ST. KILDA SOAY SHEEP PROJECT: ANNUAL REPORT 2002

J.G. Pilkington[1+3], S.D. Albon[2], K. Byrne[1], D.W. Coltman[7], T.H. Clutton-Brock[3], T.N. Coulson[3], B.Craig[1], M.J. Crawley[4], I. Gordon[5], B.T. Grenfell[3], O. Jones[4+5], L. Kruuk[1], B. Morgan[8], A. Overall[1], J.M. Pemberton[1], B. Preston[6], I.R. Stevenson[6], G. Tavecchia[3], L.Tempest[6], K. Wilson[6], B. Wimmer[1].

[1]Institute of Cell, Animal and Population Biology, University of Edinburgh.
[2]Centre for Ecology and Hydrology, Banchory.
[3]Department of Zoology, University of Cambridge.
[4]Department of Biology, Imperial College, University of London.
[5]Macaulay Institute, Aberdeen.
[6]Institute of Biological Sciences, University of Stirling.
[7]Department of Animal and Plant Sciences, University of Sheffield.
[8]Institute of Maths and Statistics, University of Kent at Canterbury.

POPULATION OVERVIEW

The sheep population on Hirta entered 2002 at a very high level and, as a result, there was substantial mortality in the first few months of the year. 478 Sheep were found dead within the study area between January and May of 2002, of which 180 were untagged. The untagged animals were made up of study area lambs not tagged during the foot and mouth crisis in 2001 and untagged animals which entered Village Bay from other parts of the island. Of the tagged animals that died, 200 were adults and 98 were juveniles. Lambing recruitment began on the 29th of March with 59% of lambs born surviving (Fig. 1).
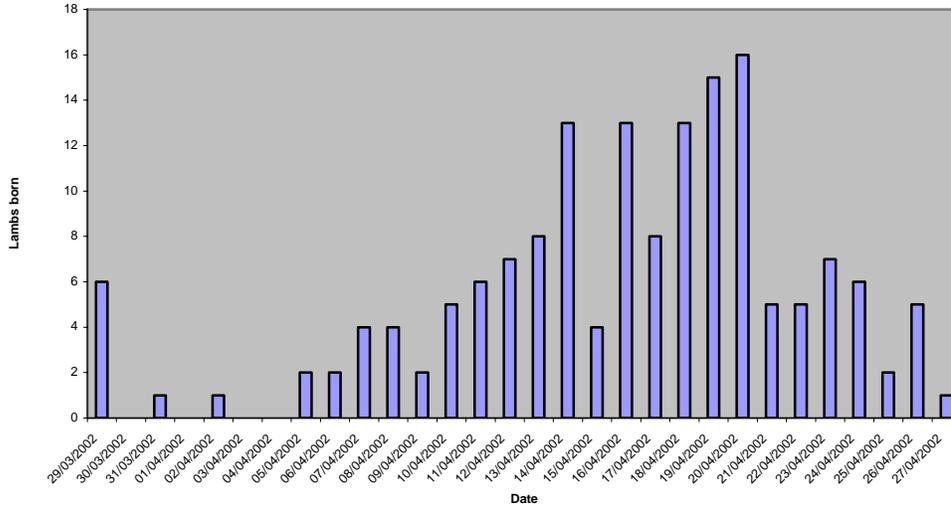
Figure 1. The temporal distribution of lamb births during 2002.

In December 2002, 652 tagged sheep were believed to be alive on Hirta, of which 333 regularly used the study area, a total decrease of nearly 49% on the previous year. The age distribution of the population is shown in Fig. 2 and changes in sheep numbers in the study area over time are shown in Fig. 3.
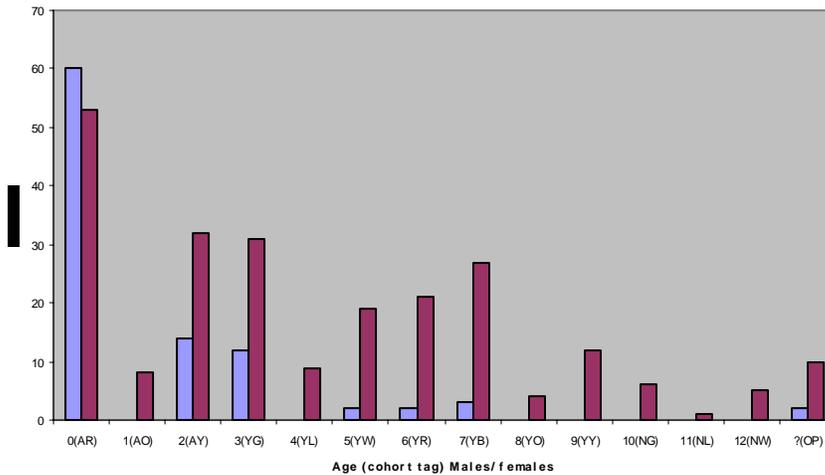

