to nearly 100% of pup mortality has been observed in Galapagos fur seals (*A. galapagoensis*), Galapagos sea lions (*Zalophus californianus wollebaeki*) and South American sea lions (*Otaria flavescens*) (Trillmich & Limberger 1985; Soto, Trites & Arias-Schreiber 2004).

Fig. 1. Subantarctic fur seal pup at Amsterdam Island (study pup V799). Due to the maternal attendance pattern, subantarctic fur seal pups must repeatedly endure exceptionally long fasts of increasing duration throughout the 10-month lactation period. How they adapt to such extreme energetic constraints while sustaining growth and development, as well as the ecological implications and evolutionary consequences of such life history trait are investigated in the present study. Photograph B. Dauteloup.

Consequently, due to the maternal attendance pattern, subantarctic fur seal pups born at Amsterdam Island face the longest inter-suckling intervals of any mammalian infant, endure among the longest fasts of any physically active mammal (on a mass-specific basis), and spend > 85% of the maternal-dependence period in repeated fasting episodes of extreme durations (Verrier *et al.* 2009). Yet, while almost constantly fasting, they have to prepare for the transition to nutritional independence (Martin 1984). This implies allocating resources into demanding processes such as somatic growth, physiological development and the acquisition of foraging skills, which are highly conflicting with the metabolic adaptations to survive prolonged food deprivation. In response to such energetic constraints, these animals have clearly evolved robust physiological mechanisms making them one of the most advanced evolutionary adaptations of any mammal to conditions of no food and no water during development (Verrier *et al.* 2009). Little is known, however, of how these physiological traits develop, and how pups handle the trade-offs between their immediate survival to prolonged fasting and their long-term fitness. The ecological implications and evolutionary consequences of such unique life history traits also remain largely unexplored.

Understanding the interactions between the physiology and the ecology of animals and their influence on individual strategies (in particular in terms of habitat selection and energy allocation) is critical for comprehending the relative importance of given traits to the animals' life history and the role of natural selection in shaping the evolution of those traits (Ricklefs & Wikelski 2002, Costa & Sinervo 2004). Such insight is also crucial for appreciating animals' capacities to respond to fluctuating environmental conditions and ultimately predicting population responses to anticipated global changes (Frankham & Kingsolver 2004; Trathan, Forcada & Murphy 2007). Subantarctic fur seals pups represent a fascinating model to address these issues pertaining to the ecological physiology of prolonged fasting. Indeed, while substantial information exists on bird and mammal physiological adaptations to long-term fasting (Castellini & Rea 1992; Cherel & Groscolas 1999), little is known, however, of the selective mechanisms that have led to their evolution. For instance, the role of ecological constraints in shaping such phenotypic features and the factors controlling the degree of physiological plasticity possible have remained largely unexplored. As development is associated with significant changes in physiological maturity, body composition, metabolism and various requirements (Brody 1945; Kleiber 1975; Schmidt-Nielsen 1997), tracking alterations in the physiological responses of subantarctic fur seal pups to maternal absences of various lengths throughout their development will contribute to the elucidation of the mechanisms and selective pressures involved in shaping animals' adaptations to food deprivation.

The specific aims of the present study therefore were to (i) investigate the metabolic responses of subantarctic fur seal pups to the natural episodes of prolonged fasting they regularly experience throughout their development and how these responses develop with age; (ii) estimate pups' fasting endurance; and (iii) assess its ecological implications and evolutionary significance in terms of pup survival, individual strategies, and phenotypic plasticity. Energy metabolism was studied through the measurement of body mass loss and resting metabolic rate. Field metabolic rate and fuel utilization were estimated from the changes in body composition, fuel utilization being also assessed through the measurement of plasma metabolites. Fasting endurance was estimated by modelling the use of body lipid and protein stores and by taking into account data from the literature on their critical exhaustion (Le Maho, Robin & Cherel 1988; Robin *et al.* 1988; Caloin 2004). Lastly, pup mortality and maternal attendance patterns were examined throughout the whole lactation period, and their inter-relationship was interpreted in the light of the ecology of the study species.

Materials and methods

STUDY SITE AND ANIMALS

All procedures involved in the present study were approved by the Ethics Committee of the French Polar Institute (IPEV) and the Polar Environment Committee of Terres Australes et Antarctiques Françaises. They complied with the Agreed Measures for the Conservation of Antarctic and sub-Antarctic Fauna and current French laws.

The study was carried out on the subantarctic fur seal breeding colony of La Mare aux Elephants, located on the north-east coast of Amsterdam Island, Southern Indian Ocean (37°55′S, 77°30′E). In this colony, adult females give birth to a single pup each year from late November to early January and weaning takes place at *c.* 10 months of age. As part of a long-term population-monitoring programme, approximately 150 pups of previously tagged females are sexed and marked each year at birth using temporary codes glued to the fur on the top of their head. At approximately 1 month of age, these pups are tagged in the trailing edge of both fore-flippers with an individually numbered plastic tag (Dalton Rototag, Nettlebed, UK) (Georges & Guinet 2000).

Following the 2003–2004 and 2004–2005 pupping seasons, four independent subsample groups were randomly selected among the known-age cohorts to study the responses to fasting at different stages of the lactation period (Table 1): (i) first period of maternal absence at the end of the perinatal attendance period in December 2003–January 2004; (ii) pre-moult in February–March 2004; (iii) moult in April–May 2005; and (iv) winter post-moult in July–September 2005. Working within logistical constraints, these stages were chosen to cover the whole range of natural fasting durations experienced by subantarctic fur seal pups across their rearing period: from the first fast (on average 4–6 days) experienced by naïve pups a week after birth to the extended winter fasts (on average 28–30 days) faced by animals preparing for weaning (Georges & Guinet 2000; Guinet & Georges 2000; Beauplet *et al.* 2004). Age and body mass ranged from 6 days to 9 months and from 4 to 20 kg, respectively (Table 1). Study groups were of balanced sex ratio (Table 1). Pup mortality among the known-age cohorts was monitored on a daily basis from December to April during the breeding season 2003/2004 and from December to October during the breeding season 2004/2005. Stillbirths and deaths that occurred during the perinatal attendance period (mostly by trauma and crushing by conspecifics) were excluded from analysis.

Table 1. Characteristics of the subantarctic fur seal pups studied during natural fasting associated with maternal absence at Amsterdam Island

Study stage	N			Age (days)	Initial BM (kg)	Fasting duration (days)	
	Total	♂	♀			Average	Range
First fast	20	10	10	9·3 (2·6)[a]	6·05 (0·15)[a]	5·2 (0·4)[a]	2–7
Pre-moult	20	10	10	70·3 (6·5)[b]	9·95 (0·30)[b]	13·9 (0·6)[b]	8–18
Moult	14	7	7	127·1 (2·9)[c]	13·60 (0·70)[c]	17·1 (1·4)[b]	8–24
Winter post-moult	20	10	10	218·1 (2·7)[d]	15·90 (0·80)[d]	33·4 (3·2)[c]	15–73

The stages 'First fast' and 'Pre-moult' were studied during the 2003–2004 pupping season, 'Moult' and 'Winter post-moult' during the 2004–2005 pupping season. Average age and BM of the study pups at the onset of the fasting periods monitored are reported. Results are presented as means and SE in parentheses. Values within a column without a common superscript are significantly different (ANOVA: $P < 0.001$).
BM, body mass.

BLOOD SAMPLING AND METABOLIC RATE MEASUREMENT PROCEDURES

Pups of each age group were serially sampled throughout one single period of maternal absence. To detect fasting bouts in the study animals, individual maternal attendance patterns were monitored at least twice daily by visual inspection of the colony. Study periods commenced at the end of a maternal attendance period ashore as the mother departed the colony on a foraging trip and continued until she returned to nurse. Within the different groups, the average fasting duration ranged from 5 to 33 days (Table 1). Pups were captured on days 0, 1, 2, 4 and 6 following maternal departure and subsequently every 4 days from day 8 until the end of the natural fast. As pups were left to move freely in the colony between sampling periods, not all animals could be located and captured on each sampling day. Maternal absence bouts varied between individuals and the fasting periods covered were therefore of unequal durations between study animals.

Upon capture, animals were placed in a large Hessian bag to facilitate manual restraint and a blood sample (5–10 mL, representing < 2% of total blood volume) was collected by venipuncture of either an inter-digital vein of the hind flipper or the brachial vein of the fore flipper within 5 min of capture. Blood was immediately transferred into lithium-heparin-treated tubes and kept on ice for < 3 h prior centrifugation at 1000 *g* for 10 min. Plasma fractions were separated and stored at −20 °C until analysis for metabolite concentrations (within 6 months). Following blood collection, pups were weighed in the bag using a spring scale (±0·05 kg) and transported to the nearby field laboratory (300–600 m from pup location in the colony) where they were allowed to rest in an outdoor enclosure (2·5 × 2 m, exposed to natural climatic conditions) for > 1 h prior to resting metabolic rate (RMR) measurements using standard open-circuit respirometry (Withers 2001).

The respirometry system used was similar to that described by Arnould *et al.* (2003). Nitrogen dilution tests (Fedak, Rome & Seeherman 1981) were used to check for any leaks within the system. Prior to each trial, the O_2 analyser was two point-calibrated using H_2O- and CO_2-free atmospheric air and pure nitrogen. The CO_2 analyser was calibrated twice daily using H_2O- and CO_2-free atmospheric air and a 5% CO_2 in nitrogen mixture (Messer France, Saint Herblain, France). On the first day of capture (day 0), RMR measurement started after a minimum period of 12 h from maternal departure to allow pups to enter a 'post-absorptive' state. Once introduced into the metabolic chamber, pups were allowed to rest and acclimatize for 1 h. Pup behaviour was monitored regularly through a small Plexiglas window installed on the chamber lid. All pups became calm within minutes if not immediately after placement. Measurements of O_2 and CO_2 concentrations, temperature, humidity, pressure and flow were recorded continuously throughout the duration of the experiment (2–3 h). However, only the values corresponding to the 15 min of minimum O_2 consumption after the hour of acclimatization were used for the calculations of resting values of CO_2 production and O_2 consumption using the equations of Withers (Withers 2001). Rates of O_2 consumption were designated as RMR, which corresponds to the maintenance requirements at thermoneutrality. Conversion from mL O_2 to kJ was made assuming a calorific equivalent of 20 kJ L^{-1} O_2 (Schmidt-Nielsen 1997). Upon completion of metabolic measurements, pups were released in the colony at the site of capture.

