Polar Biol (2002) 25: 399–403
DOI 10.1007/s00300-001-0355-6

ORIGINAL PAPER

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Behavioural and morphometric measurements of parental investment in southern elephant seals at the Falkland Islands

Accepted: 17 December 2001 / Published online: 2 February 2002

Abstract Parental investment is a key variable in the study of breeding strategies and life-histories evolution. In *Pinnipedia*, parental investment is usually calculated from direct measurements of pup weight gain or energy transfer between the mother and the pup. These direct methods always involve handling and restraining procedures that pose practical, logistical and ethical problems. To evaluate if weighing can be substituted by indirect observational estimates of parental investment, we analysed the relationship among various behavioural measures of suckling and post-natal growth in the southern elephant seal population of Sea Lion Island (Falkland Islands). Behavioural measures were in all cases a poor predictor of true investment as estimated by weighing. We concluded that there are currently no effective alternatives to direct handling, and that the best way to reduce the potential adverse impact of investment studies is the improvement of the handling protocol, which should include an estimation of the long-term effects on the health of handled animals. Further research is needed to test the validity of non-behavioural indirect methods (e.g. 3D photogrammetry).

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Introduction

Parental investment is a fundamental variable in testing of hypotheses on the evolution of life-histories and breeding strategies (e.g. Clutton-Brock 1991). Moreover, the capability of the parents to invest can be used as an index of the status of populations and ecosystems (Burton et al. 1997). In *Pinnipedia* species, parental investment is usually calculated from direct estimates of weight change or energy transfer of pups and mothers. In seal species with a short and well-defined suckling period, followed by the abrupt weaning of the pup, the weighing of pups at birth and then at weaning permits one to estimate the actual increase in weight during the suckling, which is an excellent index of maternal investment (Le Boeuf and Laws 1994). The tendency to form groups, and the low mobility of most species of seals and sea lions, encouraged researchers studying pinnipeds to use direct handling and weighing more frequently, and with much larger samples of handled animals, than in any other large mammal species. Observational methods were less frequently used (e.g. Trillmich 1986; Higgins et al. 1988).

Although direct-estimation methods permit an accurate assessment of parental investment, they are also more or less invasive. Therefore they pose, in principle, ethical problems, because of the disturbance and pain suffered by the handled subjects (e.g. Bateson 1991), and the potential impact on individual welfare and survival (e.g. Cuthill 1991) and on the population status. Moreover, handling and weighing may be cumbersome, posing practical and logistical problems, particularly with large species.

The use of a non-invasive method is in accordance with the increasing concern about the impact of scientific research on animal welfare (Animal Behaviour Guidelines for the treatment of animals in behavioural research and teaching, http://www.academicpress.com/anbehav; SCAR code of conduct for use of animals for scientific purposes in Antarctica, http://www.scar.org/

intro/animalconduct.htm). Behavioural indices of parental investment present many advantages over direct-estimation methods, because they require data-collection protocols that are easier to set up in the field, and avoid the risks of handling for both the researchers and the animals. However, the relationship between observable suckling behaviour and true parental investment is far from certain (Cameron 1998). The use of behavioural measures may lead to erroneous conclusions, in particular when testing hypotheses about theories of parental investment evolution, and when the physiology of lactation is greatly adapted to specific conditions, e.g. the reduction of the length of the suckling period typical of land-breeding marine mammals (Oftedal et al. 1987).

In southern elephant seals (*Mirounga leonina*), parental investment has been studied almost always by direct weighing of pups and/or mothers (e.g. Campagna et al. 1992; Arnbom et al. 1997; Carlini et al. 1997). Although handling of elephant seals is now common practice, it is an invasive procedure, always requiring some form of physical or chemical restraint due to the large body size of the species. Few studies have evaluated the impact of handling of elephant seals on individual stress or population status. Wilkinson and Bester (1988) showed that on Marion Island, the decline of the local elephant-seal population was homogeneous among areas of low and high human disturbance, although the quantification of the variation in disturbance was approximate. Engelhard et al. (2001) compared two areas of Macquarie Island, one with high human disturbance and one with low disturbance. Weaning weight was lower in the high-disturbance area but this effect was due to the presence of larger mothers, which produce larger weanlings (Galimberti and Boitani 1999), in the low-disturbance area. The authors were not able to form a firm conclusion about the determinants of the distribution of females, which may therefore be or not be affected by disturbance, with smaller, and less dominant (Baldi et al. 1996), females settling in sub-optimal breeding areas. Human disturbance may produce a disruption of female time budgets and suckling schedules (as social molestation does; Galimberti et al. 2000a) and may increase physiological stress. The availability of observational measures correlated with actual investment will reduce the need for direct handling. Unfortunately, no study has yet examined in detail the relationship among behavioural and morphometric measurements of investment in southern elephant seals.