Figure 2. Age distribution of tagged Soay sheep presumed to be alive at the end of 2002.
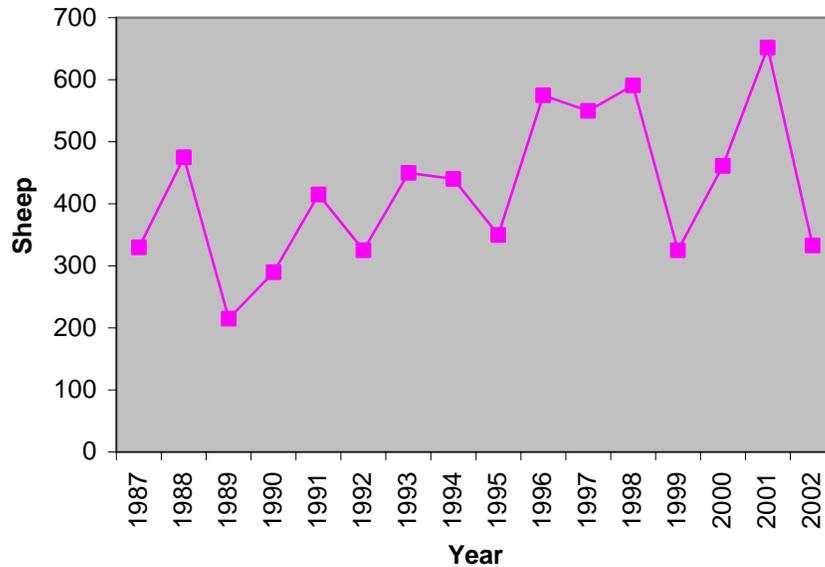
2

Figure 3. *The number of tagged sheep regularly using the study area since 1985.*

One whole-island count yielded an estimate of 907 tagged and untagged sheep on the whole island with the details displayed in Table 1. The total population had decreased by 52% since summer 2001.

Table 1. *Demographic and geographic distribution of sheep observed during the count of Hirta on August 20th 2002. Coat colours are DW = dark wild, DS = dark self, LW = light wild, and LS = light self.*

Location	Females				Males				Lambs	Total
	DW	DS	LW	LS	DW	DS	LW	LS		
Conachair/Oiseval	123	2	37	1	26	0	9	0	71	**269**
Mullach Bi/Cambir	99	8	35	5	12	3	5	0	92	**259**
Ruaival/Village	146	7	64	4	29	1	7	0	121	**379**
Total	**368**	**17**	**136**	**10**	**67**	**4**	**21**	**0**	**284**	**907**

REPORTS ON COMPONENT STUDIES

Inheritance of coat and horn polymorphisms.

Dave Coltman & Josephine Pemberton.

Long before molecular markers were used to measure the levels of genetic variation in the Soays, the phenotypic variation evident in coat colour and horn shape suggested that the Hirta population harbours considerable genetic polymorphism. However, the genetic basis of these variants has not yet been definitively worked out. Four coat colour morphs are recognised in Hirta Soays: dark wild, light wild, dark self and light self, where the 'wild' type refers to the mouflon pattern with pale belly and rump, and 'self' means having the same colour all over the body. Three horn phenotypes, normal, polled (horns absent) and scurred (stunted or deformed horns) are found on Hirta. Using observed frequencies of these phenotypes and patterns of inheritance from the Village Bay pedigree we have attempted to deduce the genetics underlying the inheritance of these phenotypes.

Coat Colour

An inheritance model was proposed by Doney (Island Survivors, 1974) who suggested one autosomal locus, known as the Brown or B locus, with two alleles, at which the dark allele B^+ is dominant to the light allele B^b, and a second locus, the Agouti or A locus, at which wild A^+ is dominant to self A^a. Frequencies of the four morphs in Soays, along with their putative genotypes, are shown below. (We are using international allele codes established since 1974).