BODY COMPOSITION MEASUREMENTS AND PLASMA METABOLITE ANALYSES

Body composition was measured by the hydrogen isotope dilution technique (Costa 1987), which has been previously validated in young otariids using tritiated water (Arnould, Boyd & Speakman 1996a). Two days after maternal departure, pups were given an intramuscular injection (*c*. 1 mL) of a weighed dose (±0·001 g) of tritiated water (3·70 MBq mL^{-1} in neonates, 7·40 MBq mL^{-1} in other age groups) after the initial blood withdrawal and weighing. Three to 4 h later, an equilibration blood sample (3–5 mL, representing <1% of total blood volume) was collected to determine the total body water pool (TBW) (Costa 1987; Arnould, Boyd & Speakman 1996a). To determine changes in body composition throughout fasting, pups were injected with a second weighed dose of tritium (1·85 MBq mL^{-1} in neonates, 3·70 MBq mL^{-1} in other age groups) after collection of a background blood sample near the predicted end of the fast and a final blood sample was collected after 3–4 h of equilibration. Dates of mothers' return to the colony were predicted based on the pup age and previous trip durations (Guinet & Georges 2000). On average, the second injection was performed at day $4·0 ± 0·0$, $12·9 ± 0·2$, $14·8 ± 0·9$ and $23·5 ± 1·3$ of fasting in naïve, pre-moult, moulting and winter post-moult pups, respectively. All blood samples were centrifuged and the plasma fraction stored frozen (−20 °C) until analysis within 6 months. Due to the relative unpredictability of maternal return coupled with freely moving pups, not all pups could be successfully recaptured for the second tritium injection prior to re-feeding. Hence, the measurement of body composition changes throughout fasting and related parameters could not be achieved in all study animals.

The specific activity of tritium in plasma water was determined using the evaporative-freeze capture technique as detailed by Beauplet, Guinet & Arnould (2003). Tritium dilution space (HTO, L) was calculated using the following equation: HTO $= (A_i \cdot V)/(A_{eq} - A_0)$,

where A_i represents the specific activity of the injectate (dpm mL^{-1}), V the injection volume (mL), A_{eq} the specific activity of the equilibration sample and A_0 the specific activity of the background sample. Total body water pool (TBW, L) was calculated from HTO as determined empirically in Antarctic fur seal pups: TBW = 0·11 + 0·97 HTO (Arnould, Boyd & Speakman 1996a). Assuming water is exclusively distributed within lean body mass (LBM, kg) and 1 L of water equals 1 kg, LBM was calculated from TBW using the equation: LBM = TBW/α, where α represents the fractional water content of LBM. The value of α was not determined empirically for this study and the value of 0·747 reported for Antarctic fur seal pups was used for calculations (Arnould, Boyd & Speakman 1996a; Beauplet, Guinet & Arnould 2003). Total body lipid (TBL, kg) was then calculated as TBL = BM − LBM, where BM represents body mass and total body protein (TBP, kg) as TBP = LBM − TBW. Average field metabolic rate (FMR) was calculated from the amounts of protein and lipid lost by pups throughout the fasting periods assuming energy equivalents of 18·0 and 39·3 kJ g^{-1}, respectively (Schmidt-Nielsen 1997), and the relative contributions of lipid and protein to FMR (ρ_{fat} and $\rho_{protein}$, respectively) were then deduced.

Plasma metabolite concentrations are good indices of fuel utilization (Nelson, Steiger & Beck 1983; Groscolas 1990). Plasma urea and creatinine concentrations were determined as indicators of protein and skeletal muscle catabolism, respectively. The plasma urea to creatinine ratio (U : C) was then used as a corrected index of whole body protein catabolism (Nelson, Steiger & Beck 1983). Plasma concentration of β-hydroxybutyrate (β-OHB), a by-product of fatty acid oxidation in the liver, was measured as indicator of lipid utilization (Robin *et al.* 1988; Groscolas 1990). Circulating concentrations of urea, creatinine and β-OHB were determined from heparinized plasma using enzymatic methods (kits UR3825, CR3814 and RB1007, respectively; Randox Australia Pty Ltd, Melbourne, Vic., Australia) on a RX Daytona© auto-analyzer (Randox Laboratories Ltd, Crumlin, Antrim, UK).

MODELLING BODY RESERVE DEPLETION AND RESISTANCE TO FASTING

Resistance to fasting is limited by a critical threshold of body reserve depletion. Indeed, due to their structural, mechanical and/or enzymatic functions, body components can be only partly used to fuel metabolism. This is particularly true for body proteins, which can be only depleted by < 40–50% before death occurs and, thus, represent a critical limiting factor to starvation survival (Le Maho, Robin & Cherel 1988; Caloin 2004). Similarly, although body lipids can theoretically be almost entirely depleted to cover maintenance requirements, a lower critical adiposity threshold (3% in rats, 9% in penguins and 5–10% in passerine birds) (Robin *et al.* 1988; Cherel *et al.* 1992, Cherel & Groscolas 1999; Schwilch *et al.* 2002) is known to end the strategy of preferential body fat mobilization and protein loss minimization adopted throughout the phase of metabolic economy (phase II fasting) and trigger the onset of phase III, which resumes reliance on protein catabolism in fasting animals (Goodman *et al.* 1980; Cherel, Robin & Le Maho 1988; Robin *et al.* 1988; Cherel *et al.* 1992; Groscolas, Lacroix & Robin 2007). While phase III fasting may act as a cue to stimulate re-feeding in some species (Koubi *et al.* 1991; Robin *et al.* 1998), the massive protein loss occurring at this terminal stage may rapidly lead to compromised muscle function and irreversible starvation (Le Maho, Robin & Cherel 1988).

Following the approach suggested by Caloin (2004) to model lipid and protein depletion during starvation, we developed a model to estimate (i) the duration required for fasting subantarctic fur seal pups to reach a lower lipid threshold; (ii) the cumulative protein loss associated with given adiposities, as limiting factor to fasting resistance; and (iii) pup fasting resistance, as defined by the sum of the fasting duration required to attain a critical level of body lipid depletion plus the duration of phase III until death occurs.

To determine how long subantarctic fur seal pups would be able to fast prior to reaching the lower critical adiposity thresholds characterizing the entrance into phase III fasting (3% and 9% as in rats and penguins, respectively) (Robin *et al.* 1988; Cherel *et al.* 1992; Cherel & Groscolas 1999; Schwilch *et al.* 2002), we calculated:

(1) the theoretical duration (D_α, days) to reach different levels of body lipid depletion:

$$D_\alpha = 39{\cdot}3{\cdot}\alpha{\cdot}TBL_i/(FMR{\cdot}\rho_{fat}),$$

where α represents the level of body lipid depletion (e.g. α ranges from 0 at the onset of the fast to 1 at complete body lipid depletion), TBL_i the initial total body lipid (g) at the onset of the fast converted into energy units assuming an energy equivalent of 39·3 kJ g^{-1} for lipids (Schmidt-Nielsen 1997) and ρ_{fat} the proportion of daily energy expenditure (FMR, kJ day^{-1}) fuelled by fat over the fasting period;

(2) the final adiposity (%TBL$_\alpha$, %) associated with the different levels of lipid depletion:

$$\%TBL_\alpha = 100{\cdot}(1-\alpha){\cdot}TBL_i/BM_\alpha$$
$$= 100{\cdot}(1-\alpha){\cdot}TBL_i/(BM_\lambda - (dm/dt{\cdot}(D_\alpha - D_\lambda))),$$

where BM_α represents the theoretical body mass (kg) at the level α of body lipid depletion, BM_λ the body mass (kg) measured on day λ of the fast ($D_\lambda < D_\alpha$) and dm/dt (kg day^{-1}) the rate of mass loss between the days λ and α of the fast.

The cumulative protein loss (CPL$_\alpha$, %) associated with the different levels of lipid depletion was calculated as: CPL$_\alpha$ = 100 TBP$_i$ (1 − (1 − ((P_c D_α)/TBP$_i$))), where TBP$_i$ represents the initial total body protein (kg) at the onset of the fast and P_c the rate of protein catabolism (kg day^{-1}) over the fasting period.

Finally, death from starvation has been shown to occur when approximately 50% of body protein has been depleted in severely obese humans and penguins (Le Maho, Robin & Cherel 1988). The theoretical duration (D_β, days) needed to reach this lethal level of protein depletion after entrance into phase III fasting was calculated as

$$D_\beta = 18{\cdot}\beta{\cdot}TBP_s/(FMR{\cdot}\rho'_{protein})$$
$$= 18{\cdot}\beta{\cdot}(TBP_i - P_c{\cdot}D_{\alpha'})/(FMR{\cdot}\rho'_{protein}),$$

where β represents the level of body protein depletion (β = 0·5) and TBP$_s$ the total body protein (kg) at the shift to phase III fasting converted into energy units assuming an energy equivalent of 18 kJ g^{-1} for proteins (Schmidt-Nielsen 1997). $D_{\alpha'}$ represents the theoretical duration to the transition to phase III fasting assuming a level of lipid depletion at this stage (α') corresponding with the attainment of lower critical adiposity thresholds (e.g. 9% and 3%), and $\rho'_{protein}$ the proportion of daily energy expenditure fuelled by body proteins in phase III fasting. $\rho'_{protein}$ was unknown and, thus, estimated as $\rho'_{protein}$ = 14 $\rho_{protein}$, for protein contribution to FMR at the end of phase III is 14 times greater than in phase II in starving emperor penguins (*Aptenodytes forsteri*) (Robin *et al.* 1988). The minimal resistance to starvation (R_{min}, days) was then

estimated as the sum of the fasting duration prior to reaching the critical lipid depletion plus the duration of phase III fasting that can be tolerated before death, i.e.: $R_{min} = D_{\alpha'} + D_\beta$.

STATISTICAL ANALYSES

Statistical analyses were performed using SPSS© (Version 12.0 for Windows; SPSS Inc, Chicago, IL, USA). Linear mixed models were used to analyse data with a repeated measure pattern (Littell, Henry & Ammerman 1998). Individuals were used as random effect and fasting days as ranks for repeated measures. For each mixed model analysis, the covariance structure was examined and the best fit selected based on lowest Schwarz's Bayesian criterion. Effects related to developmental stage, gender and fasting phase were systematically tested by mixed analysis of covariance and removed from the model if not significant ($P > 0.05$) prior to re-running the analysis. Where means were compared by mixed analysis of variance, Sidak adjustments were performed to allow for multiple pair-wise comparisons. Values are reported as means ± SE, and results were considered significantly different at $P < 0.05$.

