Research on southern elephant seals of Sea Lion Island (Falkland Islands)

Research protocol, current status and prospects for the future

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Abstract

In this report, we describe the rationale, methodology, and prospects for the future of the research project on southern elephant seals of Sea Lion Island, the main breeding colony of this species in the Falkland Islands. We provide a brief introduction to the main areas of the research, which is focused on mating tactics, breeding strategies, parental investment, and individual life-history evolution. We examine in great detail all methodological and technical aspects of the data collection protocol employed in the field, and we summarize some relevant aspects of data processing and analysis. We discuss the ethical background of the project and the impact of our research work on welfare of the population. We briefly review the results produced, providing some statistics on the field work, and a bibliography of the scientific literature produced. Lastly, we outline the main prospects for the development of the project, and we highlight the main areas of development of our research effort in the future.
Introduction

In 1995, a research project on elephant seals of Sea Lion Island (Falkland Islands; hereinafter SLI) was started by a research team (Elephant Seals Research Group, ESRG hereafter) lead by Dr. Filippo Galimberti. The project is currently carried on by two principal investigators, Filippo Galimberti (ESRG, Milano, Italy) and Simona Sanvito (Department of Biology, Memorial University of Newfoundland, St. John’s, Canada). We chose the southern elephant seal (*Mirounga leonina*) as research species because of the high level of polygyny, the strongly despotic mating system, and the ample variation in demography and socionomy of different populations (Le Boeuf and Laws 1994). All together these factors produce an ideal situation to study mating tactics, sexual selection, and breeding strategies. Moreover, elephant seals are easy to approach, mark and observe, and are, therefore, an ideal subject for a longitudinal study of recognizable individuals.

After a few years of field work on elephant seals of the Valdés Peninsula (Patagonia, Argentina: Galimberti 1995; Galimberti et al. 2000 a), we decided to move to a smaller population of elephant seals, to simplify the across-seasons tracking of individuals. At that time, the status of elephant seals in the Falkland Islands was almost unknown, no paper was available in the literature and even the estimates of the size of the population were tentative (Laws 1994). Only anecdotal information was available on the population of SLI (Mr. David Gray, pers. comm.). A pilot study carried out during the '95 breeding season produced results well over the initial expectations, and demonstrated that SLI elephant seals offer a wonderful research opportunity. The population is small and localized, but it is healthy, with high fecundity and low pup mortality rates (Galimberti and Boitani 1999; Galimberti et al. 2001).

Due to its small size and isolation, this population is suitable for an intensive research project, and presents many advantages for a research on breeding behaviour and ecology, including the following:
- it is possible to mark and recognize all the breeding individuals across years, permitting the build up of a longitudinal data base; the advantages of longitudinal sampling plans over cross-sectional ones in the study of breeding strategies is well established in the literature (Arnold and Wade 1984 a; Clutton-Brock 1988 a; Newton 1989); on purely statistical ground, longitudinal data permits a better understanding of phenomena, due to the capability to separate within-subjects effects from between-subjects ones (Diggle et al. 1994).
- the risk of loss of marked individuals due to emigration is small, because of the lack of alternative breeding sites in the rest of the Falklands (Galimberti and Boitani 1999); this permits to reduce the error in estimates of survival and breeding success (Le Boeuf and Reiter 1988)
- the likelihood of undetected mixing of different cohorts, due to immigration of unmarked individuals (Grant and Grant 1989), is also low, because of the isolation from other populations (Galimberti et al. 2001)
- the low risks of sampling bias in the observation of copulations (Drickamer 1974), the low likelihood of undetected variations in local demography and age structure (Charlesworth 1992), and the high likelihood of detection of short term fluctuations in selection (Gibbs and Grant 1987), increase the accuracy of selection pressure estimates.

SLI elephant seals are interesting also from a general point of view. They are an important component of the Falklands wildlife, being the only notable breeding colony of the species in the whole Falklands (Galimberti and Boitani 1999). They represent an asset for Falklands economy, being a main attraction for tourism (David Gray, pers. comm.). Moreover, southern elephant seals have been studied in depth in most of their breeding range, and data about present status, demography and breeding biology is available for most populations (Laws 1994). The Falkland Islands population represents a notable exception, and, hence, this project is a good opportunity to fill the gap.
Research rationale

The focus of the research project are mating tactics, breeding strategies, and individual life histories of male and female southern elephant seals. A mating tactic is a short term behavioural response to the problem of achieving a mate (Dominey 1984). In elephant seals the tactical options for females are limited, due to the scarce opportunities for female mate choice (Galimberti et al. 2000 b; but see Cox and Le Boeuf 1977), while many different mating opportunities are available for males (Baldi et al. 1996). Sexual selection by inter-male competition has probably driven adaptation of morphology and behaviour of male elephant seals (Galimberti 1995).

A breeding strategy is a long term series of decisions about more basic aspects of reproduction, like when to begin breeding, if and when to skip a breeding season, how much effort put in the current breeding attempt, and how much preserve for the next ones (Roff 1992). In elephant seals both males and females have opportunities to fine tune their breeding strategy. For example, males should put particular care in deciding at which age to start competition for mates, while females should decide how to allocate breeding effort across different breeding seasons (Deutsch et al. 1994). Notwithstanding the different temporal scale, and the different effects on individual fitness, mating tactics and breeding strategies are correlated, although in a complex manner (Dunbar 1983; 1984; Caudron 1997).

Although most targets of our original project were short term, it was clear from the beginning that only a long term study may fully exploit the potential of the research. An individual life history record becomes fully valuable information only when it’s complete: for a long living species this implies to collect data in the field for many years. Long term studies of wild mammal populations are quite rare (Clutton-Brock et al. 1982; Endler 1986), mainly due to their practical and logistic drawbacks, but they are needed for a full understanding of the variation of behavioural tactics and strategies (Dunbar 1984), for the estimation of lifetime reproductive success (Clutton-Brock 1988 b), and for the study for the study of natural (Gibbs and Grant 1987; Grant and Grant 1989) and sexual selection (Andersson 1994).

- Male mating tactics

Alternative mating tactics have been identified in many mammal species (Clutton-Brock 1989; Lott 1991). In southern elephant seals, the main mating tactic is to get control of an harem: most of the copulations are done by the holder of the harem (McCann 1981; Galimberti et al. 2002). The association with isolated females is a cheap, but non very valuable, tactic due the rarity of females that give birth away from harems and remain isolated (Galimberti and Boitani 1999; Galimberti et al. 2000 b). The mating with departing females by peripheral males is, on the contrary, quite frequent (Galimberti et al. 2000 c), but the likelihood of these copulations to result in true fertilizations is low, because departing females have in all cases copulated one or more time in the harem with the harem holder (Le Boeuf and Reiter 1988; Marzetti 1997). This is confirmed by genetic data (Wainstein 2000; Anna Fabiani, pers. comm.). Hence, the elephant seals mating system seems to put some limitations to the variability and “creativity” of male mating tactics, and alternative tactics are probably an example of “making the best of a bad job” (Grafen 1987).

On the other hand, the detailed study of a variety of harems during many breeding seasons gave us preliminary evidences that many subtle variation in the tactic of harem acquisition are possible (Galimberti 1995), and that the time of the season, the socionomy of the breeding area, and stochastic factors may modify the effectiveness of mating tactics (Baldi et al. 1996; Modig 1996; Honigman 1998). A safe recognition of such subtle differences, the estimation of true effects of mating tactics on individual fitness, and the
evaluation of the role of stochastic factors require a large sample of breeding situations (the “mating problem”) and male behavioural responses (the “mating solution”). A recurrent problem of research in behavioural ecology is the concentration on a small sample of individuals and groups, that facilitates intensive observations, but may give a misrepresentation of the social and mating system (Altmann and Altmann 1979; Sharman and Dunbar 1982). This problem is often present in research on Pinnipedia species, and is common in elephant seals studies, where there is a strong tendency towards concentrating observations on one or few harems (e.g., McCann 1981; Campagna et al. 1993).

• Phenotypic selection on males

Natural and sexual selection are key concepts of evolutionary biology, but the study of the action of selection in natural population has been a neglected area of research until recently (Endler 1986; Andersson 1994). The measurement of selection pressures in phenotypic traits is crucial to the understanding of current traits maintenance (Grafen 1988), and may give hints on their origin (Reeve and Sherman 1993; but see Grafen 1988). The most debated areas of the study of selection currently are the evaluation of the effect of stochastic factors (Sutherland 1985; Sutherland 1987), and the role of short term fluctuation of selection pressures (Grant and Grant 1989).

We wish to estimate phenotypic selection pressures on male traits and evaluate between seasons variation of these pressures. Due to the widespread correlations between phenotypic traits (Lande and Arnold 1983), and the multivariate nature of phenotypic selection (Arnold and Wade 1984 a; Charlesworth 1992), the measurement of a wide array traits is needed to achieve unbiased estimation of selection coefficients. The study of action of sexual selection on male elephant seals phenotype requires:

- the measurement of the relevant traits of the male structural phenotype (e.g., age, body length, weight, size and shape of secondary sexual characters, acoustic structure of vocal threats)
- the calculation of indices of behavioural performance, from daily records and observations (e.g., length of tenure, indices of competitive success, indices of mating efficiency)
- the calculation of measures of breeding success from behavioural data (e.g., female control, mating success, number of female fertilized)
- the validation of behavioural measures of breeding success with genetic data, through paternity analysis
- the estimation of survival rates between breeding seasons

There is now a wide array of models and statistical tools to analyze the links between phenotype and fitness, and to study selection, in purely observational, non-experimental, settings (Brodie III et al. 1995). Exploratory analysis can be carried out by non-parametric, model-free, methods in two (Schluter 1988; Trexler and Trevis 1993) and three dimensions (Schluter and Nychka 1994). A more formal analysis can be carried out using methods directly related to the theoretical equations of phenotypic selection (Lande and Arnold 1983), both univariate (selection differentials; Endler 1986) and multivariate (selection gradients; Arnold and Wade 1984 b; Phillips and Arnold 1989). Different, and alternative, models of the hierarchical relationships between fitness and phenotypic traits can be explored using path analysis (Crespi and Bookstein 1989; Mitchell 1992; Conner 1996), and compared using structural equation modelling (Focardi and Tinelli 1996; Pugesek and Tomer 1996; Grace and Pugesek 1998; but see Smith et al. 1997; 1998). Multivariate methods are more effective than univariate in the analysis of selection (Gibson 1987). Although potentially very powerful, all these methods have drawbacks (Mitchell-Olds and Shaw 1987). In particular, very large samples are required to obtain good estimates of selection gradients (Van Tienderen 1989), path coefficients, and structural equation parameters (Tanaka 1987). Even
graphical methods are affected by the number of data points. For example, the fitting of three dimensional surfaces to small number of data points is questionable (Phillips and Arnold 1989; Schluter and Nychka 1994). A rough rule of thumbs used in multivariate regression, the simplest approach to multivariate selection analysis, is to have at least ten observations for each regression coefficient to estimate (Rawlings 1988). This should be considered an absolute minimum (Green 1991), and, therefore, the collection of data on phenotype and breeding success of hundreds of individuals is required to apply multivariate methods to the complex system of structural and behavioural traits that may affect breeding in species with a complex breeding biology, mating system, and social behaviour.