Table 2. Distribution of coat colour and pattern morphs, and their proposed genotypes, in 1193 Hirta Soay sheep with paternity inferred at 80% confidence from molecular data.

	Wild			Self		
	Genotype(s)	Observed	Frequency	Genotype(s)	Observed	Frequency
Dark	$A^+A^+B^+B^+$ $A^+A^+B^+B^b$ $A^+A^aB^+B^+$ $A^+A^aB^+B^b$	805	0.675	$A^aA^aB^+B^+$ $A^aA^aB^+B^b$	43	0.036
Light	$A^+A^+B^bB^b$ $A^+A^aB^bB^b$	327	0.274	$A^aA^aB^bB^b$	18	0.015

Assuming Hardy-Weinberg equilibrium, the frequency of the B^b allele can be estimated as $(0.274+0.015)^{0.5}$ or 0.538. The frequency of the B^+ allele must therefore be 0.462. Similarly, at the A locus, the frequency of the A^a allele can be estimated as $(0.036 + 0.015)^{0.5}$ or 0.226. The frequency of the A^+ allele must therefore be 0.774.

We tested this model by comparing the observed and expected frequencies of offspring coat colour combinations using the pedigree data, based on known mother offspring relationships and paternities inferred at 95% confidence. Contingency analyses show that

the inheritance models for dark/light ($\chi^2 = 1.37$, 4 d.f., NS) and wild/self ($\chi^2 = 1.32$, 3 d.f., NS) fit the data well.

Horns

The inheritance of horn phenotypes in the Soays is made more complicated by the fact that the frequencies differ between the sexes, suggesting that there is sex-specific expression of the genes underlying this trait. We examined the fit of a model suggested originally for the Merino breed in the 1960s that proposes three alleles with differential expression in the sexes: $\mathbf{Ho^L}$ - sex-limited horns, $\mathbf{Ho^+}$ - normal horns, and $\mathbf{Ho^P}$ - polledness. According to this model, the $\mathbf{Ho^P}$ allele is recessive in rams, causing scurs, and dominant to $\mathbf{Ho^L}$ in ewes. The $\mathbf{Ho^P}$ allele is partially expressed in the presence of $\mathbf{Ho^+}$ in ewes, causing an intermediate scurred phenotype. $\mathbf{Ho^L}$ is recessive in ewes to both the other alleles, and causes the scurred phenotype when homozygous. Frequencies of the horn phenotypes in Soay ram and ewes, along with their putative genotypes, are shown below.

Table 3. Proposed genotype-phenotype relationships under a three-allele sex-specific expression model of horn inheritance, and frequencies of observed phenotypes in the Village Bay population. Note that because of the difficulty of discriminating scurred from polled ewes during early development, the ewe phenotype data shown is for ewes captured at one year or older.

	Rams			Ewes		
Phenotype	Genotype(s)	Observed	Frequency	Genotype(s)	Observed	Frequency
Polled				Ho^PHo^P, Ho^PHo^L	171	0.284
Scurred	Ho^PHo^P	151	0.151	Ho^LHo^L, Ho^+Ho^P	224	0.371
Normal	Ho^+--, Ho^L--	848	0.849	Ho^+Ho^+, Ho^+Ho^L	208	0.345

The frequency of the $\mathbf{Ho^P}$ allele can most easily be estimated from the male scurred class in rams as $0.151^{0.5} = 0.389$. Based on the frequency of polled ewes, $\mathbf{Ho^L} = 0.170$, leaving the frequency of $\mathbf{Ho^+} = 1 - 0.389 - 0.170 = 0.441$. Rechecking the predicted ewe phenotype frequencies leads to estimates of polled = 0.284, scurred = 0.372, and normal = 0.344. This model therefore matches the observed frequencies of phenotypes almost exactly.

To test the predictions of this model, we examined observed phenotypes of 666 lambs with known maternity and paternity inferred at 80% confidence from molecular data. A contingency analysis indicates that the observed and expected counts do not differ significantly ($\chi^2 = 24.51$, 17 d.f., $0.10 > P > 0.05$). In general the data fit the model acceptably well, however it is not possible to pursue this analysis among the 95% confident paternity data set at this stage, due to the reduction of sample size involved which leads to too many unacceptably low expected numbers. We investigated other possible models of inheritance, including simpler two-allele models and more complicated models involving more than one locus with sex difference in expression, however none fit the data better than the three-allele model described above. This would

therefore seem to be the most parsimonious model explaining the inheritance of horn phenotype given the available data.