Results

MASS LOSS AND METABOLIC RATES

Body mass (BM) ($F_{1,77} = 0.739$, $P = 0.393$), rate of BM loss ($F_{1,60} = 0.018$, $P = 0.895$) and metabolic rates (RMR: $F_{1,59} = 0.52$, $P = 0.474$; FMR: $F_{1,44} = 0.10$, $P = 0.759$) were not affected by pup sex. Therefore, data for male and female pups for these variables were pooled for all subsequent analysis. Body mass and absolute RMR decreased in all age groups throughout fasting ($P < 0.01$ in all cases) (Figs 2a and 3a). Mass-specific BM loss (dm/mdt) diminished by nearly 50% by 6 (naïve, pre-moult and winter post-moult) to 8 (moult) days in all pups ($P < 0.001$ in all cases) and remained low and stable thereafter for the remainder of the fasting periods studied ($P > 0.05$ in all cases) (Fig. 2b). Similarly, mass-specific RMR stabilized at 75–80% of its initial level within 4–6 fasting days in the pre-moult, moult and winter post-moult groups ($P \leq 0.001$ in all cases). In contrast, mass-specific RMR remained constant throughout fasting in naïve pups ($F_{1,45} = 0.43$, $P = 0.514$) (Fig. 3b).

Whereas fasting duration ($F_{3,70} = 94.32$, $P < 0.001$) and pup BM ($F_{3,70} = 114.24$, $P < 0.001$) increased significantly throughout development (Table 1), absolute RMR did not differ among the pre-moult, moult and winter post-moult groups ($F_{2,66} = 0.584$, $P = 0.542$). It was, however, significantly lower in naïve pups ($F_{3,72} = 32.31$, $P < 0.001$) (Fig. 3a). Consistently, pup fasting RMR scaled to $BM^{0.75}$ at significantly different levels between age groups: from 1·1 (winter) to 1·5 (moult) and 2·0 (first fast and pre-moult) times above the level predicted for adult terrestrial mammals (Kleiber 1975) (Fig. 4). Correspondingly, dm/mdt ($F_{3,254} = 102.10$, $P < 0.001$) (Fig. 2b), mass-specific RMR ($F_{3,71} = 121.94$, $P < 0.001$) (Fig. 3b) and mass-specific FMR ($F_{3,41} = 13.43$, $P < 0.001$) (Table 2) decreased significantly throughout development: up to 4-, 2·5- and 2·5-fold, respectively, between naïve and winter post-moult pups.
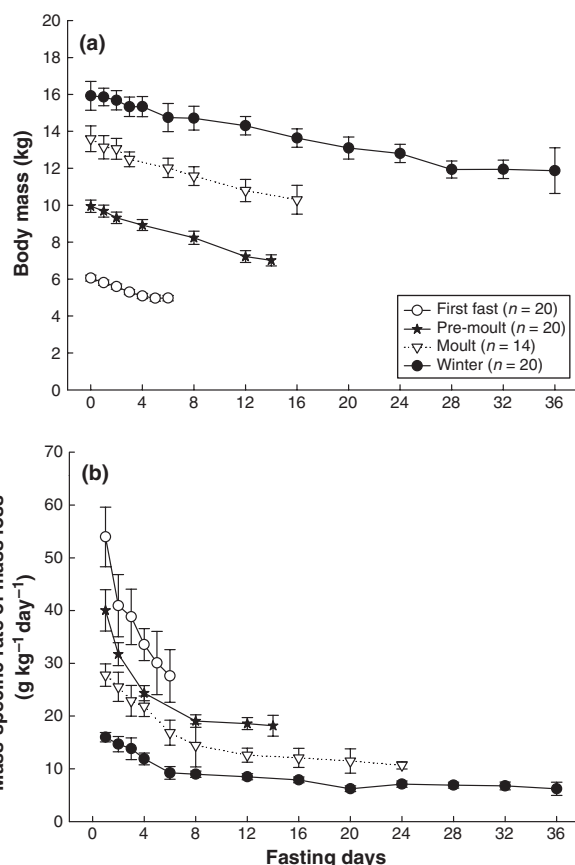

Fig. 2. Changes in body mass (a) and mass-specific rate of daily body mass loss (dm/mdt) (b) throughout natural fasting in subantarctic fur seal pups at Amsterdam Island. Data are presented as mean ± SE.

BODY COMPOSITION AND FUEL PARTITIONING

Overall, there was no sex difference in body fat content ($F_{1,79} = 1.35$, $P = 0.249$), and proportions of lipid and protein contributing to either BM loss ($F_{1,46} = 0.19$, $P = 0.666$) or total energy expenditure ($F_{1,46} = 0.17$, $P = 0.682$). Adiposity increased significantly with age ($F_{3,82} = 169.22$, $P < 0.001$) and decreased significantly throughout fasting in all groups ($P < 0.001$ in all cases) (Table 2). The relative contribution of body lipids to BM loss ($F_{3,42} = 19.30$, $P < 0.001$) and FMR ($F_{3,42} = 12.63$, $P < 0.001$) also augmented with age while that of lean BM diminished ($F_{3,42} = 19.30$ and 14.00, respectively, $P < 0.001$ in both cases) (Fig. 5 and Table 2). Furthermore, when all data were pooled, the contribution of proteins to FMR was significantly negatively related to initial adiposity ($F_{1,43} = 38.06$, $P < 0.001$) (Fig. 6b).

Concomitantly with the reduction in body fat stores, plasma β-OHB concentrations increased significantly throughout fasting in all pups ($P < 0.01$ in all cases) (Table 3). Plasma U : C either remained minimal and stable throughout fasting (first fast; $F_{13,12} = 1.60$, $P = 0.213$) or decreased by 50% within 4–6 fasting days (pre-moult, moult and winter; $P < 0.001$ in all cases) (Table 3). Thereafter, plasma U : C returned to initial value from day 12 in the pre-
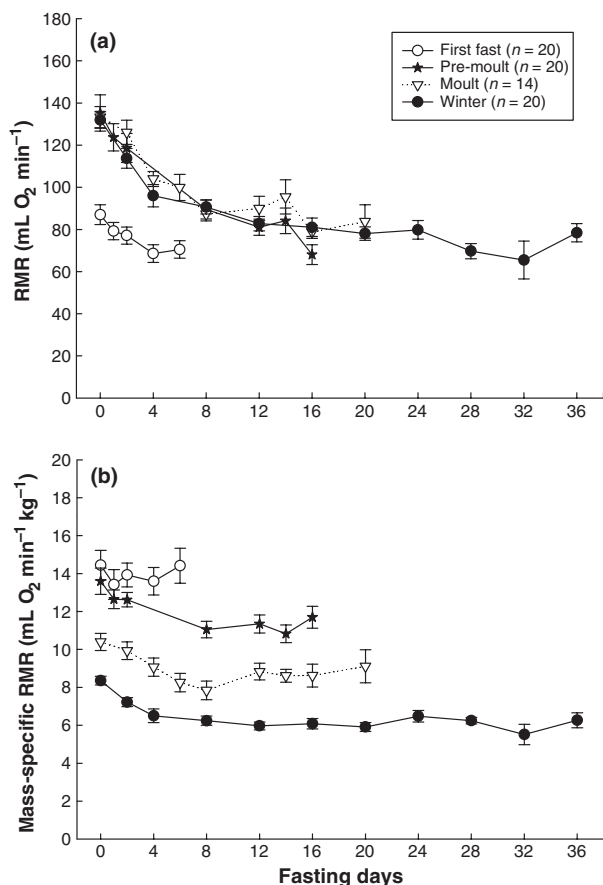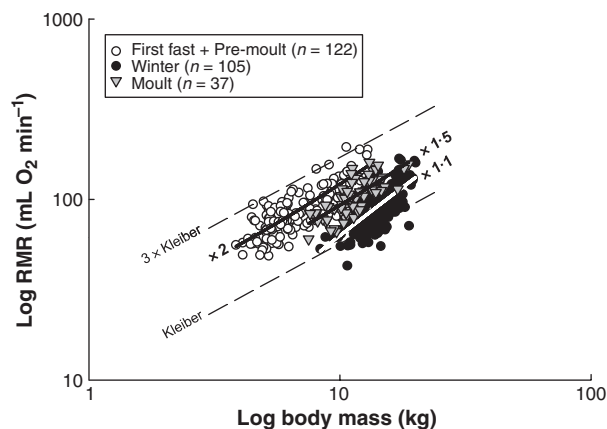
Fig. 4. Relationship between resting metabolic rate (RMR) and body mass (BM) in fasting subantarctic fur seal pups at Amsterdam Island. Linear mixed models were used to account for the repeated measure pattern, with individuals as random effect and fasting days as ranks for repeated measures. Log-log regression of RMR on BM produced significantly different results between age groups ($F_{3,80} = 45\cdot25$, $P < 0\cdot001$), except between naïve (i.e. first fast) and pre-moult pups ($F_{1,40} = 2\cdot49$, $P = 0\cdot122$). Predictive equations were: $y = 0\cdot543x + 1\cdot459$ for naïve and pre-moult pups ($n = 122$, $r^2 = 0\cdot33$, $F_{1,106} = 52\cdot75$, $P < 0\cdot001$); $y = 0\cdot606x + 1\cdot328$ for moulting pups ($n = 37$, $r^2 = 0\cdot36$, $F_{1,13} = 11\cdot33$, $P = 0\cdot005$); and $y = 0\cdot871x + 0\cdot917$ for winter post-moult pups ($n = 105$, $r^2 = 0\cdot48$, $F_{1,30} = 44\cdot19$, $P < 0\cdot001$). The dashed line annoted 'Kleiber' represents the theoretical relationship for adult terrestrial mammals (Kleiber 1975) and the multiplication factor next to each regression line, the corresponding level above Kleiber's prediction.