• Maintenance of variation in male phenotype

A striking aspect of mating systems with high level of polygyny is the maintenance of variation in male phenotype. This problem was raised, and has been much debated, in relation to lek mating system (the so called “lek paradox”; Reynolds and Gross 1990). The large variance of mating success between males should create strong selection pressures on male traits associated with high mating success, and this should in turn strongly reduce variability of these traits (Gustafsson 1986; Houle 1989; Kirkpatrick and Ryan 1991). In elephant seals, variance of mating success between males is stronger than in most species with lek mating systems (Höglund and Alatalo 1995; Galimberti et al. 2002 b). Hence, elephant seals are an ideal subject to study the problem of maintenance of variability in male traits associated with mating.

A possible solution to the lek paradox is the fluctuation of selection pressure in and between breeding seasons (Turner 1995). We plan to test this hypothesis with our long term series of data collected in different breeding situations. On the other hand, there could be no lek paradox at all (Lanctot et al. 1997). The variance in fertilization could be much lower than variance in observed copulations, and we are currently testing this hypothesis by combining precise behavioural indices of fertilization success with paternity estimation by microsatellite analysis (a summary on the genetic subproject is available online, see the Literature page of our web site, http://www.eleseal.it).

• Male and female breeding strategies

While mating tactics are strongly constrained by the short term situation in which the individual happens to be, long term strategies depend more on the basic breeding physiology of the species, the demography of the populations, and the long term balance between current reproductive effort and future expectation of reproduction (Roff 1992).

Due to the differences of basic reproductive physiology of males and females, different lifetime breeding strategies are expected in the two sexes: in fact, one of the most intriguing aspect of the study of breeding strategies is the comparison between the sexes (e.g., Clutton-Brock et al. 1982). For example, while male elephant seals are usually to fully breed during one or few breeding seasons, female elephant seals may breed for more than a dozen seasons. Hence, for males the crucial problem is to decide when to start breeding, while for females is how to allocate breeding effort across a long series of seasons (Deutsch et al. 1994).

The study of long term breeding strategies of individuals in long living species (in SESs maximum lifetime is about 15 years for males and 20 for females; McCann 1985) requires very long term research projects. In these species, each individual has a chance to breed during many different seasons. Therefore, the effectiveness of a particular strategy of allocation of breeding effort may change from year to year, and what really matters is the net lifetime payoff (Roff 1992). Studies of individual breeding strategies of long living species
are rare, and the main reason is the low initial productivity of the work, and the long delay required before getting interesting, publication-ready, results. On the other hand, long term longitudinal studies of marked animals are the only way to avoid the limitations of cross-sectional studies (Clutton-Brock 1988 a; Newton 1989).

• Female parental investment

The main component of female breeding strategies is seasonal parental investment, i.e., the resources that each female put in the production of a pup (litter size in elephant seals is equal to one, twinning is very rare). Parental investment may be divided in two parts: the gestational investment, i.e., the investment realized during the gestation by transferring of energy through the placenta, and the post-gestational investment, i.e., the investment realized after birth by transferring energy through milk and suckling. There is a notable difference between the two: females feed during the gestation, while they fast during the land phase (Le Boeuf and Laws 1994). Therefore, the energy transfer during suckling depends only on stored reserves of energy. Maternal investment in elephant seals is a complex phenomenon, that involves a huge transfer of resources from the mother to the pup (Deutsch et al. 1994), and his affect by many phenotypic traits of the mother (Boyd et al. 1997), including age, size and experience (Galimberti and Boitani 1999; Galimberti et al. 2002 c). Parental investment is easy to estimate in elephant seals, because the duration of parental cares is short (about 23 days of suckling in the southern species, Campagna et al. 1992) and pups can be easily weighed at birth and at weaning. Weaning weight is a good measure of total parental investment, and the difference between weight at birth and weight at weaning is good a measure of post-gestational investment.

• General knowledge of the population

A good knowledge of the demography and population dynamics of the study population is fundamental for the correct analysis of selection (Charlesworth, 1982), and for the study of breeding strategies (Dunbar, 1983). Therefore, we are gathering basic information on population size, sex ratios, age classes, survival and fecundity rates, and haul out patterns. Although this data is just background for our research, it is also the first base of information on the elephant seals of the Falklands, and may help in the set up conservation policies for this species.

To study the demography and the breeding biology of a small population of elephant seals, like the Sea Lion Island one, may help in the understanding of the causes of the current decline that affect the majority of the large populations of elephant seals around the world (Hindell et al., 1994). The SLI population offers the opportunity to analyze the dynamics of the demographic, behavioral and ecological parameters that affect the breeding biology of a population unit without the disturbing effects of immigration and emigration. In fact, the elephant seal population of Sea Lion Island appears to be fairly isolated during the breeding season from the larger populations of the South Georgia stock (Lewis et al., 1996). No individual marked in the Valdés Peninsula or in South Georgia has ever been re-sighted at Sea Lion Island during the breeding season, and movements are restricted to breeding males of the Valdés Peninsula population that come to SLI for moulting (unpublished data; Mirtha Lewis, pers. comm.).
Methods

- A synopsis of elephant seals biology

A synopsis of elephant seals biology is required to understand the rationale and protocol of our research. Some aspects of southern elephant seals biology are particularly relevant:

- Elephant seals are large (the largest species of the Pinnipedia order) and have an high sexual dimorphism in size (the highest in land breeding mammals); due to this large dimorphism males are able to effectively herd females, and this has a strong impact on mating tactics
- Males have also well developed secondary sexual characters (proboscis, enlarged canines, frontal dermal shield), that appears to be the result of the past action of sexual selection and the adaptation to intense male competition
- Elephant seals have a mixed life style, with two aquatic phases and two land phases (breeding and moultng). The strong adaptation to aquatic life reduce the mobility on land, and this favours the grouping of females and the male control of female groups
- They feed during the aquatic phases of the yearly cycle but completely fast during the land ones; the combination of fasting and concentrated breeding effort implies for both males and females a significant energetic stress and a serious weight loss; this high and concentrated breeding effort was probably the drive of a long series of physiological and life history adaptations (e.g., control of metabolic rate and delay of breeding in males)
- Growth of males is a two phases process, with a gradual phase of growth at rate similar to females before puberty, and a notable growth spur just after; social maturation of males is very slow: puberty is reached when they are about five years old, but true maturity is reached only many years later, because they are not able to get control of a harem until they are nine years old or older
- Pre-breeding mortality of males is high, hence just a small percentage of each male cohort reaches full maturity and starts breeding.
- Breeding is strongly colonial: females concentrate in large groups, of up to many hundreds of individuals; colonial breeding is the first requirement for the evolution of a polygynous mating system
- Reproduction is concentrated in a three months breeding season, and the most of the females breed in a small part of the season (on SLI 88% of copulations are concentrated in three weeks); a concentrated breeding season is an important requirement for the evolution of a despotic system of mate access
- Females have a predictable pattern of presence on land during the breeding season. On SLI females begin to haul out during the second week of September; almost all the females have already gone back to sea by the third week of November. The peak haul out of females is constant. A typical female stay on land for 27 days: after a mean of 5 days spent on land she gives birth; then she suckle the pup for a mean of 20 days before coming into oestrus; then she copulates for a mean of 2 days with the alpha male of the harem, while carrying on the lactation; at the end she weans the pup (after a mean of 23 days of suckling), leaves the harem and goes back to sea
- The mating system of elephant seals is the purest form of harem defence polygyny. Males compete using both conventional signals and direct fights. The results of dyadic interactions set up an almost linear dominance hierarchy between males, and rank in the hierarchy determines the breeding role. One male, the harem holder, has an almost complete control of each female group (called harem), and most dominant males are in charge of the largest harems. Secondary males (that is to say males in touch with females but subordinates to the holder) are sometimes present in larger harems, but most of males which are not able to get control of an harem are kept outside the female group.
- Behavioural interactions between male depend on acoustic communication; acoustic signals are stereotyped and present a large variation between individuals. 

More details about elephant seals biology may be found in Le Boeuf and Laws, 1994.

• The study area

Sea Lion Island (52° 26' S; 59° 05' O) is a small island of about 940 hectares at the southern extreme of the Falklands. Of the 20.3 km of perimeter of the island (estimated from aerial photographs), only the eastern tip (7.2 km of coast) is occupied by elephant seals. Harems are scattered along the beaches and their spacing is uneven, with long (up to 1 km) stretches of beach with no females. Median harem size is about 30 females at maximum haul out (median = 31-35 females in different years). A detailed account of the demography of the population may be found in Galimberti and Boitani (1999).

• The schedule of the field work

Field work was carried out during seven breeding season, 1995 to 2001, from the beginning of September to the end of November, to cover the whole length of the season (about 12 weeks; Galimberti and Boitani, 1999). There is a predictable variation of demands from different field activities along the season: in September the most demanding activity is marking, due to the arrival on land of all the breeding males and the gradual arrival of females at increasing rate; in October the biggest part of the work load is due to censuses and observations of behaviour, with a gradual increase in social interaction rates; in November the departure of females gradually reduce the count and behavioural observation work load, and activities related to weanlings predominate (checking of tags, sexing, weighing, skin sampling). Field work is carried out continuously from down to dusk every day of the breeding season.

• Marking techniques and recognition of individuals

Field research at the individual level requires safe individual recognition (Lehner, 1996). The use of natural marking for recognition is well established (Bateson, 1977; Stevick et al., 2001). On the other side, when the number of individuals is large, and fast recognition is required, like during behavioural observations, the use of artificial marks is safer (Spencer, 1995).

Long term marking, for recognition across breeding seasons, is accomplished by tagging. We apply one or more nylon cattle tags of year-specific colours and with alphanumeric codes (Jumbo Rototags, Dalton Supplies Ltd., www.dalton.co.uk, info@dalton.co.uk) to the rear flippers of all breeding males, the vast majority of breeding females (e.g., > 98% in 1997) and almost all pups (> 99%). These tags are small (length = 45 mm; weight < 2 grams) and are the preferred method to mark elephant seals (Erickson et al., 1993 a; Wilkinson and Bester, 1997). In a comparative test (Testa and Rothery, 1992) Rototags resulted the tags with the lowest tag loss rate. Males and females are tagged in an opportunistic way, in most cases as soon as they arrive on land. Pups are tagged a first time shortly after birth, and a second time after weaning; almost all pups of the population are double tagged. Between seasons tag loss rates are low for both males and females (in 1997 and 1998 the likelihood of losing both tags, as calculated from a binomial model applied to double tagged individuals, was 0.0031 for both males and females). The tag loss rates observed on SLI are similar to the rates observed during a long term study at Marion Island, that are considered enough low to permit
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an accurate study of age-specific survival and fecundity (Pistorius et al., 2000). Tags of returning individuals are systematically checked, and lost tags are replaced whenever possible.