Survival costs of reproducing vary with age and environmental conditions.

Giacomo Tavecchia, Byron Morgan and Tim Coulson.

Estimating survival costs of reproduction are a key objective of population biology. However, there are methodological problems in estimating such costs if data on the fate and reproductive performance of individuals are incomplete. Even though our data are unusually accurate, not all animals are seen in all years, which can add biases in estimates of the survival cost of reproduction.

Using recently developed statistical methods termed multi-state mark-recapture models we estimated the survival cost of reproduction by comparing the survival rates of animals that bred with those of animals that didn't breed. Furthermore we explored whether the cost of survival varied with age, density and climate.

We found that the survival cost of reproduction varied with age and was highest in young and old animals (Fig.4). We also found that within these age groups the survival cost of reproducing was greatest in years of high density and wet, windy weather and was undetectable in years of low density with less windy, wet weather (Fig.5).
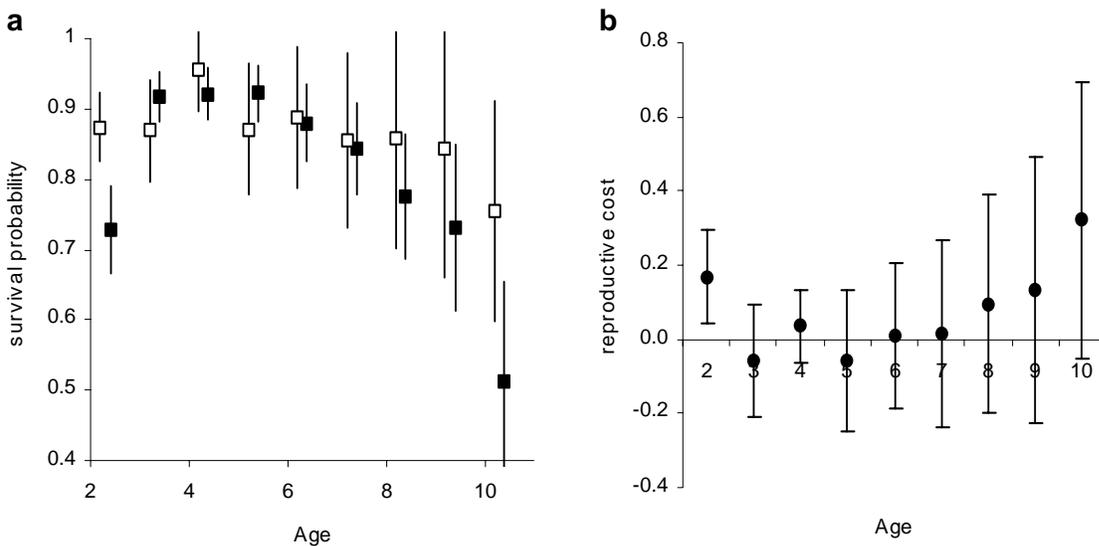

Figure 4. (a) *Age-dependent survival estimates of non-breeding (solid squares) and breeding females (empty squares).* *(b)* *The cost of reproduction (1- survival non-breeders/survival breeders) has a parabolic shape. Bars indicate the 95% confidence interval (in* *(b)* *these are estimated by δ-method).*
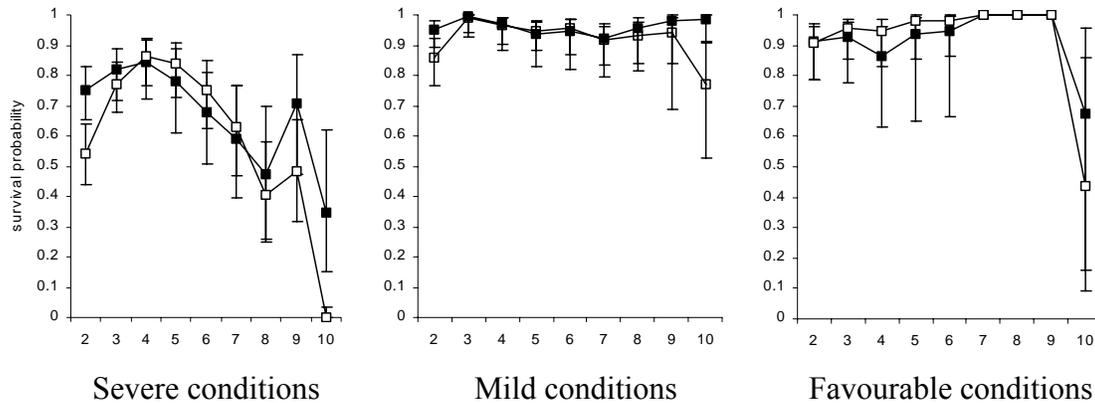
6

Figure 5. Survival probability of breeder (solid squares) and non-breeder (empty squares) females according to environmental conditions.