Fig. 3. Changes in resting metabolic rate (RMR) (a) and mass-specific RMR (b) throughout natural fasting in subantarctic fur seal pups at Amsterdam Island. Data are presented as mean ± SE.

moult and moult groups ($P < 0\cdot05$ in both cases), but remained at a reduced level for the remainder of the fast in winter pups ($P > 0\cdot05$). Winter pups also displayed greater U : C than moulting pups, which in turn had greater U : C than naïve (first fast) and pre-moult pups ($F_{3,94} = 33\cdot26$, $P < 0\cdot001$) (Table 3).

FASTING ENDURANCE AND PUP MORTALITY

Recorded fasting durations were positively correlated with pup initial adiposity ($F_{1,71} = 80\cdot68$, $P < 0\cdot001$) (Fig. 6a). Correspondingly, the theoretical durations to reach a lower critical adiposity threshold increased significantly with age ($P < 0\cdot001$ in all cases): from $7\cdot8 ± 1\cdot3$ days in naïve pups to $66\cdot2 ± 2\cdot5$ days in winter to level 9%, from $11\cdot5 ± 1\cdot5$ to $72\cdot2 ± 2\cdot7$ days to level 3% and from $13\cdot2 ± 1\cdot6$ to $75\cdot1 ± 2\cdot8$ days to complete lipid reserve depletion (Fig. 7a). Cumulative protein loss associated with the critical 3–9% threshold adiposities was on average 11% or 16% in naïve and winter pups and 28% or 36% in pre-moult and moulting animals (Fig. 7b). The model predicted that 17–25% of the pre-moult pups would reach a potentially lethal degree of protein depletion (≥50%) at the 9% and 3% adiposity thresholds, respectively, but none among the other age groups.

Whether at 3% or 9% adiposity threshold, phase III was predicted to last for a minimum of $3\cdot0 ± 0\cdot3$ days in naïve, pre-moult and moulting pups and a minimum of $20\cdot2 ± 3\cdot9$ days in winter post-moult pups.

As a result, minimal resistance to starvation increased exponentially throughout development ($F_{1,47} = 304\cdot46$, $P < 0\cdot001$), from $10\cdot9 ± 1\cdot5$ days in naïve pups to $86\cdot3 ± 4\cdot4$ days in winter pups when considering the 9% adiposity threshold (Fig. 8). Conversely, pup mortality decreased significantly with age ($F_{1,11} = 21\cdot10$, $P < 0\cdot001$) and was found minimal throughout winter in pups aged 7–10 months, although animals face the longest fasts at that stage of the rearing period (Fig. 8). The difference between minimal resistance to starvation and maternal foraging trip durations increased significantly throughout development ($F_{3,42} = 32\cdot98$, $P < 0\cdot001$).

Discussion

THE DEVELOPMENT OF EXTREME FASTING ENDURANCE: IMPORTANCE OF BODY FAT RESERVES

The results of the present study show that beginning with their first fast, subantarctic fur seal pups are able to mobilize preferentially lipid reserves while conserving protein stores during maternal absences. The extent of their physiological adaptations to prolonged fasting develops up to extreme levels throughout the maternal dependence period in response to

Table 2. Rate of mass loss, changes in body composition, total energy expenditure and fuel partitioning during natural fasting in subantarctic fur seal pups at Amsterdam Island

| Stage | N | BM loss (% day^{-1}) | Adiposity (%) | | FMR (kJ day^{-1} kg^{-1}) | $\rho_{protein}$ (%) | ρ_{fat} (%) |
			Initial	Final			
First fast	20 (12)	3·14 (0·17)[a]	16·8 (1·0)[a]	14·3 (1·2)[b]	707 (67)[a]	8·0 (1·2)[a,b]	92·0 (1·2)[a,b]
Pre-moult	20 (12)	1·96 (0·07)[b]	23·9 (0·7)[c]	19·2 (1·4)[a]	437 (46)[b]	11·8 (1·6)[a]	87·3 (1·6)[a]
Moult	14 (10)	1·34 (0·08)[c]	35·0 (1·0)[d]	24·9 (2·7)[c]	473 (68)[b]	6·6 (0·7)[b]	93·4 (0·7)[b]
Winter	20 (12)	0·78 (0·02)[d]	48·5 (0·6)[e]	38·1 (1·8)[d]	289 (10)[c]	1·9 (0·3)[c]	98·1 (0·3)[c]

Study groups were of balanced sex ratio. Results are presented as mean and SE in parentheses, except in the N column where numbers in parentheses represent the number of individuals for which final adiposity, FMR and fuel partitioning could be determined. Values within a column without a common superscript are significantly different (ANOVA: $P < 0.05$). For adiposity, values across both columns without a common superscript are significantly different (mixed ANOVA: $P < 0.05$).
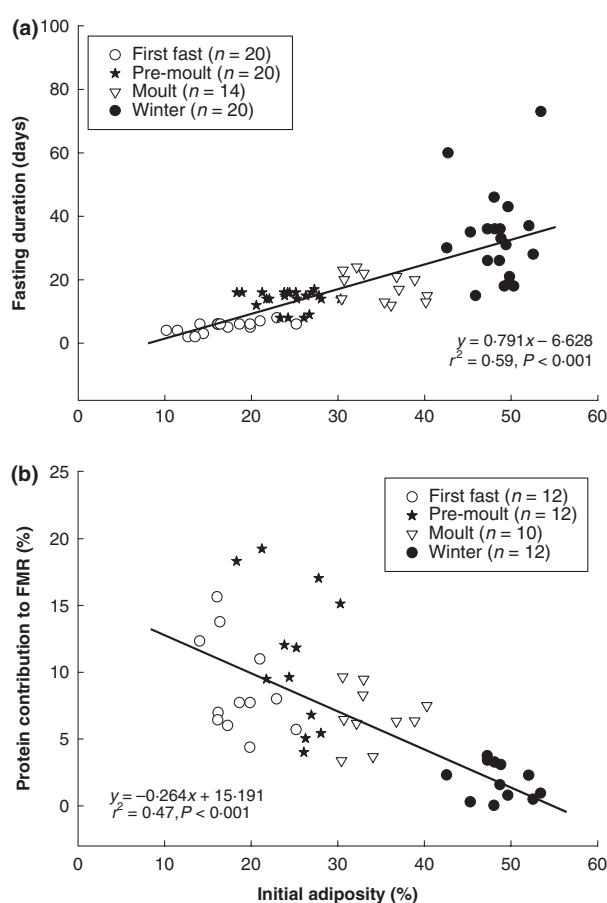BM, body mass; FMR, field metabolic rate; $\rho_{protein}$, proportion of FMR fuelled by protein; ρ_{fat}, proportion of FMR fuelled by fat.
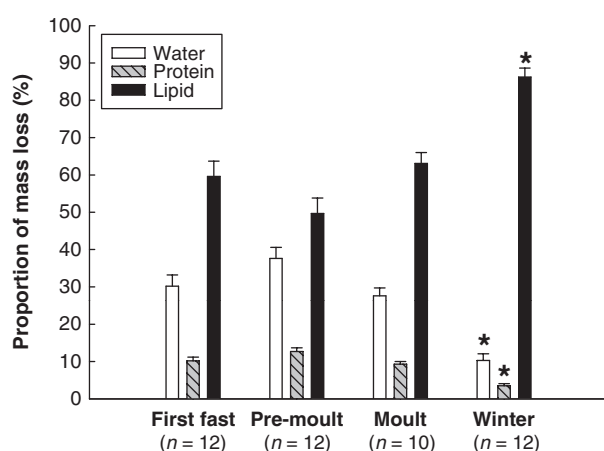
Fig. 5. Composition of body mass loss during fasting in subantarctic fur seal pups at Amsterdam Island. Data are presented as mean ± SE, *n* in parentheses. Asterisks indicate significantly different results between age groups (ANOVA and Sidak: $P < 0.05$).

Fig. 6. Relationships between initial adiposity, fasting duration (a) and the contribution of protein to total energy expenditure (b) in subantarctic fur seal pups. Each point represents one individual. Predictive equations were: $y = 0.791x - 6.628$ ($n = 74$, $r^2 = 0.59$, $F_{1,71} = 80.68$, $P < 0.001$) (a) and $y = -0.264x + 15.191$ ($n = 46$, $r^2 = 0.47$, $F_{1,43} = 38.06$, $P < 0.001$) (b).

the acute nutritional constraints experienced. The model of body reserve depletion predicted their resistance to fasting, although limited in early life, increases exponentially with age, from an average of 10 days during the first fast to the extreme durations of 12–13 weeks in winter. These predictions were supported by field observations among the known-age cohorts. The ontogenetic changes in pup fasting endurance were concurrent with profound changes in metabolic rates (e.g. decrease in mass-specific RMR and FMR), body composition (e.g. increase in adiposity) and metabolic fuel use (e.g. remarkable reduction in body protein use) occurring throughout development, which contribute to enhance pup survival during the increasing durations of maternal absence.

An animal's capability to resist starvation is determined by its ability to store energy and control its allocation during periods of food restriction (Wang, Hung & Randall 2006). Hence, accumulating large energy stores in anticipation of periods of food shortage is of high survival value (Cherel & Groscolas 1999). Correspondingly, subantarctic fur seal pups (i) increased their body fat content as they aged and confronted fasts of increased duration; and (ii) exhibited greater

adiposities and experienced longer fasting durations than any other otariid at any stage of the development (Oftedal, Iverson & Boness 1987; Arnould, Boyd & Socha 1996b; Arnould, Green & Rawlins 2001; Arnould & Hindell 2002; Donohue *et al.* 2002). This is consistent with the positive relationship observed in many vertebrate species between the amount of

Table 3. Changes in plasma β-hydroxybutyrate (β-OHB) concentration and plasma urea to creatinine ratio (U:C) throughout natural fasting in subantarctic fur seal pups at Amsterdam Island