In 1997 we tested an electronic identification system based on implanted passive integrated transponders (PITs; Galimberti et al., 2000), to improve long term marking of weanlings. The system consists in a sensing device which transmits a radio-frequency signal to a specially designed implanted tag, which responds with another radio message containing its ID code. We used Trovan Ltd (www.trovan.com, inform@trovan.com) ID100 implantable transponders, encapsulated in bio-compatible glass (2.2 x 11.5 mm) and LID500 portable readers (reader frequency: 128 MHz; PIT frequency: 64 MHz). Trovan transponders are endorsed by the Conservation Breeding Specialist Group of IUCN (www.cbsg.org). They are individually packaged in a disposable needle, pre-sterilized and ready-to-use, and are implanted with a special applicator on which the needle is fitted. We fitted 510 weanlings with one transponder, implanted between the tail and the begin of the right hind flipper. We also marked with transponders a sample of 37 adult breeding males, to test effectiveness of the marking procedure on adults. Transponders are now widely used to mark wild animals (Schooley et al., 1993) and marine mammals in particular (Thomas et al., 1987; Matsuki and Testa, 1991; Wright et al., 1998), but, to our best knowledge, there was no previous experience of use of these devices on elephant seals. The main advantage of passive transponders is the reduction of the risk of tag failure, while their obvious disadvantages are cost (about 6-12 times the cost of a plastic tag) and the need to get close to the animal and use a sensing device to read the code. In 1998, we checked transponders in a sample of 45 individuals; 42 transponders were easily read one or more time, and in consecutive days; on two of the remaining individuals, transponders were not read the first time because reading conditions were not optimal, but were read on next tries with the individual resting or sleeping; on just one individual we were not able to read the transponder in optimal reading conditions and after many tries. In all, failure rate was very low (97.8% of success). In most cases, reading was easy accomplished, and, with resting individuals, the distance of reading wand from surface of the body was very short (< 5 cm), well below the maximum operating distance of the reader (18 cm). In one case the transponders was crucial in recognition of the identity of a breeding male who lose both tags.

To be able to fast recognize individuals during social interactions even during phases of high activity, we marked all breeding males and as much females as possible (70-85%), by painting an identification code, usually a name, on their back and/or flanks using commercial black hair dye (New Rolcolor Creme Professional 1N: Nuova Ropel, Genova, Italy). Occasionally, we marked females that are difficult to dye paint with paint pellets (Nel-Spot oil based pellets: The Nelson Paint Company, Kingsford, USA) shot by a CO₂ gun (Nel Spot model 007 or ) or a slingshot. This marking method is commonly used in pinnipeds (e.g., Campagna and Le Boeuf 1988). The joint use of tags, dye marks and paint pellets ensured safe recognition of all breeding animals during counts and observations.

Any marking plan should balance the advantages of safe and fast recognition, and the potential adverse effect on animal health, including short term stress and long term reduction of survival (Murray and Fuller, 2000; Gales, 2000). All our marking activities are accomplished by trained operators, without any kind of restrain of the animal (while the individual is sleeping or when its attention is directed towards another person), and have non evident detrimental effect (see “Ethical aspects of the research” section below).

- Census techniques

The relative success of a mating tactic, or a breeding strategy, depends on the quantity and quality of the competitors (Austad, 1984), and, therefore, the demography and socionomy of
the local population may shape the effectiveness of behavioural tactics of mate acquisition and reproduction (Altmann and Altmann, 1979; Dunbar, 1983; Baldi et al., 1996; but see Twiss et al. 1998). Moreover, when animals are organized in social units, the composition of the mating group, which depends at least in part from stochastic factors (Altmann and Altmann, 1979), may significantly change the relative payoffs of different tactics and strategies (Dunbar, 1984).

Each day of the breeding season (84 in all), one observer counts the population while walking along all breeding beaches at low tide. We use standard census techniques (Erickson et al., 1993 b), identifying all males and all isolated females, counting females in harems (using manual tally counters), and identifying as much females as possible in each harem. From daily censuses three kinds of data are obtained:
- counts of the number of individuals on land by sex and age/size class, to be used to study the demography and population dynamics
- data on the structure of breeding units (number and identity of females, number, status and position of males in or near the harem), to be used to study the socionomy of the population
- data on marked males and females (age class, size class, breeding status and location), to update the daily individual records

To check for presence of breeding individuals outside our main study area we also carry out weekly censuses by walking along the cliff-tops of the entire perimeter of the island.

Weanling sex ratio (number of females per male) is estimated by recording the presence/absence of a penile opening in every (marked) individual lying on its back. This sexing method is very effective in elephant seals (Campagna et al., 1993).

- Definitions and classification of individuals

An important part of the information collected during daily counts and behavioural observation regards the qualitative classification of individuals, that requires precise definition and congruence between observers. An harem is defined as a group of two or more females. Females are considered grouped when their distance is less than 10 standard body length (SBL; American Society of Mammalogists, 1967). Breeding status of males is defined at two time scales, seasonal and daily. We classify males on a seasonal basis as:
- principal, if they stay on land for long continuous periods (> 2 weeks) and they get control of an harem for 3 or more consecutive days when at least one female is receptive
- secondary, if they stay on land for long periods as for principal males but never get control of an harem for more than 2 consecutive days
- tertiary, if their presence on land during the breeding season is occasional and short term (< 2 weeks).

During daily censuses males are classified by their distance from females as: holder (a males within the female group, distance = 0 standard body length), beta (a second male within the female group, but with less females on his side than the holder), peripheral (1-5 SBL from the closest female of the harem), marginal (6-10 SBL), solitary (> 10 SBL). A similar classification has been adopted in northern elephant seal (Deutsch et al., 1990). In the SLI population an established holder has an unrestricted control of the harem and an uncontested access to the females (Galimberti et al. 2002b; confirmed by genetic data, unpublished results). By harem control of the holder we mean both the active control of female movements by herding, and the exclusion of other males by direct and conventional competition.

We classified males into age classes, based on the external morphology, in particular the development of the proboscis, the appearance of the facial area, including the canines, and the spread of scars on the frontal shield and neck. Body size was not included in the criteria to obtain an age estimate independent from size estimate. Eight age classes were recognized:
yearlings (Y), 1 year old (i.e., > 1 year and < 2 years); 2 years old (2Y); juveniles (JUV), 3 years old; class 1 subadults (SAM1), 4 years old; class 2 subadults (SAM2), 5 years old; class 3 subadults (SAM3), 6 years old; class 4 subadults (SAM4), 7 years old; adults (AD), 8 years old or older. Since we have no data on actual age of males, our age classification is only tentative, and has only a descriptive purpose. Internal consistency of our classification is more relevant than agreement with actual ages. To check the consistency of various observers in classification of male age, we chose a random sample of the censuses of the 1996 breeding season (10 censuses of week VII-X, when most males were on land), we tabulated the age of males as recorded in censuses and found a high and significant concordance between classification of male ages in different censuses by different operators (Kendall coefficient of concordance: W = 0.93 [0.99 when excluding one of the four observers], p < 0.0001; we found analogous results for 1995 censuses). The final age classification of males was very concordant among observers (Sanvito and Galimberti, submitted). For males present in different seasons, age class attributed during one season was almost always consistent with that attributed during the following ones. A comparable system is routinely used in northern elephant seals (Clinton, 1994; Deutsch et al., 1994).

In southern elephant seal, there is a threefold variation in female size (Fedak et al., 1996) which allows a rough classification of females in size classes by visual inspection (Campagna et al., 1992). Females were classified into five classes by repeated comparison with adjacent individuals. To evaluate the accuracy of size classification, we compared the size classes independently scored by two observers for all the females breeding in an intensively observed harem. Concordance among observers was high (rho = 0.97; n = 63 females; p < 0.0001; see also Galimberti et al., 2000 a).

• Behavioural observations

We collected data on social behaviour during two-hours periods from fixed observation points overlooking one or more harems. During observations we recorded detailed information about behavioural events on log sheet (Lehner, 1996). To record information about agonistic and reproductive interactions we used an all occurrence sampling norm and a continuous recording norm (Altmann, 1974; Martin and Bateson, 1986). This choice was driven mainly by necessity to collect data for efficient estimation dominance indices (Boyd and Silk, 1983; Jameson et al., 1999), that requires large samples of interactions collected on a standard time base, and without bias among males. To gather data on individual time budgets we used a scan sampling norm and an instantaneous recording norm. To collect data on mother-pup behaviour of maternal investment we used focal sampling with continuous recording of behavioural modules. We augmented data on rare events (arrivals on land, departures to sea, births and weanings, heavy harassment episodes, fights, harem take overs) by ad libitum recording of any occurrence of these events. Intensive marking assured an high recognition rate during behavioural interaction. The percentage of interactions with both interactors recognized was very high (e.g. 97.3% of 5422 interactions in 1995, 96.4% of 3188 interactions in 1996).

The majority of observations were carried out between 06:00 and 20:00 local time, but a limited number of observations was also done during the night using spot lights and night viewing equipment. We have preliminary evidences from a small sample of observations carried out with nigh-viewing equipment that activity of elephant seals is almost equal during the day and the night (unpublished data; see also Baldi et al., 1996), as in the northern species (Shipley and Strecker, 1986).

Some typical pitfall of field research on social behaviour were effectively address in our research protocol:
- to reduce the adverse effects of observing just one or few social groups (Sharman and Dunbar, 1982), we observed all the harems in our study population; sampling was balanced across harems as much as possible, although the different duration of different harems reduce the likelihood to have fully balanced periods of observation
- to reduce the risk of a bias in observation of males adopting different mating tactics and having different observability (Drickamer, 1974), we tried to balance the observational effort between different males; due to the openness of the breeding habitat, and the conspicuousness of elephant seal intromission, it’s very unlikely that mating by secondary males goes undetected, as frequently happens in primate species
- to get unbiased estimates of behavioural indices (Altmann and Altmann, 1977), we collected data during standard length observation periods, and avoided to use ad libitum data for estimation of rates, or indices that are sensitive to non-random sampling
- to reduce the risk of pseudo-replication (Hurlbert, 1984) and pooling fallacy (Machlis et al., 1985; Martin and Kraemer, 1987; Jenkins, 2002; but see Leger and Didrichsons, 1994), we collected behavioural data for many individually recognized animals, and we used the individual as our main sampling unit
- to allow for the variation in phenotype due to physical and psychological growth, we are trying to analyze as much as possible multi-years trajectories (Dunbar, 1984), instead of year-specific measurements

The most valuable aspects of our behavioural data collection plan is the contemporaneous coverage of many harems. A large amount of the behavioural research was concentrated on individual from one or few groups, but it is now common knowledge that the quantity, quality and effects of behavioural interactions on fitness depend on the social milieu in which they happen (Altmann and Altmann, 1979).

• Spatial structure and individual movements

To study the spatial structure of the populations, the movements of individuals, and the effect of harem size and shape on mating strategies (Galimberti, 1995; Modig, 1996), we firstly divided the study site in zones and areas, defined by natural and artificial landmarks. Then, we used GPS receivers to map individuals and harems (Galimberti and Sanvito, 1999).

GPS is now a well established method to collect wildlife positional data (Leptich and Beck, 1994; Phillips et al., 1998), also because it offers more accurate positioning that other system (Burns and Castellini, 1998). We used a meter-precision GPS system composed by 3 Magellan ProMarkX receivers (Magellan Systems Corp.; http://www.magellangps.com/), one to be used as base station and the other two as rovers, and dedicated software for differential post-processing of data (MSTAR, Magellan). To map position of individuals, we simply approached the resting individuals from the back and gathered 90 seconds of position at 1 position per second rate. To map harems we used mobile differential and walked around the harem trying to stay as much close to the peripheral females as possible (distance always < 1 meter), collecting a few minutes of positions at 1 position per second. We mapped every 3-4 days all the harems of the population, while one harem was mapped one or more times per day. This setup guaranteed a precision close to the practical limit of the system, i.e., < 3 m RMS (Magellan System Corporation, 1995).