We studied parental investment by direct weighing in southern elephant seals at Sea Lion Island (Falkland Islands, SLI hereafter). On SLI, the adoption of the least invasive research protocol is of particular importance because the island is the only notable breeding colony of elephant seals in the Falklands, and it shelters a small, isolated, and non-increasing population of seals (Galimberti et al. 2001). Moreover, being more accessible than other elephant-seal colonies located on sub-Antarctic islands, it suffers from other sources of human disturbance apart from research activities (e.g. an ever-increasing "nature-oriented" tourism). Therefore, in the context of a wider effort to implement methods that may reduce research impact (e.g. passive transponders for marking: Galimberti et al. 2000b), we collected data to analyse the relationship between observable suckling behaviour and true investment, as estimated from weighing pups at birth and weaning.

Materials and methods

Data collection was carried out on Sea Lion Island, the main breeding colony of elephant seals of the Falkland Islands (Galimberti and Boitani 1999), during the 1998 and 1999 breeding seasons. Mothers were double-tagged in the rear flippers (Jumbo Rototags, Dalton Supplies, Oxon, UK) and marked by black hair dye (Rolcolor Creme Professional, Nuova Ropel, Genoa, Italy). All pups of each cohort were double-tagged (the first tag was put in place at birth, and the second at weaning; weaning = return to sea of the mother, followed by expulsion from the harem of the weaned pup). Tag loss rate between birth and weaning, as estimated from observation of tag-loss wounds, was almost zero. In the study population, the likelihood of mismatch of mother-pup pairs is almost null, as confirmed by microsatellite analysis (unpublished data). After being weighed at birth, pups were also marked by hair bleach, to permit an easy recognition at weaning.

We weighed 121 pups (55 females and 66 males) within 24 h of birth, and again within 24 h from weaning. We calculated three measures of maternal investment from pup weights: (1) the weight increase (weight at weaning–weight at birth, kg); (2) the growth rate (weight increase divided by the number of days between birth and weaning, kg); (3) the relative growth rate (the growth rate divided by the birth weight, g). The intensive observation protocol and the small size of the breeding area (Galimberti and Boitani 1999) permitted observation (or recording with at least 12-h precision) of all births and all weanings. Sex of pups was determined at birth, and checked at weaning, by observing the presence/absence of the penile opening (Campagna et al. 1992). Details on the marking and weighing protocol were presented elsewhere (Galimberti and Boitani 1999). Mothers were classified by three different observers into three size classes (small, medium, large). This classification is repeatable and concordant among observers (Galimberti et al. 2000a), and it is a good approximation of true female size (see also Campagna et al. 1992).

We analysed four behavioural measures of suckling behaviour. The first measure, the total length of the suckling period in days, was calculated from daily records of marked mothers. The other 3 measures were calculated from 704 focal observation periods of 15 min length (9.6 ± 3.2 periods per mother-pup couple, 73 pairs). For each mother-pup couple, we calculated: (1) the total duration of suckling, standardised to 1 h of observation; (2) the mean number of suckling bouts per focal period; (3) the mean duration of the suckling bout. A suckling bout was defined as the continuous period of time in which the pup was attached to the teat and was clearly observed suckling. A break of 5 s or more in the attachment to the teat was considered the end of the bout. Only focal periods in which there was a continuous observation of the mother's teats and pup's face were retained for the analysis.

The distribution of the parental investment variables was symmetric, not significantly different from the normal (Shapiro-Wilks test), and without any clear outlier. Therefore, we assumed a normal error distribution, and we applied parametric statistics. We checked relationships for non-linearities by visual inspection of scatterplots with LOWESS smoother (Trexler and Travis 1993). Regression models and general linear models were run in StatView 5 (SAS Institute). The main goal of the analysis was to evaluate how effective suckling measures were in predicting parental investment measures. We used the coefficient of determination, which is the percentage of the variation in the dependent variable explained by the independent, as measure of effectiveness. We calculated 95% confidence limits for the coefficient of determination using the R2 software (http://www.interchg.ubc.ca/steiger/r2.htm).