Fasting days	First fast	Pre-moult	Moult	Winter
β-OHB (mmol L^{-1})	*	*	*	#
0	1·54 ± 0·17 (20)[a]	1·59 ± 0·20 (20)[a]	1·66 ± 0·26 (14)[a]	1·39 ± 0·12 (20)[a]
2	2·12 ± 0·11 (19)[b]	1·73 ± 0·16 (20)[b]	1·54 ± 0·17 (14)[a]	1·87 ± 0·14 (20)[a]
4	2·22 ± 0·08 (14)[b]	1·95 ± 0·14 (20)[b]	1·82 ± 0·12 (9)[a]	1·71 ± 0·13 (20)[a]
6	2·43 ± 0·34 (12)[b]	—	1·47 ± 0·10 (12)[a]	—
8	—	1·90 ± 0·18 (19)[b]	1·72 ± 0·20 (9)[a,b]	2·05 ± 0·13 (17)[b]
12	—	1·97 ± 0·10 (19)[b]	2·58 ± 0·27 (12)[b]	3·21 ± 0·17 (18)[c]
16	—	2·66 ± 0·34 (10)[b]	2·93 ± 0·30 (8)[b]	3·56 ± 0·17 (16)[c]
20	—	—	3·53 ± 0·14 (5)[b]	4·01 ± 0·24 (14)[c]
28	—	—	—	3·85 ± 0·23 (8)[c]
36–40	—	—	—	4·86 ± 0·15 (8)[c]
U:C	*	*	#	§
0	88·3 ± 13·9 (20)[a]	188·7 ± 26·0 (20)[a]	145·6 ± 10·4 (14)[a]	290·1 ± 16·1 (20)[a]
2	78·3 ± 8·9 (19)[a]	119·4 ± 16·0 (20)[b]	90·4 ± 9·1 (14)[b]	216·1 ± 18·4 (20)[b]
4	77·0 ± 12·5 (14)[a]	86·4 ± 8·4 (20)[b]	85·9 ± 15·0 (9)[b]	154·9 ± 9·3 (20)[c]
6	84·7 ± 5·3 (12)[a]	—	79·0 ± 8·0 (12)[b]	—
8	—	119·8 ± 8·1 (19)[b]	87·6 ± 12·0 (9)[b]	129·8 ± 9·7 (17)[c]
12	—	142·2 ± 9·7 (12)[a]	107·6 ± 5·1 (12)[a]	163·1 ± 10·3 (18)[c]
16	—	168·1 ± 9·5 (10)[a]	118·0 ± 9·0 (8)[a]	175·3 ± 15·2 (16)[c]
20	—	—	119·4 ± 11·3 (5)[a]	145·0 ± 20·3 (14)[c]
28	—	—	—	167·8 ± 22·9 (8)[b,c]
36–40	—	—	—	158·0 ± 28·9 (8)[b,c]

Study groups were of balanced sex ratio. Results are presented as mean ± SE, *n* in parentheses. For each parameter, values within a column without a common superscript are significantly different (mixed ANOVA: $P < 0.05$) and different symbols in the head row (*, #, §) indicate significant global differences between age groups (mixed ANCOVA: $P < 0.001$).

body fat stores at the beginning of the fast and the duration of fasting (Robin *et al.* 1988; Cherel *et al.* 1992; Cherel & Groscolas 1999; Noren *et al.* 2003, 2008a,b; Caloin 2004).

Limiting energy expenditure can also delay the depletion of energy reserves. Correspondingly, the rates of energy expenditure (RMR and FMR) recorded in the present study were amongst the lowest reported for otariid pups to date (Oftedal, Iverson & Boness 1987; Thompson *et al.* 1987; Arnould, Green & Rawlins 2001; Donohue *et al.* 2002; Beauplet, Guinet & Arnould 2003). Furthermore, the marked reductions in the rates of BM loss (4-fold), RMR (2·5-fold) and FMR (2·5-fold) (on a mass-specific basis) with age confirm the higher metabolic costs incumbent upon the youngest animals (Brody 1945; Schmidt-Nielsen 1997) and the adoption of an efficient energy saving strategy throughout development (Verrier *et al.* 2009). Increasing body size and thermoregulatory capabilities with age in young pinnipeds (Donohue *et al.* 2000; Noren *et al.* 2008a,b), and decreasing behavioural activity throughout the period of maternal dependence in the study animals (Guinet *et al.* 2005; Verrier 2007) could partly contribute to that substantial reduction in energy expenditure.

The deposition of abundant subcutaneous adipose tissue as adiposity increases throughout development is also likely to act as a thick insulative layer, similar to the blubber of phocid seals and cetaceans, with both quantity (i.e. thickness) and quality (i.e. fatty acid composition) impacting on thermoregulatory performances (Dunkin *et al.* 2005; Castellini *et al.* 2009). In addition, with adipose tissue being less metabolically active than lean tissues such as skeletal muscles and the digestive tract, the proportion of metabolically active tissues decreases with increasing adiposity, possibly contributing to the diminution in mass-specific RMR observed as animals age. A similar relationship between increasing adiposity and decreasing mass-specific RMR has also been shown in elephant seals (*Mirounga angirostris* Gill) (Rea & Costa 1992). Fattening also modifies body shape and reduces the surface area to volume ratio, thus further reducing metabolic costs per body mass unit accordingly (Schmidt-Nielsen 1997).
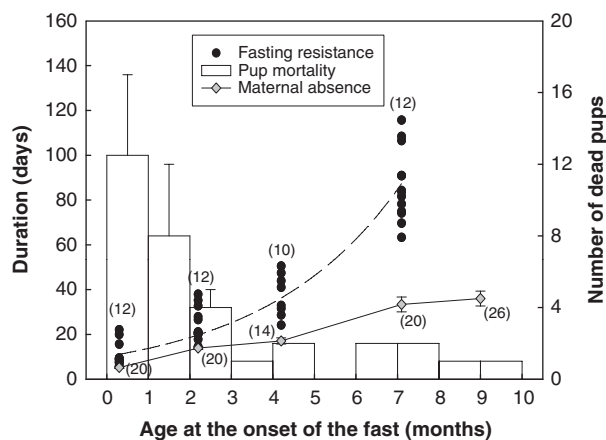
Furthermore, the contribution of protein to total energy expenditure, a critical limiting factor to starvation survival (Le Maho, Robin & Cherel 1988; Caloin 2004) ranged 2–12% only, indicating the adoption of an efficient protein-sparing pathway in all pups, regardless of age (Cherel & Groscolas 1999; Verrier *et al.* 2009) and decreased with age as body fat content increased, in agreement with the inverse relationship between adiposity and protein use found in fasting humans (Dulloo & Jacquet 1999), penguins (Robin *et al.* 1988; Cherel & Groscolas 1999), seals (Carlini *et al.* 2001; Crocker *et al.* 2001; Noren *et al.* 2003; Rea, Rosen & Trites 2007; Rea *et al.* 2009) and bears (Atkinson, Nelson & Ramsay 1996). Hence, with their exceptionally high body fat content, subantarctic fur seal pups were found to display exceptionally low rates of protein catabolism and contribution of proteins to total energy expenditure in comparison with other fasting-adapted species (Verrier *et al.* 2009). The use of body fat as the principal metabolic fuel and limited whole body protein catabolism were supported by an increase in β-OHB concentrations throughout fasting and low-maintained or early decreasing plasma U : C, respectively, in all pups.
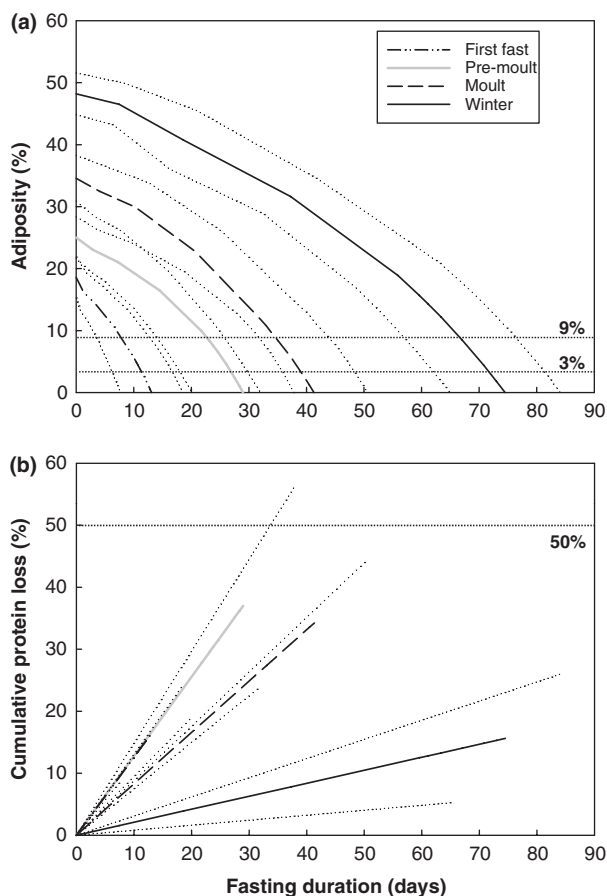
Fig. 7. Model of lipid and protein depletion in fasting subantarctic fur seal pups. (a) Predicted changes in adiposity throughout fasting. Dotted lines represent ±SD. The intercept with the horizontal dotted lines represents the theoretical fasting duration for achieving a 9% or 3% adiposity threshold. (b) Predicted cumulative protein loss throughout fasting. Dotted lines represent ±SD. The horizontal dotted line shows the 50% cumulated loss considered as lethal in fasting mammals.

Fig. 8. Predicted fasting resistance, maternal absence and pup mortality at different developmental stages in subantarctic fur seal pups. Predictions are presented for the achievement of 9% adiposity thresholds and therefore correspond to predicted minimal fasting resistance. Individual values are presented at four developmental stages: first fast ($n = 12$), pre-moult ($n = 12$), moult ($n = 10$) and winter ($n = 12$). Predicted minimal fasting resistance was related to age according to: $y = 10.154e^{0.303x}$ ($n = 46$, $r^2 = 0.89$, $F_{1,43} = 375.59$, $P < 0.001$). Pup mortality was measured among two known-age cohorts of 150 pups each, from December to April during the breeding season 2003/2004, and from December to October during the breeding season 2004/2005. Maternal foraging trip durations represent the durations of maternal absence observed in the study pups. Additional data are given on maternal absences among the known-age cohort 2005 at 9 months of age. Maternal foraging trip durations and pup mortality are reported as ±SE. Numbers in parentheses denote the sample size for fasting resistance estimations (above black circles) and maternal foraging trip durations (next to grey diamonds) for the corresponding sampling period.

The high level of protein sparing attained may be of crucial survival value. Indeed, our model predicted that the average cumulative protein loss associated with complete body fat depletion would be <17% in the naïve and post-moult cohorts and <38% in pre-moult and moulting pups. Such protein depletion is theoretically still compatible with life, especially as young pinnipeds are thought to have a greater tolerance to protein depletion than terrestrial mammals (Bennett *et al.* 2007). Hence, in contrast with obese humans, rats and phocid seals that die from excessive protein depletion before having exhausted their body fat reserves (Le Maho, Robin & Cherel 1988; Cherel *et al.* 1992; Cherel & Groscolas 1999; Bennett *et al.* 2007), starving subantarctic fur seal pups would theoretically reach a lower triggering point for the onset of phase III fasting and lethal cumulative protein loss.