These results are one of the first applications of these methods, and one of the first demonstrations of a survival cost of reproduction in a wild population. The methods are still in their comparative infancy and need to be expanded to incorporate variables like parasite egg loads that vary within individuals from year to year. We are currently working to expand these methods.

What happened to the expected post-crash flowering?

Mick Crawley.

You might imagine that the most palatable plant species would do particularly well in the summer following a crash in the sheep population. We now have botanical data for two crashes (1999 and 2002) and it is clear that there is no post-crash mass-flowering for *any* of the most palatable plants (let alone most, or all, of them). We have still not seen any flowering by *Festuca rubra* or *Rumex acetosa*, despite the fact that they flower happily on ungrazed cleit roofs every year. Only two of the plant species (*Leontodon autumnalis* (r = -0.70, n = 9) and *Potentilla erecta* (r = -0.83) show a long-term significant negative correlation between sheep density and flower density in August. The figure below shows the average flower density for 8 of the most abundant plants species from the two pre-crash summers (1998 and 2001; labelled "pre") and the two post-crash summers (1999 and 2002; labelled "post")
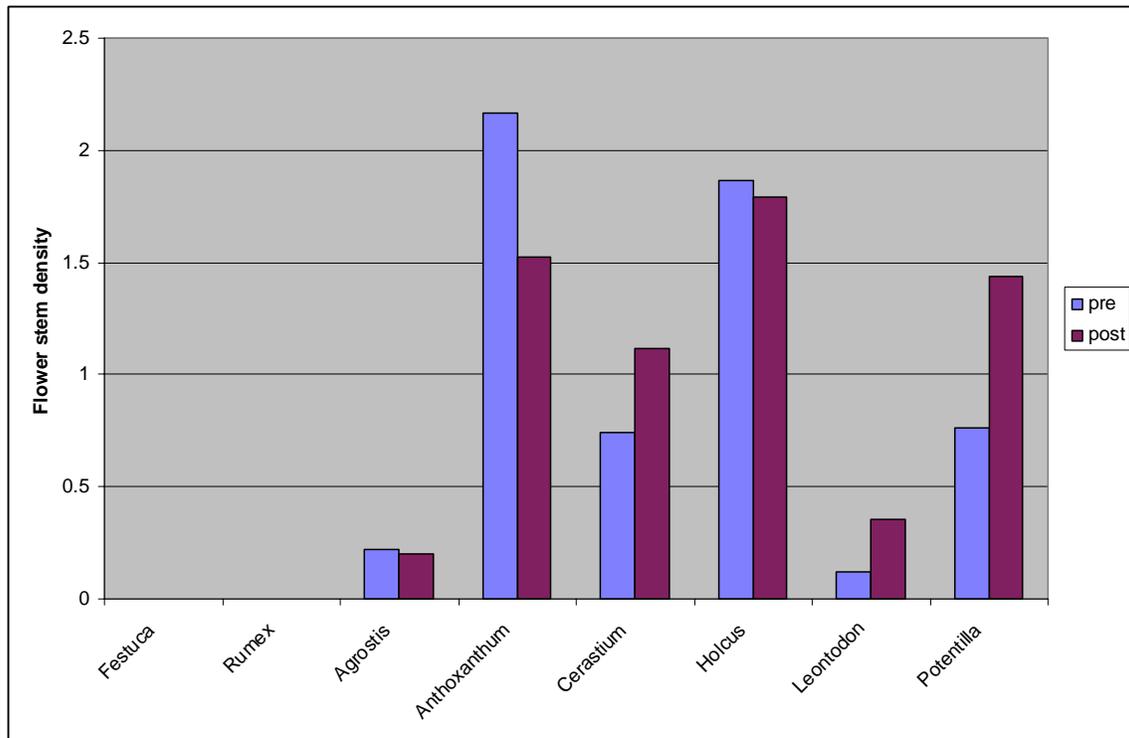
Figure 6. Flower stem density for 8 abundant plant species in the summers before and after sheep population crashes.