The suppression of selective availability (i.e., the intentional degradation of precision introduced in the signal by the U.S.A. DOD), which was the main source of GPS error (Van Dyke, 2000; http://www.edu-observatory.org/gps/check_accuracy.html), on 1st May 2002 greatly improved GPS accuracy (http://www.ngs.noaa.gov/FGCS/info/sans_SA/), reducing the need of expensive receivers and differential correction for applications that requires a low precision. Therefore, we tested a simple, commercial-grade, receiver, the Garmin 12 (Garmin Ltd., www.garmin.com) for mapping of individuals. We measured the precision of
positioning using SA Watch (http://www.hunting.com/sawatch/index.html), a program that interfaces with Garmin receivers (and any other receiver capable to output data in NMEA format), and produces logs, statistics, and graphics of the positions collected. By collecting long sequences of positions (at 1 sec interval, 6-12 hours), we discovered that, after suppression of SA, even commercial-grade receivers are very precise and accurate in positioning, with 50% of the positions falling within 2.6 meters and 95% within 6.9 meters (unpublished data). These results are in line to results using similar receivers and settings in other geographic locations (http://www.wsrec.com/wolfgang/gps/accuracy.html). Therefore, the positions obtained with such receivers are enough accurate to place individuals in a 10 by 10 m grid. This precision is not enough for topographic or harem mapping, but is more than adequate for studying movement patterns of individuals. In particular, we successfully mapped weanlings during the post-weaning land phase, to model dispersion pattern and analyse the effect of research handling and human disturbance on movements.

Data collected with GPS receivers is transferred to computer, and cross-referenced to field notes about the mapped individuals and social units. General processing of spatial data is carried out using standard GIS software (ArcInfo, ESRI, http://www.esri.com/). Harem mapping data is analysed using programs developed in Revolution xTalk programming language (Runtime Revolution Ltd., http://www.runrev.com/), that permit the calculation of a wide array of statistics of harem size and shape, and to related them to socionomic measures.

• Phenotype of males

Mating tactics may be conditional to the structural phenotype of individuals, being influenced by age, size and other traits that differ between individuals and change with growth (Grafen 1987). Moreover, phenotypic selection is an intrinsically multivariate phenomenon (Lande and Arnold 1983). Therefore, the simultaneous measurements of many different aspects of the local breeding situation, and of many different traits of individual phenotype are required. Hence, we used a wide array of field techniques, along with the basic marking/censusing/observing protocol, to measure potential correlates of copulations.

Age

Having begun our tagging plan just in 1995 we are not yet able to exactly determine age of most breeding males. Male southern elephant seals have a slow growth (McLaren 1993). They become mature from a physiological point of view at five years of age, but they need to become much bigger to have a chance to successfully breed. Therefore, most current breeding males were born before 1995, and their age can be just approximately estimated. Therefore, we are currently resorting to an observational classification in age categories, that are based on external morphology (see “Definitions and classification of individuals” section above). The situation will gradually improve, as long as new males, tagged as pups, will enter the breeding age.

Body size

Body size and weight have a strong effect of male competitive abilities (Fabiani 1996; Modig 1996). Direct measurements of adult elephant seals are quite complicated to obtain due to the large size. Hence, we classify male size by visual inspection, and we estimate body length and body weight using a simple photogrammetric method.

Males are ordered by repeatedly comparing size among dyads of males resting one close to the other. Every year 2-4 observers classify male size. The full set of paired comparison of each observer is converted to a square matrix containing for each dyad of male the number of comparison in which row male is classified as bigger of the column male. With perfect classification all entries below the diagonal should zeros. On the contrary, matrices often
have entries below the diagonal, because in the same dyad sometimes one male is judged bigger and sometimes the other. Therefore, matrices are fed to a computer algorithm that rearranges males to minimize entries below the diagonal (the same method is usually applied to dominance matrices, De Vries 1995; Lehner 1996). The final ordering of each matrix is used to attribute size ranks to males (increasing from the bigger to the smaller), and a consensus rank is then calculated by averaging ranks among observers. Agreement between observers on size ranks is high (e.g., three breeding season, Spearman rank correlation, with randomization test: r_s ranging from 0.972 to 0.985, P always < 0.0001).

For the photogrammetric method, pictures are taken, with a reference scale included in the frame, and size is then measured on pictures (Haley et al. 1991; Galimberti 1995; Bell et al. 1997 a). Although roughly valid for any age and sex class, the method is particularly suitable for large sub-adult and adult males (Bell et al. 1997 a). In the past, we took black and white 35mm pictures (Canon EOS1 camera, fitted with a Canon 35-70 mm lens). Now, we are using 3 mega-pixels compact digital cameras (Canon S20). The use of digital cameras greatly simplifies the processing of picture. We take side and front pictures of animals resting on packed sand after checking that they are lying straight. A standard surveying pole is put in the sand on the back of the animal, or held over the animal by an assistant. Black and white pictures were automatically coded on the negative with a unique 4 digits number for safe recognition of the individual. Digital images are identified by the file name automatically assigned by the camera. Black and white negatives were then transferred to CD-ROMs (Kodak PhotoCD service, or manual scanning) for computer access. All pictures are then read and analyzed using a specialized software for the processing and analysis of scientific images (Object Image, http://simon.bio.uva.nl/object-image.html). The surveying pole provides the reference scale, and measurements are carried out on the image using the tools provided with the software, that permits to save, together with the image, the paths of the features measured. From lateral pictures we take the following measures:
- nose to tail length
- maximum lateral height
- area of the lateral outline

Repeatability of body length of SES males as measured using the photogrammetric method was high both in 1996 ( 90 measurements for 24 males, 3.8 ± 1.9 per male; R = 0.839, P = 0.0000, 95% confidence interval = 0.736-0.942) and 1997 (76 measurements for 26 males, 2.9 ± 1.3 per male; R = 0.869, P = 0.0000, 95% confidence interval = 0.783-0.954). The same method is used to measure male secondary sexual traits, in particular of the muzzle area (proboscis, snout, trunk, canines).

- Vocal communication

Vocal communication has fundamental role in elephant seals societies (Bartholomew and Collias 1962; Shipley et al. 1986) and aggressive vocalizations are the single most important component of male agonistic behaviour in southern elephant seals (Sanvito, 1997). To study acoustic communication we recorded vocalizations of males, females, and pups using portable DAT recorders (Tascam DA-P1, http://www.tascam.com/; Sony TCD-D8 and TCD-D100, http://www.sonystyle.com/home/) and directional microphones (Sennheiser MD441-U and ME88; http://www.sennheiser.com/sennheiser/icm_eng.nsf).

Every season we recorded many samples of agonistic vocalizations from all the breeding males and some moulting males of all age classes (1995: 77; 1996: 74; 1997: 70; 1998: 72) by directly stimulating them. We also opportunistically recorded males vocalizations during agonistic interactions, female vocalizations during reproductive interactions, and mother-pup calls. We measured sound pressure levels of emissions, that may in principle be used as indication of size and stamina of the emitter (Prestwich et al. 1989), using a SPL meter.
In 1998 and 1999, all breeding males were recorded on a regular weekly basis, to study variation of sound structure with progress of the season. If vocalizations are to be used as a reliable signal of male resource holding potential, they should change with variation in breeding status, hormonal levels, and weight (Grafen 1990), and hence should vary along the breeding season.

Sound are transferred to computer in the digital domain, without any D/A conversion. In the past, sounds were manually transferred using an Audiomedia II/III audio card and ProTools software (Digidesign Inc., http://www.digidesign.com/). Currently, sounds are automatically transferred using an audio capable computer DAT (SGI CTD8000) and the DAT2WAW software (Computall Services, http://www.ncf.carleton.ca/~aa571/index.html). Sound files are then processed using the Canary sound analysis software (Cornell Laboratory of Bioacoustics, http://www.birds.cornell.edu/brp/SoundSoftware.html), custom scripts written in HyperTalk (Apple Computer Inc.) and custom analysis modules written in Igor Pro (Wavemetrics Inc., http://www.wavemetrics.com/). A detailed summary on the acoustic sub-project may be found online (http://www.eleseal.org/es_lit.htm).

• Male breeding success

Many aspects of our research project depend on estimation of individual fitness, and in particular its mating components. We are currently using three main measures of individual fitness:
- an index of female control (female/days index), calculated as the sum of the females controlled by each male throughout the breeding season, as observed during the daily census (Clutton-Brock et al. 1982); a male is “controlling” females if he is the holder of an harem, or is associated to a solitary female
- an index of mating success (MS100), calculated as the number of copulations made by a male in 100 hours of (Campagna and Le Boeuf 1988, Fabiani 1996); only copulations observed during standard observation periods are considered, to avoid bias, and consecutive intromissions by the same male are counted as a single copulation
- an index of fertilization success (ENFI), calculated as the product between the proportion of copulations achieved by the male in one harem and the number of females that bred in the harem, summed over all harems in which the male was observed to mate (Le Boeuf 1974, Deutsch et al. 1990).

These indices are positively correlated, and can be considered successive approximations to true individual fitness (Galimberti 1995). Various other measures of breeding inequality are also calculated (for details see Galimberti et al. 2002 b).

To verify the quality of our behavioural measures of mating success and its correlation with true breeding success, in 1996 we started a paternity analysis program (Burke 1989). We collected skin samples (few grams in weight) from the rear flippers of elephant seals using ear notchers (Pemberton et al. 1992; Hoelzel 1993) or from the rump using biopsy heads (ø = 4 mm; Karesh et al. 1987, Gemmell and Majluf 1997) mounted on a 1.6 metres pole. Both sampling protocol hare accomplished by surprise without any kind of restraint or immobilization, and sampling results in a modest and short term pain for the animal. Samples were preserved in pure ethanol until DNA extraction and analysis, which is accomplished by standard protocols (Hoelzel 1992; described in details in Fabiani 2002). Every season from 1996 to 2001, we collected skin samples from about a half of breeding females (sampling all harems of one of the two main breeding zones), all pups and all breeding males. Sampling of mothers and putative fathers during one season is coupled with sampling of pups born during the next season. We have complete series of samples for 1996-2000, while 2001 will be completed by collecting samples from pups born in 2001. The same samples and extracted DNA used for paternity analysis will be used also to study relatedness of harem females, and
the genetic structure of the population, in particular regarding gene flow between SLI and other population of the South Georgia stock. Until now DNA has been extracted from 367 samples (74 males, 115 females of 1996, 115 pups born in 1997 and 63 pups in 1998).

During 1999 moulting season, we collected samples from marked adult and sub-adult males that moulted in the past seasons on Sea Lion Island, but never bred there. These males should come from other breeding populations of the South Georgia stock, and this will be verified by mtDNA comparison between these samples and samples from other populations of the stock (South Georgia and Valdés Peninsula in particular). More details on genetic sub-project may be found online (http://www.eleseal.org/es_lit.htm). The first stage of the analysis of genetic material is now completed, and the first results are being presented to meetings (Fabiani et al. 2001 a,b) and submitted for publication (Fabiani et al. submitted). Mark-recapture data shows that the Falklands population is almost isolated during the breeding season (Lewis et al. 1996; unpublished data), but the genetic results show that it acts as a conduit for genetic flow among the main populations of the South Georgia stock.

**Female breeding success and parental investment**

A key aspect of female breeding strategies is parental investment in pups. We studied parental investment using both behavioural and morphological cues. We collected daily serial records of as much females as possible to estimate length of permanence on land, timing of parturition, and length of suckling. Moreover, we carried out focal sampling observations (Altmann 1974) of mother-pup couples to study maternal behaviour, we calculate frequency and duration of suckling bouts, and we analyzed the effect of the mother phenotype and social habitat on suckling (Gallastroni 2001). In 1998, we carried out 411 focal observation periods on 37 mother-pup couples (median = 8 periods, 5-22). In 1999, we carried out 433 focal periods. Unfortunately, we discovered that behavioural measures of maternal investment are not correlated with true investment, and that the only effective way to estimate investment currently is the direct weighing and measurement of pups (Galimberti et al. 2002 c).