Hence, by both reducing protein use and providing a greater amount of energy stores as lipid to rely on while fasting, the progressive accumulation of abundant adipose tissue throughout the maternal dependence period prepares pups to resist fasts of extreme and increasing duration from birth to

weaning. Increased body fat storage efficiency (Mellish, Iverson & Bowen 1999) across development and as compared with other otariids (Mellish & Loughlin 2003) allows the implementation of a positive feedback cycle (i.e. greater body fat content offers a better insulation and decreased metabolic costs, which reduces energy expenditure and in turn allows for more fat storage), which appears to play a central role in the ontogeny of fasting abilities, and by extension in the animal's growth strategy in response to the nutritional pressures exerted.

ECOLOGICAL IMPLICATIONS AND EVOLUTIONARY SIGNIFICANCE OF PUP FASTING ABILITIES

Our model of pre-weaning fasting abilities estimated that subantarctic fur seal pups' resistance to fasting increases throughout the maternal dependence period from an average of 10 days in naïve pups to up to 90 days in winter. Interestingly, the average durations of maternal foraging trips tended to match the lowest range of pup fasting endurance in summer (i.e. first fast and pre-moult), but were much lower than fasting durations later in the rearing period (Fig. 8). As a correlate, pup mortality was at the highest in summer during the first 3 months of life (summer), although pups were facing the shortest fasting durations, and minimal later (winter), when

maternal absences were the longest and higher mortality would, intuitively, have been expected. Indeed, substantial mortality is recorded in king penguin chicks throughout the prolonged winter fasts when both parents forage at sea for extended periods of time (Cherel, Stahl & Le Maho 1987). In the early development of subantarctic fur seal pups, mortality by starvation could notably be attributed to the high demands of growth and the relative physiological immaturity of the animals resulting in greater metabolic costs (Brody 1945; Schmidt-Nielsen 1997) while body lipid reserves are less abundant.

These observations confirm the limited fasting abilities of pups early in development, which may constrain the amount of time lactating females can spend at sea and result in substantial mortality if maternal return is delayed. At that stage, coming back to the colony as soon as possible to enhance their offspring survival may be given priority by the mother over the replenishment of her body reserves. Pup fasting endurance therefore could be a major constraint upon maternal provisioning strategy in early lactation, dictating the maximum time allocated to foraging and, thus, the maximum foraging range attainable during feeding trips. In contrast, the extreme fasting resistance of pups in winter represents a buffer of considerable survival value against the unpredictability of maternal provisioning. In late lactation, maternal attendance patterns are indeed strongly influenced by alterations in environmental factors (Beauplet *et al.* 2004; Costa 2007) and appear to be mostly controlled by maternal metabolic needs to allow for the replenishment of energy stores in spite of the increased costs of concurrent gestation (Georges & Guinet 2000). This illustrates how the physiological needs and limitations of offspring can exert a strong constraint on maternal strategy, which needs to satisfy both the conflicting demands of offspring growth and maternal own maintenance requirements (Gittleman & Oftedal 1988).

On the other hand, these observations also show how the physiological flexibility selected in pups in response to peculiar maternal foraging patterns acts as a buffer against variations in food availability and, thus, allows species to exploit patchy food resources fluctuating at different spatial and temporal scales, and eventually expand their distribution range. The substantial mortality of pups that was observed early in the maternal dependence period is likely due to individuals having low fat stores and fasting endurance and/or poor quality females not provisioning sufficiently. Indeed, the results of the present study stress the importance of body fat reserves in determining extreme fasting abilities and, thus, promoting pup survival in both the short-term (i.e. resistance to repeated fasts, compensation for the unpredictability of maternal provisioning) and long-term (i.e. accumulation and preservation of body fat stores crucial for subsequent fasts and transition to nutritional independence). Hence, only the pups that are optimally prepared in terms of body condition and, thus, capable of adopting the optimal physiological responses reported (Verrier *et al.* 2009; the present study), might be able to survive up to and throughout winter. The narrow ranges of pup adiposity in the colony at each developmental stage, the reduction in inter-individual variability in pup responses with age (e.g. in fuel selection) (Fig. 6), and the extent of the selective pressure exerted, with 88% of total pre-weaning mortality occurring before the onset of winter, tend to support this assumption.

These observations suggest the occurrence of a strong selection for body fat storage in the study population. In addition to fat storage, body size could be another trait subjected to a strong selection in response to the extreme energetic constraints encountered. Indeed, body size has recently been identified as a phenotypic trait exerting a strong influence on maternal fitness in this species, with females of high quality (i.e. with highest lifetime reproductive success) also being the largest (Beauplet & Guinet 2007). As greater body size potentially correlates with greater energy storage capacity, higher metabolic efficiency (Kleiber 1975; Peters 1983) and better diving proficiency (Kooyman 1989), larger mothers are more likely to raise successfully a young in such a challenging environment. In addition, body size is highly heritable (Silventoinen *et al.* 2003; Rønning *et al.* 2007) and due to allometric scaling, larger offspring experience reduced maintenance costs and heat loss, and can store proportionally more fat (Kleiber 1975; Peters 1983), which possibly enhances their resistance to starvation and survival, at both the pre-weaning (the present study) and post-weaning (Beauplet *et al.* 2005) stages. Correspondingly, subantarctic fur seals breeding at Amsterdam Island are known to be larger in body size than in other locations throughout their breeding range where they make shorter foraging trips (Bester & Vanjaarsveld 1994; Dabin *et al.* 2004).

Such phenotypic variations could result from micro-evolutionary changes linked to contrasted environmental conditions (Frankham & Kingsolver 2004). The fact that high quality females (representing one-third of the breeding-age females) produce nearly four quarters of the viable offspring constituting the next generation in the study population (Beauplet & Guinet 2007) highlights the strength of the selective processes involved. Hence, in the context of our current knowledge of the selective forces driving animals' adaptations to the fluctuations of their environment (Trathan, Forcada & Murphy 2007), the outcomes of the present study illustrate how an ongoing process of natural selection on morphological, behavioural and/or physiological traits in response to specific environmental constraints could be favouring the physiological flexibility needed for individuals to survive and populations to persist against sustained modifications of their environment (Frankham & Kingsolver 2004).

In highly polygynous and sexually dimorphic species such as otariid seals, there is strong selection for large body size in males to facilitate access to females and high reproductive success (Andersson 1994). Consequently, male otariid infants are expected to favour lean body mass protection and growth over fat mass storage, whereas female pups can afford to rely more on protein catabolism (Arnould, Boyd & Socha 1996b; Ono & Boness 1996, Arnould, Green & Rawlins 2001; Arnould & Hindell 2002; Donohue *et al.* 2002). In marked contrast with that commonly observed pattern in other otariid

seals, the results of the present study confirm the absence of any differences in pup body composition and fasting energetics between the sexes in subantarctic fur seal pups at Amsterdam Island (Verrier *et al.* 2009). In addition, they show that pups progressively adopt a common strategy, which enhances their survival in response to the nutritional constraints endured through (i) a state of energy economy promoting pup survival in both short-term and long-term; and (ii) a high adiposity and predominant reliance on body fat permitting a great degree of protein sparing, which in turn extend fasting abilities. These findings suggest a convergent strategy that could have evolved to promote offspring survival in response to repeated prolonged fasting, regardless of gender. This phenomenon could also be amplified by a density-dependent effect suspected to have been causing a deterioration of growth performances over the years at Amsterdam Island and could, thus, contribute to reduce differences in growth strategy between the sexes (Guinet & Georges 2000; Chambellant *et al.* 2003).

The outcomes of the present study also pose the question of how growing animals resolve the conflict between the necessity to conserve energy to be able to survive drastic energetic constraints (Wang, Hung & Randall 2006) and the necessity to allocate substantial amount of energy into physiological and behavioural development to prepare for nutritional independence (Martin 1984). Indeed, limiting energy expenditure implies the curtailment of costly activities such as growth, development, immunity and physical activity (Nelson & Demas 1996). Although it has been previously shown that subantarctic fur seal pup growth is not arrested during the longest winter fasts (Verrier *et al.* 2009), a reduction in resource allocation into physiological and behavioural development could be predicted, as fasting durations become extreme. For instance, the lack of swimming and diving practice observed in these pups (Guinet *et al.* 2005; Verrier 2007), presumably to minimize energy expenditure, is likely to represent a considerable disadvantage for newly weaned individuals commencing to forage independently in the marine environment.

In addition to being crucial for pre-weaning survival (e.g. the present study), body fat stores have been shown to promote post-weaning survival in young pinnipeds for the amount of fat available at weaning will determine the time available to locate a favourable food patch once animals start foraging independently (McMahon, Burton & Bester 2000; Hall, McConnell & Barker 2001; Rutishauser *et al.* 2004). Therefore, in this context, the high level of body lipid stores available at weaning in subantarctic fur seal pups could in fact be a trade-off in the pre-weaning behavioural development (i.e. practice of swimming and diving) for the subsequent post-weaning survival. However, such a trade-off will remain beneficial after weaning only if the abundance of prey in the located patches suffices to support nutritional requirements and compensates for suboptimal diving performances. In contrast, it would increase the vulnerability of newly weaned individuals facing unpredictable changes in environmental conditions and food availability (Beauplet *et al.*

2005). This skewed trade-off between pre- and post-weaning pup survival could represent the price to pay for exploiting remote food resources in central place foraging mothers.

The present study shows how infants of a pinniped species adapt to the extreme nutritional conditions they naturally experience throughout their development due to the maternal attendance pattern. Data reveal the progressive implementation of an efficient strategy of fat storage, metabolic economy and lean body mass preservation, enabling them to survive recurrent periods of food deprivation of up to 3 months while their mother is foraging at sea. These findings stress the central role of body fat storage in the species growth strategy, which is of high survival value in both short-term and long-term. Resulting trade-offs between pre- and post-weaning survival could represent major factors driving population dynamics (Blumstein & Foggin 1997; Beauplet *et al.* 2005) and therefore require further attention. Further research should also focus on the endocrine regulations governing the development of the extreme adaptations reported. Our current understanding of the mechanisms controlling energy balance and nutritional transitions in free-ranging animals would clearly benefit from it.