Results

Descriptive statistics of all the variables (plus weight at birth and weaning) are reported in Table 1. Suckling variables were homogeneous among female and male pups (*t*-test, suckling per hour: mean diff. = –60 s, t_{37} = –0.55, $P = 0.59$; number of bouts per period: mean diff. = 0.14, $t_{37} = 0.40$, $P = 0.69$; mean bout length: mean diff. = –8 s, $t_{37} = -0.34$, $P = 0.73$) and among mothers of different size classes (ANOVA, suckling per hour: $F_{2,36} = 0.27$, $P = 0.77$; number of bouts per period: $F_{2,36} = 1.62$, $P = 0.21$; mean bout length: $F_{2,36} = 0.62$, $P = 0.54$).

The length of the suckling period in days was related to the weight increase ($b = 4.28$, $t = 5.78$, $P < 0.0001$), but only 23% of variance in weight increase (95% confidence limits: 10–37%) was explained by suckling length. The relationship was similar in female ($R^2 = 0.19$, 95% CL = 3–40%) and male pups ($R^2 = 0.26$, 95% CL = 8–45%). None of the three variables calculated from focal observations was able to predict parental investment. In a series of linear regression analysis, we found coefficient of determinations almost equal to 0 for all direct-measurement variables versus suckling per hour of observation ($N = 39$, $R^2 = 0$–2%), mean number of suckling bouts per focal period ($R^2 = 1$–5%) and mean length of the suckling bout ($R^2 = 0$–3%). None of the regression coefficients was significant ($P > 0.37$ in all cases).

Parental investment may be affected by the sex of the pup and by the size of the mother. In the study population, males are larger than females at birth but not at weaning (unpublished data), and the weaning weight increases with increase of the mother's size (Galimberti and Boitani 1999). To check the effects of these two factors on the relationship between suckling behaviour and parental investment, we ran two series of general linear models, one including sex of the pup (two-level fixed factor: female/male), and the other including the size of the mother (three-level fixed factor: small/medium/large). Due to the sample size, it was not possible to run a single series of general models with both factors. In none of these models were any of the suckling variables significantly related to any of the parental investment measures ($P > 0.20$ in all cases).

Discussion

Only the simplest behavioural measure of investment, the length of the suckling period in days, was related to total weight gained, but it had a small predictive capability. The other behavioural measures were not related to parental investment as calculated from weight measures, with a very small effect size in all tests. Moreover, the main goal of the analysis was prediction, not inference, and, therefore, the relevant measure was the coefficient of determination, which was close to 0 in all cases.

These results could be due to an inadequate behavioural sampling protocol (Cameron 1998). Focal observations can produce a weak estimate of suckling intensity because of observer-induced disturbance, because they cover too small a proportion of mother-pup time budget, or because they are biased with respect to the true suckling activity. Due to the ease of observing elephant seals, and the lack of reaction to humans at the distance from which we observed them (10–20 m), we are inclined to exclude an inhibitory effect due to observer presence. Focal periods were randomly allocated to the various mother-pup pairs. Behaviour was observed only during the daylight hours, but in southern elephant seals, activity is similar at day and night (Baldi et al. 1996; Galimberti et al. 2000a). Moreover, the time budgets calculated from focal observation were in accordance with the ones calculated from a very large database of scan samples (unpublished data). Therefore, we tend to exclude a systematic bias in the behavioural data.