Parental investment may be divided in two components: gestational and post-gestational. To estimate gestational investment we weighed pups at birth (always within 24 hours, and within 12 in most cases), and to estimate post-gestational investment we re-weighed them at weaning to calculate the increase in weight during suckling (Campagna et al. 1992; Fedak et al. 1996). Weight at weaning is an excellent measure of total investment. The pups were weighed using a simple canvas bag and a 500 kg digital dynamometer (model DIN-1R/TS - accuracy ± 0.5 % - C.A.M.I. Pavignaniti, Trezzano sul Naviglio, Italy; http://www.cami-it.com/), held up by hand by two people. The weanlings were weighed using a weighing bag (a cotton sheet with straps cut out to fully enfold the weanling, held up by two horizontal aluminium poles) connected by steel chains and springs to a dynamometer (same model used for pups, or same model but 1000 kg). At first, we attached the weighing bag to a half ton crane (model GP5/DE - OMCN, Villa di Serio, Italy; http://www.omcn.com/default000.htm). In 1998, we switched from the crane to an aluminium tripod fitted with an hoist, that was easier to move in the field. For weanlings not weighed within 24 hours from weaning, we corrected the observed weight by the weight lost between weaning and weighing, as estimated as the product of the number of days between weaning and weighing and a sex-and week-dependent correction factor ranging from 0.78 -1.02 kg lost per day (proposed by Campagna et al. 1992 for the Valdés Peninsula population, confirmed by SLI data).

In 1996, we weighed 100 weanlings (60% males) to test the weighing apparatus, and get a basic estimate of weight at weaning for comparison with other populations. In 1997, we were forced to stop weighing operations just after 25 weanlings due to lack of personnel. In 1998, we implemented a more intensive weighing plan for both pups and weanlings. We
weighed 82 pups (51.2 % males), 23.9% during the 24 hours interval after birth, 93.0% during a five days interval. Pups were sampled from 6 harems (2-27 pups per harem). Weanlings were sampled from 9 harems (5-69 per harem). We weighed at least one time 179 weanlings (46.4% males), 16.4% during the 24 hours interval after birth, 46.3% during a seven days interval. To test effectiveness of the weighing procedure, we reweighed most pups (n = 81) and weanlings (n = 128) three consecutive times, and calculated repeatabilities, which resulted very high (0.995 for pups and 0.985 for weanlings. The weighing plan was carried on in 1999, when we weighed 122 pups (belongings to 7 harems), at a rate of 1 to 15 pups per day, depending on the presence of freshly born pups, and 192 weanlings (108 males and 83 females), at a rate of 3 to 26 weanlings per day; 77 weanlings were weighed two or more times, to estimate weigh loss rate during the post weaning fast. After a stop in 2000, the weighing plan was resumed in 2001, when we obtained a total of 733 weights. We weighed 163 pups and 293 weanlings; moreover, we re-weighed 43 pup at one week of age and 22 pups at one and two weeks, to study growth patterns.

- Hormone studies

Hormone studies are becoming an important area of research on marine mammals, because they are linked to social behaviour (Creel 2001), have important implications for physiology (e.g. fasting, Ortiz et al. 2001), and are an excellent index of human induced stress (Engelhardt et al. 2002).

We are currently working on two hormonal sub-project. The first one regards the study of cortisol in weanlings, to estimate the stress due to handling and weighing operations. The data collection for this project was completed in 2001. We collected 75 blood samples from 69. Blood samples were collected from weanlings either after restraint and weighing (50.7% of the samples; mean handling time = 466 ± 132 sec, n = 28); or immediately upon capture (49.3% of the sample; mean handling time = 49 ± 14 sec, n = 36). The total number of all weanlings included 36 females and 33 males. Blood was collected from the rear flippers using Vacutainers (5 ml, red top, no additive) and 20 gauge, 1.5 inch needles (Becton Dickinson). Each sample was subjected to three different handling procedures immediately after collection. First, four drops of blood were spotted on a filter paper blood collection card (Sigma Diagnostics, Inc, Catalog No. 160-C). Cards were placed in individual, sealable plastic bags. Second, a sub-sample of unclotted blood was transferred to a siliconized microcentrifuge tube (Costar Brand, Catalog No. 3207). The remainder was left in the original Vacutainer. All these samples were kept at a temperature between 15 and 25° C for a few hours (2 to 8) after collection. Samples were then processed as per the description below. Blood spots. The spotted filter paper was removed from the plastic bag and dried overnight in an airtight container with silica gel. The dried filter paper was transferred into a paper coin envelope to avoid heat build-up or moisture accumulation that has been reported for samples stored in plastic bags (Knudsen, R. C., W. E. Slazyk, J. Y. Richmond and W. H. Hannon. 1993. Guidelines for the Shipment of Dried Blood Spot Specimens, www.cdc.gov/od/ohs/biosfty/driblood.htm). Each envelope was stored at temperatures ranging from 4 to 15 °C for 15 to 60 days in an airtight container with silica gel. After transportation from the field samples were frozen at –20° C. Microcentrifuge tubes. The blood in the microcentrifuge tube was centrifuged for 20 minutes at 6000 rpm (VWR Mini Centrifuge). The serum was pipetted into a new microcentrifuge tube and then stored frozen at –20o C within 24 hours. Vacutainer tubes. Blood left in the original Vacutainer sat undisturbed overnight (temperature 10 – 15° C). The next day, and with no centrifugation, the serum was pipetted into a new microcentrifuge tube. The serum was frozen at –20° C within 24 hours from separation. All the samples were then transported from the Falklands to Canada in thermal resistant containers (with ice packs for the frozen serum samples), and
stored at – 20º C upon arrival. Samples were analyzed 6 to 7 months after blood collection with standard radioimmunoassay techniques (details in Sanvito et al. submitted, available online from the Literature page of our web site, http://www.eleseal.org/es_lit.htm).

The second, yet ongoing, project is focused on the effect of testosterone on male agonistic behaviour (male threat vocalizations and fights in particular). Testosterone present a notable seasonal variation in southern elephant seals (Griffiths 1984). In this case, we are using only the blood spots method, i.e., the collection of blood drops on filter paper. Blood is collected opportunistically from bleeding natural wounds, or from small (2 cm length) incisions made above the rear flippers using a scalpel. A preliminary analysis demonstrated that this is a very effective method to analyze testosterone adult elephant seals with any form of restrain and with a minimum of invasiveness (unpublished data).

Ethical aspects of the research

Any research project should respect a careful balance between the scientific results obtained and the impact on the study population and subjects (Dawkins and Gosling 1992; Boitani and Fuller 2000; Gales 2000). Moreover, there is an increasing awareness of the importance reduce invasiveness of research methods and techniques (Whitten et al. 1998), in particular when fragile habitats, small populations, or endangered species are involved. On the other hand, we think that the value and the adverse impact of scientific research should be evaluated on factual, and not emotional, ground (see, e.g. Gales 2000 for an excellent example of scientific evaluation of research impact on animal welfare).

We are using standard field methods widely accepted within research on seals and sea lions (for a general reference see Laws 1993). We have been using the same methodology since the beginning of the study in 1995, however we have introduced various improvements to specific techniques (e.g., in weighing and skin sampling protocols) during the years. Our research is carried out under a license released by the Falkland Islands Government (1995-1998 by the Secretariat, 1999-now by the Environmental Planning Officer) in accordance with the Conservation of Wildlife and Nature Ordinance 1999. We strictly adhere to the following research ethics guidelines:
- the SCAR code of conduct for use of animals for scientific purposes in Antarctica (Laws 1993; http://www.scar.org/intro/animalconduct.htm)
- the Canadian Council on Animal Care guidelines for the care and use of research animals (http://www.ccac.ca/)

The acoustic sub-project is carried out after referral by the Animal Care Committee of the Memorial University of Newfoundland (St. John’s, Newfoundland, Canada).

• Invasive techniques

The following techniques have an invasive component or involve handling of animals.

1) Marking
We are using three marking procedures: tagging, dye marking and implantation of transponders. Marking is the single most important technique of our research. Our goal is to obtain long-term data on individual behaviour and ecology. Without reliable identification of individuals the whole research project will lose most of its value and interest.

We are trying to mark as many individuals as possible. A correct identification of all individuals of the population permits to avoid sampling bias and to reduce the error in data collection and recording. This high data reliability is obviously relevant for all research.
targets, but it is of particular importance in demography and population dynamics studies. The accurate identification of all individuals permits to greatly reduce the error in parameter estimates. For example, the very accurate estimate of the number of females hauled out on SLI permits to achieve a notable power in detection of population trends notwithstanding the small size of the population (Galimberti and Sanvito 2001; Galimberti 2002). This is a crucial aspect in forecasting and population viability analysis (Forcada 2000). We wish to emphasize that marking is currently carried out without any kind of restraint of the animals, by simply approaching them from the back while they are resting.

1.1 Tagging
About 99% of the females and 100% of the breeding males of the population are marked by cattle tags (see “Methods” section for details). The tags are placed in the inter-digital web of the rear flippers and each individual is at least double-tagged. Double tagging is fundamental to reduce the risk of lack of recognition in following seasons, and to estimate tag loss rates. In the study population tag loss rate is very low (likelihood to lose two tags = 0.36% for females, 0.37% for males, unpublished data; see also Wilkinson and Bester 1997). Obviously, the loss of all marks by an individual means to loose all his data from the previous seasons and to introduce a bias in parameter estimates. With the double-tagging strategy, and the low tag loss rate, each year the majority of the animals are already tagged from the previous breeding seasons, and we need only to replace lost tags.

In most animals tagging produces a brief reaction (a few seconds) and a very small pain, although animal pain is obviously difficult to assess (Bateson 1991). Tagging may produce a wound in the inter-digital membrane and a local infection, but this rarely happens in our population, as showed by the very low tag loss rate (a bad tag, that produce local infection, tend to pass through the hole and get lost). After many years of continuous tagging (more than 13000 tags put in place) we have no indication of any adverse effect of the procedure. Tagging of elephant seals seems to have no effect on their survival rate (Testa and Rothery 1992).

1.2 Dye marking
We paint an identification code on animals’ backs and flanks using commercial black hair dye in order to have a fast and easy recognition of individuals. Examples of marks and pictures of the dye-marking procedure are available on our web site (http://www.elseal.org). We mark with hair dye all breeding males, and 70-85% of the breeding females of the breeding area. We do not mark with dye individuals that come to the island only for moulting. We mark pups with hair bleach when they are weighed at birth. These bleach marks are put on about 1/5 of the pups, and are almost completely lost with the first change of the fur, that happens within a month from birth.

We understand that dye marks could be criticized from an esthetical point of view, but we firmly reject any complain on the ethical ground. If the basic principle of research is accepted, i.e. animals should be recognized, dye marks are a very effective way to reduce disturbance on animals. A dye mark permits the sure recognition of animals from distance, reducing the needing to come close and read the tag. They are put with a minimum disturbance to the animal, approaching it from the back: the best marks are put without waking-up the subject, not during the approach nor after it, to have a clearly readable mark and a safe setting of the dye. The use of dyes tested for human hairs exclude any risk of toxicity. Moreover, dye marks last just for a few weeks or months, and even the best marks disappear during the moult.