References

Andersson, M. (1994) *Sexual Selection*, Princeton University Press, Princeton, NJ.

Arnold, J.P.Y., Boyd, I.L. & Socha, D.G. (1996b) Milk consumption and growth efficiency in Antarctic fur seal (*Arctocephalus gazella*) pups. *Canadian Journal of Zoology*, **74**, 254–266.

Arnould, J.P., Boyd, I.L. & Speakman, J.R. (1996a) Measuring the body composition of Antarctic fur seals (Arctocephalus gazella): validation of hydrogen isotope dilution. *Physiological Zoology*, **69**, 93–116.

Arnould, J.P.Y., Green, J.A. & Rawlins, D.R. (2001) Fasting metabolism in Antarctic fur seal (Arctocephalus gazella) pups. *Comparative Biochemistry and Physiology A*, **129**, 829–841.

Arnould, J.P.Y. & Hindell, M.A. (2002) Milk consumption, body composition and pre-weaning growth rates of Australian fur seal (Arctocephalus pusillus doriferus) pups. *Journal of Zoology*, **256**, 351–359.

Arnould, J.P.Y., Luque, S.P., Guinet, C., Costa, D.P., Kingston, J. & Shaffer, S.A. (2003) The comparative energetics and growth strategies of sympatric Antarctic and subantarctic fur seal pups at Iles Crozet. *Journal of Experimental Biology*, **206**, 4497–4506.

Atkinson, S.N., Nelson, R.A. & Ramsay, M.A. (1996) Changes in the body composition of fasting polar bears (*Ursus maritimus*): the effect of relative fatness on protein conservation. *Physiological Zoology*, **69**, 304–316.

Beauplet, G. & Guinet, C. (2007) Phenotypic determinants of individual fitness in female fur seals: larger is better. *Proceedings of the Royal Society Biological Sciences*, **274**, 1877–1883.

Beauplet, G., Guinet, C. & Arnould, J.P.Y. (2003) Body composition changes, metabolic fuel use, and energy expenditure during extended fasting in subantarctic fur seal (Arctocephalus tropicalis) pups at Amsterdam Island. *Physiological and Biochemical Zoology*, **76**, 262–270.

Beauplet, G., Dubroca, L., Guinet, C., Cherel, Y., Dabin, W., Gagne, C. & Hindell, M. (2004) Foraging ecology of subantarctic fur seals *Arctocephalus tropicalis* breeding on Amsterdam Island: seasonal changes in relation to maternal characteristics and pup growth. *Marine Ecology Progress Series*, **273**, 211–225.

Beauplet, G., Barbraud, C., Chambellant, M. & Guinet, C. (2005) Interannual variation in the post-weaning and juvenile survival of subantarctic fur seals: influence of pup sex, growth rate and oceanographic conditions. *Journal of Animal Ecology*, **74**, 1160–1172.

Bennett, K.A., Speakman, J.R., Moss, S.E.W., Pomeroy, P.P. & Fedak, M.A. (2007) Effects of mass and body composition on fasting fuel utilisation in grey seal pups (*Halichoerus grypus* Fabricius): an experimental study using supplementary feeding. *Journal of Experimental Biology*, **210**, 3043–3053.

Bester, M.N. & Vanjaarsveld, A.S. (1994) Sex-specific and latitudinal variance in postnatal growth of the sub-Antarctic fur seal (*Arctocephalus tropicalis*). *Canadian Journal of Zoology*, **72**, 1126–1133.

Blas, J., Bortolotti, G.R., Tella, J.L., Baos, R. & Marchant, T.A. (2007) Stress response during development predicts fitness in a wild, long lived vertebrate. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 8880–8884.

Blumstein, D.T. & Foggin, J.M. (1997) Effects of vegetative variation on weaning success, overwinter survival and social group density in golden marmots (*Marmota caudate aurea*). *Journal of Zoology*, **243**, 57–69.

Bonner, W.N. (1984) Lactation strategies in pinnipeds: problems for a marine mammalian group. *Symposium of the Zoological Society of London*, **51**, 253–272.

Brody, S. (1945) *Bioenergetics and Growth, With Special Reference to the Efficiency Complex in Domestic Animals*. Hafner, New York.

Caloin, M. (2004) Modeling of lipid and protein depletion during total starvation. *American Journal of Physiology*, **287**, E790–E798.

Carlini, A.R., Marquez, M.E.I., Ramdohr, S., Bornemann, H., Panarello, H.O. & Daneri, G.A. (2001) Postweaning duration and body composition changes in southern elephant seal (*Mirounga leonina*) pups at King George Island. *Physiological and Biochemical Zoology*, **74**, 531–540.

Castellini, M.A. & Rea, L.D. (1992) The biochemistry of natural fasting at its limits. *Experientia*, **48**, 575–582.

Castellini, M.A., Trumble, S.J., Mau, T., Yochem, P. & Stewart, B. (2009) Body and blubber measurements of Antarctic pack-ice seals: implications for the control of body condition and blubber regulation. *Physiological and Biochemical Zoology*, **82**, 113–120.

Chambellant, M., Beauplet, G., Guinet, C. & Georges, J.Y. (2003) Long-term evaluation of pup growth and preweaning survival rates in subantarctic fur seals, *Arctocephalus tropicalis*, on Amsterdam Island. *Canadian Journal of Zoology*, **81**, 1222–1232.

Cherel, Y. & Groscolas, R. (1999) Relationships between nutrient storage and nutrient utilisation in long-term fasting birds and mammals. *Proceedings of the 22nd International Ornithological Congress, Durban* (eds N.J. Adams & R.H. Slotow), pp. 17–34. Birdlife, Johannesburg, South Africa.

Cherel, Y., Robin, J.P. & Le Maho, Y. (1988) Physiology and biochemistry of long-term fasting in birds. *Canadian Journal of Zoology*, **66**, 159–166.

Cherel, Y., Stahl, J.C. & Le Maho, Y. (1987) Ecology and physiology of fasting in king penguin chicks. *Auk*, **104**, 254–262.

Cherel, Y., Robin, J.P., Heitz, A., Calgari, C. & Le Maho, Y. (1992) Relationships between lipid availability and protein utilization during prolonged fasting. *Journal of Comparative Physiology B*, **162**, 305–313.

Costa, D.P. (1987) Isotopic methods for quantifying material and energy intake of free-ranging mammals. *Approaches to Marine Mammals Energetics* (eds A.C. Huntley, D.P. Costa, G.A.J. Worthy & M.A. Castellini), pp. 43–66, Allen Press, Lawrence, KS.

Costa, D.P. (1991) Reproduction and foraging energetics of pinnipeds: implications for life history patterns. *The Behavior of Pinnipeds* (ed. D. Renouf), pp. 301–344. Chapman and Hall, London.

Costa, D.P. (2007) A conceptual model of the variation in parental attendance in response to environmental fluctuation: foraging energetics of lactating sea lions and fur seals. *Aquatic Conservation: Marine and Freshwater Ecosystems* **17**, S44–S52.

Costa, D.P. & Sinervo, B. (2004) Field physiology: physiological insights from animals in nature. *Annual Review of Physiology*, **66**, 209–238.

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

Dabin, W., Beauplet, G., Crespo, E.A. & Guinet, C. (2004) Age structure, growth and demographic parameters in breeding-age female subantarctic fur seals, *Arctocephalus tropicalis*. *Canadian Journal of Zoology*, **82**, 1043–1050.

Donohue, M.J., Costa, D.P., Goebel, M.E. & Baker, J.D. (2000) The ontogeny of metabolic rate and thermoregulatory capabilities of northern fur seal, Callorhinus ursinus, pups in air and water. *Journal of Experimental Biology*, **203**, 1003–1016.

Donohue, M.J., Costa, D.P., Goebel, E., Antonelis, G.A. & Baker, J.D. (2002) Milk intake and energy expenditure of free-ranging northern fur seal, *Callorhinus ursinus*, pups. *Physiological and Biochemical Zoology*, **75**, 3–18.

Dulloo, A.G. & Jacquet, J. (1999) The control of partitioning between protein and fat during human starvation: its internal determinants and biological significance. *British Journal of Nutrition*, **82**, 339–356.

Dunkin, R.C., McLellan, W.A., Blum, J.E. & Pabst, D.A. (2005) The ontogenetic changes in the thermal properties of blubber from Atlantic bottlenose dolphin *Tursiops truncatus*. *The Journal of Experimental Biology*, **208**, 1469–1480.

Fedak, M.A., Rome, L. & Seeherman, H.J. (1981) One-step N_2-dilution technique for calibrating open-circuit VO_2 measuring systems. *Journal of Applied Physiology*, **51**, 772–776.

Festa-Bianchet, M., Jorgensen, J.T., Berube, C.H., Portier, C. & Wishart, W.D. (1997) Body mass and survival of bighorn sheep. *Canadian Journal of Zoology*, **75**, 1372–1379.

Frankham, R. & Kingsolver, J. (2004) Responses to environmental change: adaptation or extinction. *Evolutionary Conservation Biology* (eds R. Ferrière, U. Dieckmann & D. Couvet), pp. 428. Cambridge University Press, Cambridge.

Gentry, R.L. & Kooyman, G.L. (1986) *Fur Seals: Maternal Strategies on Land and at Sea*. Princeton University Press, Guildford, NJ

Georges, J.Y. & Guinet, C. (2000) Maternal care in the subantarctic fur seals on Amsterdam Island. *Ecology*, **81**, 295–308.

Gittleman, J.L. & Oftedal, O.T. (1988) Energy allocation in mammalian reproduction. *American Zoologist*, **28**, 868–875.

Goodman, M.N., Larsen, R.P., Kaplan, M.M., Aoki, T.T., Young, V.R. & Ruderman, N.B. (1980) Starvation in the rat. II. Effect of age and obesity on protein sparing and fuel metabolism. *American Journal of Physiology*, **239**, E277–E286.

Groscolas, R. (1990) Metabolic adaptations to fasting in emperor and king penguins. *Penguin Biology* (eds L.S. Davis & J.T. Darby), pp. 269–296. Academic, San Diego, CA.

Groscolas, R., Lacroix, A. & Robin, J.-P. (2007) Spontaneous egg or chick abandonment in energy-depleted king penguins: a role for corticosterone and prolactin? *Hormone and Behavior*, **53**, 51–60.