Repeatability was significant, but low, for duration of suckling per focal period ($R = 0.24$, $P < 0.0001$) and for the mean length of the suckling bout ($R = 0.16$, $P = 0.04$); it was close to zero and non-significant for the number of suckling bouts. Therefore, an accurate estimate of the mother-pup couple mean values of these variables requires a very large number of focal periods, and this obviously poses practical problems. In our study, the focal observations had a low productivity. In large harems, it was not always possible to observe mother-pup pairs from a distance and a position that permitted a safe recording of suckling bouts. Moreover, the frequent change in position of the focal individuals, or the nearby ones, frequently forced us to discard the focal observation and start again. Notwithstanding the large

Table 1. Descriptive statistics for the morphometric and behavioural variables (*SD* standard deviation, *CV* coefficient of variation)

Variable	Mean	SD	CV	Min	Max
Birth weight (kg)	44.6	7.3	0.164	28.9	56.0
Weaning weight (kg)	134.9	25.6	0.190	81.0	172.8
Weaning weight–birth weight (kg)	90.3	20.5	0.227	49.6	121.2
Growth rate (kg)	3.9	0.77	0.196	2.1	5.2
Relative growth rate (g)	88.1	15.4	0.175	60.4	115.9
Suckling per hour of observation (s)	629	339	0.538	29	1530
Mean number of suckling bouts	1.7	1.1	0.615	0.1	5.0
Mean length of suckling bout (s)	122	75	0.613	33	333

global effort put in focal observations, we were able to collect a mean of just ten valid focal periods per mother-pup couples. A preliminary assessment of scan sampling on multiple mother-pup pairs demonstrated that a safe assessment of true suckling requires a focal observation protocol, instead of potentially more productive ones (e.g. scan sampling).

In conclusion, and contrary to our initial expectation, measures of observed suckling behaviour are not a valuable replacement for direct weighing in southern elephant seals. They are inaccurate, and they may require an unreasonable increase of observational effort to be improved. This general result is in agreement with a recent review of the relationship among observational and structural measures of suckling in mammal species, which showed a low correlation between observed suckling and true maternal investment (Cameron 1998).

The only ways to reduce the intrinsic drawbacks of the estimation of investment by weighing are to improve the handling protocol, or to use indirect methods to estimate weight. Even simple modifications of the handling protocol may improve animal welfare, by reducing the handling time or disturbance. For example, in elephant seals the use of closed canvas bags instead of nets, although less easy to implement, reduces the intensity of the behavioural reaction during weighing, improving at the same time the accuracy and repeatability of the measures (personal observation). At the same time, the short- and long-term effects of handling should be carefully studied. Weight and growth rates may be compared among areas with different levels of research-induced disturbance (Engelhard et al. 2001). Movement and spatial distribution of weaners, which may be easily estimated using GPS technology (unpublished data), may be compared among years with and without handling disturbance. The availability of non-invasive blood sampling techniques and effective hormone essays (Theodorou and Atkinson 1998; Whitten et al. 1998) may permit a quantitative estimation of the stress induced by handling.

More research is needed on the alternatives to direct weighing to estimate weight. Two-dimensional photogrammetric methods have been used to estimate weight in elephant seals (Haley et al. 1991; Bell et al. 1997). The technique is simple to implement in the field, requiring a photograph of the animal to be taken from the front or the side with some reference object (usually a calibrated survey pole) included in the frame. Although two-dimensional photogrammetry is an effective way to estimate length or weight in adult elephant seals, and males in particular (Haley et al. 1991; Galimberti 1995), it seems less suitable for other age classes (Bell et al. 1997), for which repeatability of measures is lower and confidence limits of the weight estimates are larger. A proper application of the technique requires the subject to be located on a flat and compact surface, to rest in a straight posture, and to remain motionless and unaware of the person carrying the survey pole when approached. In our experience, these requirements are rarely met in the case of weaners, and almost never in the case of pups. An alternative is three-dimensional photogrammetry, which is frequently used to build models of objects and estimate their dimensions without direct measurements, but has rarely been applied to pinnipeds (Waite 2000). Three-dimensional photogrammetry should permit effective volumetric models of elephant seals to be made, due to their simple shape, and these models could be well correlated to actual weight. Although three-dimensional photogrammetry is not easy to implement in the field (personal observation), requiring at least four photographs of the same animal to be taken at the same time, it significantly reduces the need to have the resting subject motionless in a straight posture, because the modelling software allows for bent or distorted body parts. Therefore, this technique may provide a viable alternative to direct weighing of elephant seal pups.

Acknowledgements We wish to thank the following: Carla and Alberto Galimberti for their support of our research on elephant seals; Daniela Marasco and Henry Guala for their help with data collection; the Falkland Islands Government for the research licence; the Falkland Islands Development Corporation; David Gray and Jenny Luxton for their help with the logistics; and Strachan Visick and Sea Lion Island Company for funding.

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