1.3 Implantation of transponders
To improve the quality of long term marking of pups, we employed an electronic identification system based on passive transponders (see “Methods” section for details). Transponders are packaged in disposable needles, pre-sterilized and ready-to-use, and are implanted with a special syringe-like applicator in a way similar to a subcutaneous injection. The harassment of the animal is short. At the beginning we were used to restrain the weanling
by hands, but then we developed a technique to implant the transponder by surprise without any kind of restrain: with this last protocols the implantation takes just few seconds. No detrimental effect of the implantation was detected (Galimberti et al. 2000). Transponders are now widely used to mark wild animals (e.g. Thomas et al. 1987; Schooley et al. 1993).

1.4 Paint pellets

We sometimes use paint pellets shoot by a CO2 gun, to mark specific females. Less than 5% of females are marked with pellets at any time. Paint pellets are a widespread marking method for sea lions (Campagna and Le Boeuf 1988). Although pellets marking produces a short-term disturbance, and in particular a reaction to the noise and to the sudden impact of the pellet, it permits to rapidly core females in large harems. In fact, a very short and limited disturbance is much more desirable than repeated tries with other methods. In five years of use of pellets we never observed a single case of pup abandonment or premature departure by females marked with paint pellets.

2) Weighing of pups and weanlings

Weighing of pups and weanlings is carried out without any form of chemical anesthesia, pups are restrained by hand and handling time is very short (Galimberti and Boitani 1999). Mean handling is 2’ 30” for pups (from the separation form the mother to the return to her), and 3’ 30” (single measure) or 5’ 30” (three consecutive measures, to calculate weighing error) for weanlings.

Weighing of pups after birth never resulted in physical damage of pup or abandonment by the mother. All weighed pups were successfully weaned. Weighing of weanlings also had no effect: every weighed individual resumed his previous activity (usually resting) a few minutes after being released. To study the physiological effects of weighing we analyzed plasma cortisol, which is an excellent index of handling stress (Morton et al. 1995). Cortisol of weighed weanlings was not different from cortisol of resting weanlings (unpublished data). Evidences collected in other populations (e.g., Engelhardt et al. 2002) demonstrated that only a very intensive handling, with many repetitions for each individual, produces a measurable adverse effect in southern elephant seal pups.

3) Sampling of adults and weanlings

We collect skin samples from females, pup and putative fathers for genetic studies. Samples are taken from the inter-digital web of the hind flippers of each animal using Dalton ear-notchers, or from the back using a biopsy head mounted on a pole to reach core females of large harems (see “Methods” section for details). Notchers and biopsy heads are carefully cleaned after each sample is taken. The samples are about 8-10 cube millimetres in size and 1-2 grams in weight. The scars left by the ear-puncher and the small holes produced by the biopsy head are barely recognizable after few hours. We never observed any sign of infection due to skin sampling.

Blood sampling is carried out mostly with the blood spots method (collection of blood on filter paper), that is the least invasive technique to collect blood for hormone studies (Whitten et al. 1998), and is becoming very popular for primates and human blood sampling (Worthman and Stallings 1997). In 2001, we sampled liquid blood of weanlings using Vacutainers (Sanvito et al. submitted) to validate the blood spots methods. The handling time for blood collection from resting weanling was almost always under 60 seconds (see “Methods” above). The blood collection during weighing operation produced just modest increase (10-15%) in handling time. Blood collection elicited a modest behavioural reaction, analogue to the reaction observed after tagging or skin sampling.

4) Stimulation for communication studies

To study male acoustic communication we use two different methods, recording of vocalizations in natural contest, and standardized recording with artificial stimulation. The
stimulus is just one of the researcher who approaches the subject to elicit a vocal display. This method is very successful (Sanvito and Galimberti 2000 a), permitting to collect a large amount of recordings from most of the males when the recording conditions are optimal, something that rarely happens due to SLI weather. Although with this stimulation we add an extra-load to the time budget and breeding cost of the subject, the low frequency of stimulation of each individual make us confident that no long-term effect will result. To check this point, we are currently implementing a non-invasive blood collection method that, through hormone essays, should permit us to evaluate the human-induced stress on males.

In conclusion, we firmly believe that our method techniques cause either no disturbance or very short term disturbance to the animals. Moreover, our techniques are of minimal impact if compared with much more invasive procedures adopted by other research teams. These include repeated weighing of pups and adults, chemical restrain of pups and mothers, application of labelled water techniques, invasive biological sampling, implantation of instruments for physiological studies, implantation or gluing of devices and instruments for tracking at sea, experimental translocation (see Laws 1993 for general references).

• Impact of the research on the study population

We have no evidences of any adverse effect of our research on the study population. Population size has been almost steady during 1989-2001 period (Galimberti and Boitani 1999; Galimberti  2001; unpublished data), and, notwithstanding the very accurate monitoring, there is no indication that our presence changed the dynamics of the population. The current lack of increase in population size seems to depend completely on the aquatic phase of the yearly cycle (Galimberti et al. 2001). Moreover, we have three direct indications of the good health of the population (Galimberti and Boitani 1999; Galimberti et al. 2001):
• female fecundity is close to 100% for the females coming to land to give birth (mean of 6 years = 97%), and reduces to 88% including females that skip breeding; these values are equal to or higher than other SES populations (McCann 1985)
• pup mortality is very low (mean of six years = 3.1%), among the lowest reported for any SES population (Galimberti and Boitani 1999)
• weight at weaning (mean = 136 kg), which is good index of the status of the population, is on the high side of the range observed in southern elephant seals (Burton et al. 1997)

Despite these clear evidences, we are always trying to improve our methodology and the evaluation of the impact of our research. For the future, we plan to evaluate the effect of disturbance on parental investment by comparing weaning weight among areas with different level of disturbance, and to examine the effect of handling on individual stress by hormone essays.

• Impact of the research on the local ecosystem

Our field work may in principle have a general adverse effect on the local ecosystem. This seems unlikely because the work is limited in space and time. Our study areas are the sandy beaches of the eastern tip of the island, where elephant seals breed. Our presence in the rest of the island is spotty and not invasive at all, we just carry out weekly counts of seals on the full island perimeter. Therefore, the most of our activity is carried out far away from the nesting sites of most bird species. Moreover, our work is limited to the breeding season (September-November), well before the peak breeding of other species. Unfortunately, a full evaluation of the impact of the research on the local ecosystem requires time series on abundance and productivity of other species, which are almost completely lacking. Only the
set up of regular monitoring of Sea Lion Island wildlife may permit a full understanding of the impact of our research and of other sources of human disturbance, including for example nature-oriented tourism and wildlife photography.

**Results achieved**

- **Some statistics on field work**

  We summarize here a few statistics on the field work carried out during the whole breeding season for five years (1995-2001) by a team of four people (with a mean of 12 hours per person of presence in the field every day of the season). More than 13000 tags were put in place to mark 2295 adult females (543-579 per season), 404 non-moulting males (92-112 per season), 2180 pups (525-560 per season), and 1106 moulting individuals of various sex/age class (272-284 per season). Tagging was very successful, with high rate of retention of tags (tag loss rate was estimated by double tagging in 1997 as a 0.0035 likelihood to lose both tags for males and 0.0033 for female). During the four seasons all breeding males and also the majority of breeding females (from 70% in 1995 to 85% in 1996) were dye marked. In 1997, 510 weanlings (91% of the total) were fitted with a passive transponder.

  We carried on 5722 hours of standard behavioural observation (862-1294 per season), during which we recorded data about 15003 male-male interactions (3188-5422 per season), 11484 male-female interactions (2764-3171 per season), 1732 copulations (366-529 per season). Also on the quality side the project was successful: for example, rate of recognition of individuals was very high, if compared to the standard of behavioural studies on free ranging animals, with 95-98% of male-male interactions with both individuals identified, and 93-96% of male-female interactions with both the male and the female identified.

- **Scientific literature produced**

  We put below a list of the publications of the research team that appeared in the period 1999-2002 on peer reviewed scientific journals (abstract included, PDF files of most papers available from the Literature page of our web site, http://www.eleseal.org/es_lit.htm).


  Southern elephant seals have been studied in depth in most of their breeding range. One notable exception is the Falklands Islands population. We present data on demography and breeding biology of elephant seals of Sea Lion Island, the main breeding site of this species in the Falklands. Sea Lion Island shelters a small, localized population of southern elephant seals (516 breeding females in 1995 and 518 in 1996). Comparison with the few available census data collected prior to our study suggests that the population has been stable in the short term (1989-1996). Females produced pups at maximum rate and pup mortality was low (2.13%). Breeding sex ratio was strongly unbalanced, with about 14 females per breeding male and 47 females per harem-holding males at peak haul-out. Survival rate between breeding seasons was very similar to that recorded in other populations and was in accordance with clinical variation with latitude. Sex ratio at birth was balanced, and no significant weight dimorphisms at weaning between sexes was detected (males: 135.4 kg; females: 132.0 kg). Weaning weight was correlated with size class of the mother.


  Notwithstanding the important role of male harassment of females for theories of the evolution of mating systems, accurate estimates of its frequency and costs are available for only a few species. In this paper, we quantify the frequency of harassment in southern elephant seals, compare occurrences of harassment inside and outside harems, and estimate the costs of harassment in two populations at Sea Lion Island (Falkland Islands).
and Punta Delgada (Valdés Peninsula). Southern elephant seal males are much larger than females, have enlarged canines, and are much more agile on land; hence, females have a small probability of escape from approaching males and may suffer intense molestation. Most males had limited access to females due to the despotic mating system, and their libido was high. Females were approached by males at high frequency, mostly when out of oestrus. The harassment level was negatively related to the ratio of breeding females to breeding males, and females breeding at the peak of the season suffered a lower level of harassment. Females of large harems were harassed less, and their likelihood to interact with secondary males was lower. The activity of harem females was less disrupted, and females in large harems had an higher proportion of resting time. Isolated females suffered more herding episodes, and were approached more frequently by secondary males. The main short-term cost of harassment was disruption of the females’ activity schedule; harassment level and total active time were positively related. Suckling bouts were rarely interrupted by male harassment. Mother and pup separations caused by males interaction were rare, short-lasting, and rarely permanent. There was little effect of harassment on weaning weight, physical damage of females was rare, and there was only a slight non-significant negative relationship between harassment level during one season and the likelihood of surviving to the next.

Female southern elephant seals are expected to adopt behaviours that reduce the costs of male harassment. We studied the strategies and tactics of harassment reduction in two populations, at Punta Delgada (Valdés Peninsula, Argentina) and at Sea Lion Island (Falkland Islands) during five breeding seasons in all. Females synchronized their breeding activities to reduce harassment risk, and rarely bred alone to reduce the likelihood of encounters with subadult males. Females showed a clear preference for larger harems, that guaranteed a reduced harassment risk: movements between arrival on land and parturition were mostly from smaller to larger harems, and the likelihood of abandonment was lower for large harems. Females protested against approaching males in the vast majority of interactions, regardless of the social context and the status of the interacting male, but protest varied with female breeding status and male phenotype. Frequency of protest of individual females decreased linearly from the beginning of oestrus to departure to sea. Interactions with mature males were less protested. The frequency of protest linearly decreased with increase in age class, and mating attempts by males of higher status and dominance rank were less often protested. Most of this variation with male phenotype, however, was due to the higher probability of older and more dominant males to interact with oestrus females that had an intrinsically lower tendency to protest. Protest variation in relation to male phenotype was more parsimoniously explained as a consequence of differential access of males to oestrus females rather than of female selectivity. Protests had a role in disruption of mating attempts, although the phenotype of interactors was more important: adult, large and dominant males disrupted interactions regardless of incitation by female protest.