Guinet, C. & Georges, J.Y. (2000) Growth in pups of the subantarctic fur seal (Arctocephalus tropicalis) on Amsterdam Island. *Journal of Zoology*, **251**, 289–296.

Guinet, C., Servera, N., Deville, T. & Beauplet, G. (2005) Changes in subantarctic fur seal pups' activity budget and diving behaviours throughout the rearing period. *Canadian Journal of Zoology*, **83**, 962–970.

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

Kellogg, N.D. & Lukefahr, J.L. (2005) Criminally prosecuted cases of child starvation. *Pediatrics*, **116**, 1309–1316.

Kleiber, M. (1975) *The Fire of Life. An Introduction to Animal Energetics*. Robert E. Krieger Publishing Co. Inc., New York.

Kooyman, G.L. (1989) *Diverse Divers: Physiology and Behaviour*. Springer-Verlag, Berlin.

Koubi, H.E., Robin, J.P., Dewasmes, G., Le Maho, Y., Frutoso, J. & Minaire, Y. (1991) Fasting-induced rise in locomotor activity in rats coincides with increased protein utilisation. *Physiology and Behavior*, **50**, 337–343.

Le Maho, Y., Robin, J.P. & Cherel, Y. (1988) Starvation as a treatment for obesity – the need to conserve body protein. *News in Physiological Sciences*, **3**, 21–24.

Lindström, J. (1999) Early development and fitness in birds and mammals. *Trends in Ecology and Evolution*, **14**, 343–348.

Littell, R.C., Henry, P.R. & Ammerman, C.B. (1998) Statistical analysis of repeated measures data using SAS procedures. *Journal of Animal Science*, **76**, 1216–1231.

Martin, P. (1984) The meaning of weaning. *Animal Behaviour*, **32**, 1257–1259.

McCafferty, D.J., Boyd, I.L., Walker, T.R. & Taylor, R.I. (1998) Foraging responses of Antarctic fur seals to changes in the marine environment. *Marine Ecology Progress Series*, **166**, 285–299.

McMahon, C.R., Burton, H.R. & Bester, M.N. (2000) Weaning mass and the future survival of juvenile southern elephant seals, *Mirounga leonina*, at Macquarie Island. *Antarctic Science*, **12**, 149–153.

Mellish, J.E., Iverson, S.J. & Bowen, W.D. (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.

Mellish, J.E. & Loughlin, T.R. (2003) Lipoprotein lipase in lactating and neo-natal northern fur seals: exploring physiological management of energetic conflicts. *Comparative Biochemistry and Physiology A*, **134**, 149–158.

Mellor, D.J. & Cockburn, F. (1986) A comparison of energy metabolism in new-born infant, piglet and lamb. *Quarterly Journal of Experimental Physiology*, **71**, 361–379.

Mrosovsky, N. & Sherry, D.F. (1980) Animal anorexias. *Science*, **207**, 837–842.

Naef-Daenzer, B., Widmer, F. & Nuber, M. (2001) Differential post-fledging survival of great and coal tits in relation to their condition and fledging date. *Journal of Animal Ecology*, **70**, 730–738.

Nelson, R.J. & Demas, G.E. (1996) Seasonal changes in immune function. *Quarterly Review of Biology*, **71**, 511–548.

Nelson, R.A., Steiger, D.L. & Beck, T.D.T. (1983) Neuroendocrine and meta-bolic interactions in hibernating black bear. *Acta Zoologica Fennica*, **174**, 137–141.

Noren, D.P. & Mangel, M. (2004) Energy reserve allocation in fasting Northern Elephant Seal pups: inter-relationships between body condition and fasting duration. *Functional Ecology*, **18**, 233–242.

Noren, D.P., Rea, L.D. & Loughlin, T.R. (2009) A model to predict fasting capacities and utilization of body energy stores in weaned Steller sea lions (*Eumetopias jubatus*) during periods of reduced prey availability. *Canadian Journal of Zoology*, **87**, 852–864.

Noren, D.P., Crocker, D.E., Williams, T.M. & Costa, D.P. (2003) Energy reserve utilization in northern elephant seal (Mirounga angustirostris) pups during the postweaning fast: size does matter. *Journal of Comparative Physiology B*, **173**, 443–454.

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

Noren, S.R., Pearson, L.E., Davis, J.W., Trumble, S.J. & Kanatous, S.B. (2008b) Different thermoregulatory strategies in nearly weaned pup, year-ling, and adult weddell seals (*Leptonychotes weddelli*). *Physiological and Biochemical Zoology*, **81**, 868–879.

Oftedal, O.T., Iverson, S.J. & Boness, D.J. (1987) Milk and energy intakes of suckling California sea lion *Zalophus californianus* pups in relation to sex, growth, and predicted maintenance requirements. *Physiological Zoology*, **60**, 560–575.

Olsson, O. (1997) Effects of food availability on fledging condition and post-fledging survival in king penguin chicks. *Polar Biology*, **18**, 161–165.

Ono, K.A. & Boness, D.J. (1996) Sexual dimorphism in sea lion pups: differen-tial maternal investment, or sex-specific differences in energy allocation? *Behavioral Ecology and Sociobiology*, **38**, 31–41.

Owen, O.E. (1989) Starvation. *Endocrinology* (ed L.J. De Groot), pp. 2282–2293. W.B. Saunders Company, Philadelphia.

Owen-Smith, N. (2004) Allocation of food gains to storage versus growth in a variable environment. *International Congress Series*, **1275**, 321–326.

Peters, R.H. (1983) *The Ecological Implications of Body Size*. Cambridge Uni-versity Press, Cambridge.

Rea, L.D. & Costa, D.P. (1992) Changes in standard metabolism during long-term fasting in Northern elephant seal pups (*Mirounga angustirostris*). *Physiological Zoology*, **65**, 97–111.

Rea, L.D., Rosen, D.A.S. & Trites, A.W. (2007) Utilization of stored energy reserves during fasting varies by age and season in Steller sea lions. *Canadian Journal of Zoology*, **85**, 190–200.

Rea, L.D., Berman-Kowalewski, M., Rosen, D.A.S. & Trites, A.W. (2009) Sea-sonal differences in biochemical adaptation to fasting in juvenile and suba-dult Steller sea lions (Eumetopias jubatus). *Physiological and Biochemical Zoology*, **82**, 236–247.

Ricklefs, R.E. & Wikelski, M. (2002) The physiology/life history nexus. *Trends in Ecology and Evolution*, **17**, 462–468.

Robin, J.P., Frain, M., Sardet, C., Groscolas, R. & Le Maho, Y. (1988) Protein and lipid utilization during long-term fasting in Emperor penguins. *Ameri-can Journal of Physiology*, **254**, R61–R68.

Robin, J.P., Boucontet, L., Chillet, P. & Groscolas, R. (1998) Behavioral changes in fasting emperor penguins: evidence for a "refeeding signal" linked to a metabolic shift. *American Journal of Physiology*, **274**, R746–R753.

Rønning, B., Jensen, H., Moe, B. & Bech, C. (2007) Basal metabolic rate: heri-tability and genetic correlations with morphological traits in the zebra finch. *Journal of Evolutionary Biology*, **20**, 1815–1822.

Rutishauser, M.R., Costa, D.P., Goebel, M.E. & Williams, T.M. (2004) Eco-logical implications of body composition and thermal capabilities in young Antarctic fur seals (Arctocephalus gazella). *Physiological and Biochemical Zoology*, **77**, 669–681.

Schmidt-Nielsen, K. (1997) *Animal Physiology: Adaptation and Environment*. Cambridge University Press, Cambridge, UK.

Schwilch, R., Grattarola, A., Spina, F. & Jenni, L. (2002) Protein loss during long-distance migratory flight in passerine birds: adaptation and constraint. *Journal of Experimental Biology*, **205**, 687–695.

Silventoinen, K., Sammalisto, S., Perola, M., Boomsma, D.I., Cornes, B.K., Davis, C., Dunkel, L., De Lange, M., Harris, J.R., Hjelmborg, J.V., Luci-ano, M., Martin, N.G., Mortensen, J., Nisticò, L., Pedersen, N.L., Skytthe, A., Spector, T.D., Stazi, M.A., Willemsen, G. & Kaprio, J. (2003) Heritabil-ity of adult body height: a comparative study of twin cohorts in eight coun-tries. *Twin Research*, **6**, 399–408.

Soto, K.H., Trites, A.W. & Arias-Schreiber, M. (2004) The effects of prey avail-ability on pup mortality and the timing of birth of South American sea lions (*Otaria flavescens*) in Peru. *Journal of Zoology*, **264**, 419–428.

Swiatek, K.R., Kipnis, D.M., Mason, G., Chao, K.L. & Cornblath, M. (1968) Starvation hypoglycemia in newborn pigs. *American Journal of Physiology*, **214**, R400–R405.

Thompson, S.D., Ono, K.A., Oftedal, O.T. & Boness, D.J. (1987) Thermoregu-lation and resting metabolic rate of California sea lion (*Zalophus californi-anus*) pups. *Physiological Zoology*, **60**, 730–736.

Trathan, P.N., Forcada, J. & Murphy, E.J. (2007) Environmental forcing and Southern Ocean marine predator populations: effects of climate change and variability. *Philosophical Transactions of the Royal Society B*, **362**, 2351–2365.

Trillmich, F. & Limberger, D. (1985) Drastic effects of El Niño on Galapagos pinnipeds. *Oecologia*, **67**, 19–22.

Verrier, D. (2007) *Extreme Fasting in Subantarctic fur Seal (Arctocephalus trop-icalis) pups: Physiological Adaptations and Ecological Implications*. PhD the-sis, University of Melbourne, Melbourne, Australia.

Verrier, D., Groscolas, R., Guinet, C. & Arnould, J.P.Y. (2009) Physiological response to extreme fasting in subantarctic fur seal (*Arctocephalus tropicalis*) pups: metabolic rates, energy reserve utilization and water fluxes. *American Journal of Physiology*, **297**, R1582–R1592.

Wang, T., Hung, C.C.Y. & Randall, D.J. (2006) The comparative physiology of food deprivation: from feast to famine. *Annual Review of Physiology*, **68**, 223–251.

Withers, P.C. (2001) Design, calibration and calculation for flow-through respi-rometry systems. *Australian Journal of Zoology*, **49**, 445–461.