During the breeding season female elephant seals spent most of their time on land inside harems. When they arrive on land before joining harems, and when they leave harems to return to sea, they are exposed to secondary males and may suffer intense harassment. Hence, arrival and departure present an ideal opportunity to test hypotheses concerning female tactics of harassment reduction. We studied harassment during arrival and departure in two southern elephant seal populations at Punta Delgada (Valdés Peninsula; DEL hereafter) and Sea Lion Island (Falkland Islands; SLI hereafter). Females were less likely to be intercepted by males during arrival than during departure. They also arrived mostly at high tide, thereby reducing the distance from water to the harems. Interception rate and harassment during departure were higher at DEL, where male density and the breeding sex ratio affected the likelihood of interception; on SLI, the sociology had a small effect. Harassment was higher at low tide at DEL but not at SLI, because tide level variation was larger at DEL and this resulted in a larger variation in the distances of the harems from the water. Females departed more often than expected at high tide at DEL but not at SLI. In both populations females departed directly to sea, rarely stopping before reaching the water, and they never sought contact with males. Social distraction during departure significantly reduced the likelihood of interception. Departures were more frequent during periods of high social activity, and females departing just after other females were less prone to harassment. Accepting copulations with secondary males may reduce the dangerous effects of harassment: interactions occurring during departure were less frequently protested, but we found no indication that departing females were facilitating copulations in a special manner. Quantity and quality of protest during departures was similar to protest during the last days of residence of the females in the harem.

Southern elephant seal (Mirounga leonina) males have a complex and stereotyped system of access to breeding females. The single most important component of male behaviour is vocal signalling, which is used to settle
agonistic encounters in most cases. Most aspects of the breeding biology of the species have been studied in depth, but detailed information about structure of vocalizations is not readily available. Here, we present data about the acoustic structure of aggressive male vocalizations collected in the Falkland Islands, and we compare these data to published data on the northern elephant seals. Our main goal is to describe the structure of sounds as a preliminary and indispensable step towards analysis of their functional significance. Male vocalizations were low pitch sounds, made up by pulse trains, with scarce frequency modulation: low frequency of emission and high sound pressure level are typical of male vocalizations in Pinnipedia, but they were particularly evident in southern elephant seals, probably due to the unusually large body size. The comparison with published data on northern elephant seals was not very easy, due to differences in acoustic terminology and methodology, but it revealed many similarities between the species. We also carried on a detailed analysis of variability of different acoustic variables, and we discovered that frequency and intensity measures have lower variability than temporal ones, and should hence be the most effective way to convey information about the individual who emits the vocalization.


In traditional studies of animal communication individual variability was sometimes considered less relevant than species specific aspects, mostly because the goal was the classifications of sounds in repertoires. On the other side, individual variability seems to have a significant role in signal function and evolution. In this paper, we analyze individual variation of structure of aggressive vocalizations of male southern elephant seals, and we compare sounds from our main study population, Sea Lion Island (Falkland Islands), with sounds recorded in the nearby population of the Valdés Peninsula (Patagonia, Argentina). We firstly analyzed repeatability of acoustic parameters at vocalization and male level. Repeatability of bouts of the same vocalization was extremely high, and this confirmed that vocalization is the fundamental level of organization of male acoustic communication in this species. Also repeatability of vocalizations of individual males was very high, and hence sounds may effectively convey information about identity of the individual who emits the sound. Male aggressive vocalizations were classified in a small number of types, and each male emitted always the same type of vocalization. We compared the typology of sounds emitted by Sea Lion Island males with vocalizations by Valdés Peninsula males, and we found striking differences. None of the sound types was shared by the two populations, and, although similar in fundamental acoustics, sounds from the two populations had different macrostructure. We conclude that these two populations present dialects in male acoustic communication, although scarcity of recordings from other populations limit the scope of this conclusion.


In 1995 we started a long-term research project on southern elephant seals (Mirounga leonina) at Sea Lion Island, Falkland Islands. In 1997 we implanted Trovan PIT tag (model ID 100, Trovan Inc.; http://www.trovan.com/) transponders in 510 weanlings (= 93.2% of the full cohort of survivors). These small glass-encapsulated transponders (length 11.5 mm, diameter 2.15 mm, weight 0.1 g), operating at 128 KHz, are widely used in zoos and are endorsed by the Captive Breeding Specialist Group of the IUCN (Wright et al. 1998). We chose Trovan Inc. because they supplied a hand-held reader (model LID 500) that, although large (length 22 cm, width 17.5 cm, height 27 cm) and awkwardly shaped, had the greatest scanning distance (18-20 cm, depending on battery charge) of the models available on the market. The reliability of PITs in the long term remains unclear, but preliminary evidence reported in the literature (Wright et al. 1998) indicates that, if the transponder is properly placed, it should remain in that particular position without migration into deeper tissues. PIT tagging has many advantages, but it is expensive (6-12 times the cost of a plastic tag), marked individuals are not easily recognized at first encounter, and reading of the ID code requires an awkward battery-operated device. Hence, we consider PITs not as a replacement for plastic tags but as an effective back-up system, especially when lifetime identification is required. In this role, PITs are an effective way to mark elephant seals and probably seals in general.


Accurate long-term series of demographic data are available for most populations of southern elephant seals. However, research on elephant seals of the Falkland Islands began only recently, and information for an accurate forecasting of the future of this population is lacking. In this paper, we present data on the current status and of the population and its trend in size during the last 11 years. We built an age-structured model of the population and analyzed the effect of variation in demographic parameters on population growth. Elasticity analysis demonstrated that variation in mortality has a more pronounced effect on instantaneous growth rate than equivalent variation in fecundity. We examined the effects of environmental variability, inbreeding, and catastrophes on population viability by computer simulation using the VORTEX PVA program. In the stochastic model, the most important factor affecting extinction risk was variability in mortality rates, in particular of the adult classes. We concluded that, although the population does not appear to be at immediate
risk of extinction, its small size and isolation make compelling an accurate monitoring of population trend and the acquisition of additional information on life history and feeding strategies.


In Pinnipedia species is difficult to achieve good estimates of population size by direct counts, because a part of the population is at sea at any time. In southern elephant seals (Mirounga leonina) the estimation of population size is carried out starting with the number of females hauled out during the breeding season, by applying a correction factor calculated from life tables. Different models were proposed in the literature to estimate the total number of females hauled out. In this paper, we consider the model proposed by Rothery & McCann (1987) for the South Georgia population, applying it to a five years data set for the population of Sea Lion Island (Falkland Islands). We test the assumptions of the model, finding them reasonable. We fit the model to our data set, obtaining an excellent fit in all cases, better than or equal to other models proposed in the literature. The precision of the estimation depended mostly on the length of presence on land of females, which is a constant of the model. A 1 day variation in length produced a 4% variation in the estimated total number of females. When a good model of the haul out process is already available for the population, even a single count close to the peak of the season is enough to estimate total females in the ± 2% range. When such a model is to be estimated from data, at least 8 counts are needed to have a good estimate. The model was not only a good description of the haul out process at population level, but its application to a set of daily counts of single harems demonstrated its usefulness also at sub-population level.


The opportunity for selection, I, calculated as the variance in relative fitness, sets an upper limit to the amount of adaptive change that selection may produce. Therefore, it is a potentially valuable, and frequently used, measure of the potential of action of phenotypic selection. Although many different aspects of I calculation and analysis have been explored, the effect of the spatial scale chosen for calculation received little attention, notwithstanding the growing evidence that natural populations are not homogeneous and present a hierarchical spatial structure. The effect of scale on the estimation of I was examined from data collected in two populations of southern elephant seals (Mirounga leonina), an easily observable and strongly polygynous species. A significant effect of spatial scale on three important aspects of I calculation and analysis was found: dependence of I on mean fitness, between population variation of I, and effect of local demography on I.


Inequality in distribution of resources is a key aspect of evolutionary biology, in particular in relation to distribution of mates an copulations. Nevertheless its important role, inequality is not easily defined, and its measurement is complicated by theoretical and methodological issues. Although the formal treatment of inequality has been mostly limited to the evolution of lek mating system, a methodologically correct approach to measurement of inequality has a general validity for the study of any kind of mating system. In this paper, we analyze inequality in a large set of southern elephant seals harems. The observed distribution of fertilizations was significantly different from both the expected distribution with equal shares of resources, and the expected distribution with equal propensities to get resources. We calculate and compare various measures of inequality, observing a wide variation, in particular among unbounded and bounded indices. We check the effect of choosing a specific measure of inequality by considering the effect of two aspects of harem socionomy, the number of females in the harem (i.e., the total amount of resources to be shared) and the number of males associated to the harem (i.e., the number of competitors). The choice of a specific measure of inequality had a strong impact on the results obtained, and should be considered a critical step in every study of functional and evolutionary correlates of inequality. Not bounded indices showed a strong relationship with both harem size and number of males, while no effect was evident in the analysis of bounded indices. This demonstrates that, in this species, the despotism of the mating system remains high even in large harems and with many competitors, i.e., with the worst conditions for monopolization.


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).


The analysis of linearity is a key aspect of the study of dominance hierarchies. To study the effect of the choice of socio-spatial level of analysis, we calculated linearity in a large set of southern elephant seal (*Mirounga leonina*) hierarchies from two populations (Valdés Peninsula and Falkland Islands). The socio-spatial level of analysis affect the observational effort, the completeness of matrices, and the frequency of unknown relationships. These factors, in turn, have a notable effect on linearity. We conclude that dominance should be studied at local level, where the absence of structural zeros, and the low incidence of observational zeros, produce complete matrices, well rooted in the true spatial and social structure of the population. Depending on the specific social system, the extrapolation of dominance from the local level to higher levels may result in sparse matrices, and in biased estimates of linearity. The variation of the socio-spatial level of analysis may in part explain the contrasting results obtained in different studies of linearity of dominance hierarchies.

- **Degree thesis**

Members of the research team and field helpers produced four degree thesis on elephant seals of Sea Lion Island (abstracts available online, http://www.eleseal.org/es_lit.htm):
- a full honours MSc thesis on the comparison of male competition between Sea Lion Island and the Valdés Peninsula (Argentina), with results on variation of agonistic behaviour between the two population, on structural correlates of fighting success, and on relationship between dominance and mating (Fabiani 1996)
- a full honours MSc thesis on structure, ontogeny and function of male aggressive vocalizations, that represents the first detailed description of acoustic structure of male sounds, and the first evaluation of hypotheses of signal evolution in southern elephant seals, a species previously neglected by bioacousticians (Sanvito 1997)
- a full honours MSc thesis on the effect of male mating tactics on female breeding performance, that explored for the first time the effect of male breeding behaviour on health and survival of mothers and pups in southern elephant seals (Marzetti 1997)
- a full honours MSc thesis on the effect of the phenotype of the mother and the social habitat on maternal investment (Gallastroni 2001)

Three ongoing PhDs are currently focused on Sea Lion Island elephant seals (the first one is close to completion; summary of PhD projects available online, see above):
- a PhD on genetics of the Sea Lion Island population, including paternity, relatedness and relationship with the South Georgia stock (Anna Fabiani, Department of Biology, University of Durham, Durham, UK)
- a PhD on male vocalizations, including the effects of structural phenotype, and the relationship with competition and breeding success (Simona Sanvito, Department of Biology, Memorial University of Newfoundland, Newfoundland, Canada)
- a PhD on male fights, including structural, hormonal, and functional correlates (Chiara Braschi, BAU, Università degli Studi di Roma “La Sapienza”, Italy)

- **Submitted manuscripts**

We currently have four manuscript submitted to various scientific journals:
- a manuscript on timing of breeding of females (submitted to *Marine Mammal Science*)
- a paper on sound pressure level of male vocalizations in the genus *Mirounga* (submitted to *Bioacoustics*)
- a paper on the use of blood spots for cortisol analysis in elephant seal weanlings (submitted to *Marine Mammal Science*)
- a paper on genetic evidences of very long distance migration of southern elephant seal breeders (submitted to *Science*)

A manuscript on philopatry, breeding site fidelity, and genetic relatedness of female southern elephant seals in currently in preparation.

**Prospects for the future**

- **Short term prospects**

Prospects of the research for the future may be divided in two sections, short term and long term. On the short term side, during the next breeding season, we wish to take to an end the collection of data for the acoustic sub-project. We employed as often as possible power analysis techniques (Cohen 1988; Erdfelder et al. 1996) to evaluate the sample size required to carry on meaningful statistical analysis, and decide when to stop data collection. Moreover, we are starting a new project, with a new PhD student, on the structural, physiological, and functional correlates of fighting, the most striking aspect of elephant seals behaviour.

**Male acoustic communication**

During the 2002 breeding season our main goal is to complete the data collection for the acoustic sub-project. The work will be focused on four main areas:

1. Structural and functional correlates of male vocalizations

   An extensive set of behavioural observations (Fabiani 1996; Sanvito 1997) demonstrated the fundamental role of vocal signals in competition between elephant seal males. The analysis of structure of vocalizations demonstrated a large variation between vocalizations of different males (Sanvito and Galimberti 2000, b). Hence, the structure and frequency of vocalizations may have a significant correlation to male structural phenotype, behavioural performance in competition with other males, and breeding success. The study the structural and functional correlates of vocalizations requires:

   - the measurement of a large set of acoustic variables on each vocalization, due to the very complex structure of vocal signals, that can’t be reduced to a small number of simple measures or to a small number of components obtained by standard data reduction methods (Sanvito and Galimberti, 2000, a & b)

   - the unbiased estimation of acoustic parameters to characterize vocalizations of each individual male, that may be accomplished only by using a large sample of good quality recordings (preliminary power analysis suggest a minimum of 10 vocalizations per male)

   - the analysis of a large sample of males, to cope with the requirements of multivariate analysis techniques, the only ones that may provide a good assessment (the standard rule of thumbs of ten cases per variable suggest a minimum sample size of a few hundreds of males)

   The main structural correlates of vocalizations should be size (Sanvito and Galimberti, submitted). Therefore, a large effort will be dedicated to photogrammetric estimation of size. The actual sample size is yet too small to permit a meaningful evaluation of selection pressure (power calculations revealed that a sample size of about 200 males is required to achieve the desired level of protection in analysis). To improve the quality of size estimation we are trying to implement a three dimensional photogrammetric method (Waite 2000).

2. Ontogeny of male vocalizations.
It’s important to keep in mind that vocalizations are not an instantaneous product of the structural phenotype and of social conditions, but, on the contrary, they result from a development process, that is particularly evident in elephant seals when examining differences in vocalization structure between age classes (Sanvito 1997). Hence, we expect to expand as much as possible our base of recording of immature individuals (and we already started this phase by recording moulting juveniles and sub-adults during March 1999). A full understanding of ontogeny may probably take advantage of a good knowledge of female acoustic communication during phases of aggression towards other females, to look for possible evolutionary pathways from monomorphic signals to the actual dimorphic ones. Therefore, subject to availability of time, we plan to opportunistically record female vocalizations.

3. Hormonal control of male vocalizations

We observed a notable variation of tendency to emit vocalizations of individual males along the breeding season. We also collected preliminary evidences of a corresponding variation in acoustic structure. We wish to test the hypothesis that this variation is related to hormonal status, and in particular to testosterone level. This will be accomplished by recording breeding males, and, at the same time, by sampling blood to be used for testosterone assays. We carried out blood spot collection (•) and testosterone analysis on sample of males of the genus Mirounga (both southern and northern species), and preliminary results are encouraging (unpublished data).

**Male fights**

Male fights are probably the most conspicuous aspect of elephant seal social behaviour, both in the northern (Sandegren 1976; Haley 1994) and in the southern species (Mc Cann 1981; Fabiani 1996). The goal of this sub-project, that we are just beginning, is to determine the structural, physiological, and functional correlates of elephant seal fights. In particular, we wish to examine the effect on the likelihood to win a fight of asymmetries in:
- structural traits, including age, body mass, body length, size of canines
- vocal traits, including sound pressure level and frequency structure of vocalizations
- behavioural traits, including aggressiveness, experience, tenure, and fighting record
- physiological traits, including testosterone level and heart rate
- value of the resource, and reproductive payoffs (short and long term)

Moreover, we wish to examine the effect of the social context, which depends from the number and identity of the other males, which are in turn affected by stochastic factors. Finally, we wish to estimate the direct and indirect costs of fighting behaviour, including the incidence of wounds and the effect on energy consumption and weight loss (although preliminary evidences cast some doubts on the real costs of fighting).

**Mapping of individuals and breeding units**

We are having encouraging results with the use of GPS to map elephant seals (Galimberti and Sanvito 1999). The next step, subject to additional funds availability, should be the integration with GPS of a laser rangefinder with built-in magnetic compass (Leica Laser Locator, Leica Geosystems; http://www.leica.com/optronics/index.htm), that permits determination of 3D position of remote points without having the operator actually reach each point. With this kind of positioning system, an operator may easily map the position of animals with an accuracy of about 1 metre at up to 1000-1500 metres of distance from the target with a modest effort, by firstly determining his own position with the GPS receiver, and then collecting 3D positions (relative to his own position) of animals by simply pointing them with the Vector GIS binocular. Due to the very short measurement time (the rangefinder
requires the target to be pointed for 0.3 second to collect the positional data) the operator may quickly map large quantities of animals.

**Study of female parental investment**
The first part of the parental investment project is now completed, and data are now being processed and published. The next obvious development of this part of the project should be the direct estimation of costs of parental investment. Behavioural measures of lactation effort (e.g., number and length of suckling bouts) are not correlated to true female investment (Cameron 1998; Galimberti et al. 2002 c). Therefore, the best way to produce estimates of costs of reproduction for mothers is to calculate their energetic expenditure during lactation, by measuring weight loss (Arnbom et al. 1997) or energetic transfer from the mother to the pup (Costa et al. 1986). Direct measurement of parental investment requires extensive handling of mothers and pups, and it may affect the handled individuals (Engelhard et al. 1998; 2002). This part of the project will require collaboration with veterinary personnel with experience in elephant seals immobilization. Moreover, chemical restrain of animals implies a very high level of invasiveness, and, therefore, needs to be evaluated in the context of the ethical rules of the project, and discussed with the local authorities.

**Sexual selection on male phenotype**
The study of sexual selection requires collection and analysis of data on many phenotypic traits, due to the ubiquitous phenomenon of traits correlation. One caveat of multivariate set of traits is that their analysis requires a very large sample size. Hence, the first reason to carry on a selection study in the long term is purely statistical: to put together a sample of breeding males large enough to carry on sophisticated analysis many breeding seasons are required. From a more theoretical point of view a long term study of selection permits the evaluation of fluctuations of selection pressures in different seasons: this fluctuation may be stochastic, or may depend on a variation of demography and social milieu. Our plan is to carry on the selection study as long as possible to permit a throughout evaluation of phenotypic selection pressures.

**Lifetime breeding performance**
The study of individual breeding strategies and life histories requires accurate tracking of individual across their whole life span. This research target is somehow complicate to achieve in long living species. In elephant seals the following of a whole cohort of individuals from birth to death will require more about twenty years, with few chances to get hard results in the meanwhile. Despite this may seem a bit daunting, we wish to carry on the collection of data on breeding histories of marked individuals in the long term, to get estimates of lifetime breeding success of males and females, that with greatly help in the understanding of the selection pressures that act on the two sexes.

**Mark-recapture study and survival estimation**
The estimation of survival rate of different sex and age classes has a double value. From a theoretical point of view, survival rates, and their dependence on individual phenotype, are a key component in the evolution of life histories. From a practical point of view, survival rates are a instrumental in the estimation of demography and population dynamics, and essential for the forecasting of the future status of populations. The best approach to the study of survival rates are mark-recapture studies, that in long living species require long term research projects. We plan to intensify our marking plan, by tagging individuals out of SLI in the rest of the Falklands, by expanding the use of passive transponders, and by testing new ways of marking (e.g., Hall et al. 2000).

The survival rate of young individuals is a fundamental aspect of elephant seals population dynamics (Hindell 1991; McMahon et al. 1999). Our research team carries on
field work on Sea Lion Island for the whole length of the breeding season, but not during the moult. Therefore, we are not able to fully collect data about survival of younger individuals, that haul out mostly during the moult season and not during the breeding (McMahon et al. 1999). The re-sighting of these individuals will permit:
- to estimate survival rates of younger age classes (yearlings, two years old males and females, juvenile males, nulliparous females)
- to complete our record of individual life histories, improving our understanding of individual breeding strategies

Survival of the young individuals, and in particular survival during the first year of life, is the single most important demographic parameter of elephant seals population (Bester and Wilkinson 1994), and is a crucial phase of individual life histories (Bell et al. 1997b). Variation in first year survival is the most relevant factor in the control of population size. Therefore, the proper forecasting of the status of the Sea Lion population will require an exact estimation of survival rate of yearlings (Boyd et al. 1996; Galimberti et al. 2001a).

We tagged all pups born on Sea Lion Island from 1995 to 1998 (Galimberti and Boitani, 1999) and the tag loss rate in yearlings and 2 years old individuals was low (unpublished data). Hence, what we really need is a good re-sighting coverage of the moult season (November-March). We already cover November, but we have just scanty information for the rest of the moult season (data courtesy of Mr. Henry Guala). Each elephant seal stays on land for moulting for at least 20 days (Le Boeuf and Laws 1994): hence, resightings during the moult can be collected with a small effort. Data collected in November gave us preliminary information on yearling and juvenile survival, but good estimates of actual survival rate may be gathered only with a full coverage of the whole moult season, than can perhaps be carried out with the collaboration of local volunteers.

Predicting the future of mammalian populations requires long time series of demographic and life history parameters (Hindell, 1991). For example, the short term stability of the effective size of our seal population may be confirmed in the long term only when long time series of number of breeding females, pup mortality rate, and adult survival rate will be available. One of our long term goal is to obtain this kind of evidence.

The distribution of breeding elephant seals should be assessed.
The population of Sea Lion Island seems to represent what is left of the formerly large population of the Falkland Islands. Preliminary information from surveys of the entire Falklands coast for marine birds census, carried out at the end of the elephant seal breeding season, showed very limited signs of breeding (Mike Bingham, pers. comm. 1996; Mike Morrison and Robin Woods, pers. comm. 1998). Therefore, the future status of the Sea Lion Island population could have a significant role in the conservation of the entire Falklands population.

A full assessment of elephant seals distribution in the Falklands presents significant problems due to the length and intricacy of the coast line, that potentially offers many suitable place for elephant seals breeding. The best way to proceed, should be to do a preliminary selection of likely sites, based on historical and anecdotal evidence, and then carry out a complete aerial survey of these sites around peak haul-out (i.e., between third and fourth week of October). From counts of females, and by applying correction factors from mathematical models derived on SLI data, a total estimate of pup production could be estimated. From pup production is easy to calculate total population size using a correction factor calculated from a generic elephant seal life table.
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