The significant positive effect of sheep density on flowering in *Anthoxanthum* is interesting; this suggests that the grass is relatively unpalatable to Soay sheep and suffers increased levels of interspecific competition in post crash years, leading to reduced flowering.

Perhaps the lack of a response is due to time lags? It might take more than one season's growth for plants to build up sufficient reserves for flowering. To test for this, we plotted flowering in year t against sheep population in years t-1, t-2 and t-3. There is clear evidence of 1-year lag effects for 3 of the plant species (*Trifolium repens, Ranunculus acris* and *Agrostis capillaris*) but no evidence for 2-year or 3-year lag effects on flowering for any of the species.
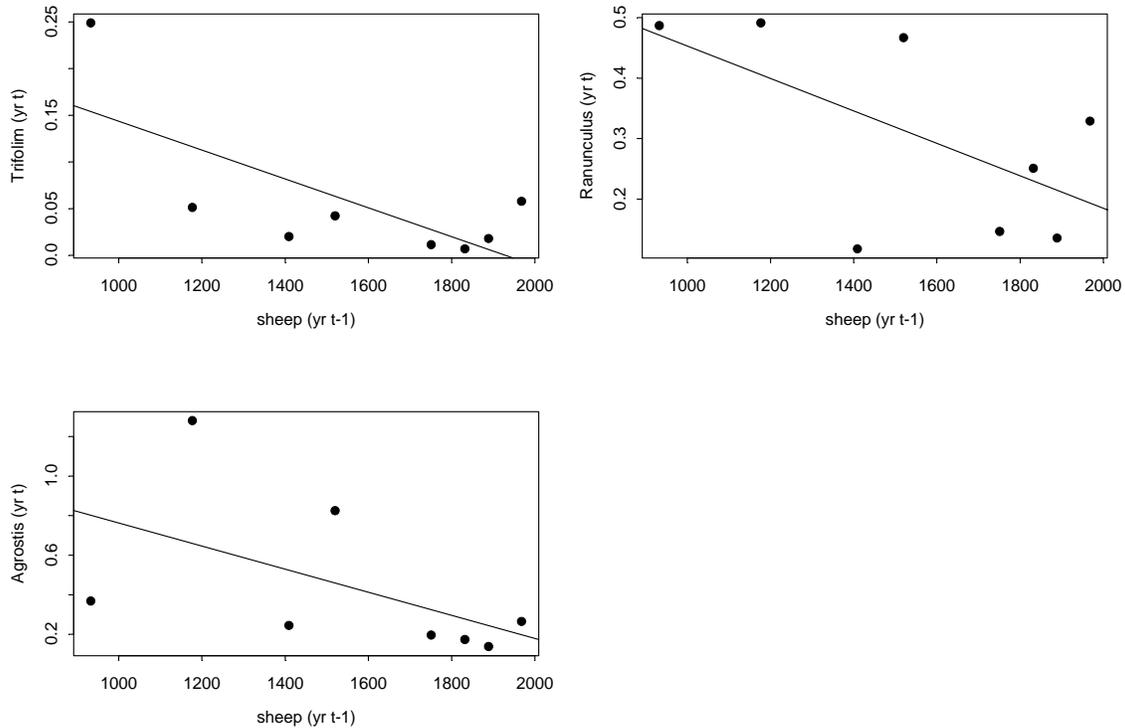
Figure 7. *Flower stem density in year t plotted against sheep numbers in year t-1. (Trifolium, r = -0.71 , Ranunculus, r = -0.61 , Agrostis r = -0.53 , n = 8).*

Protozoan parasite prevalence of the St. Kilda Soay sheep.

Barbara Craig, Josephine Pemberton, and Jill Pilkington.

Last year's report on the coccidia of the Soay sheep related that eleven species of *Eimeria* and *Cryptosporidium parvum* had been identified based on the morphology of oocysts found in faecal samples, collected in August 2001. This is the first report of a thirteenth protozoan, this time a flagellate named *Giardia duodenalis*, identified from cysts in the same sample set. Prevalence of this recent addition and of the *Eimeria* species can now be given and a comparison of prevalence of *C. parvum* between August 2001 and August 2002 reveals how a crash in the Soay sheep population has caused a consequent crash in the prevalence of this parasite in lambs.
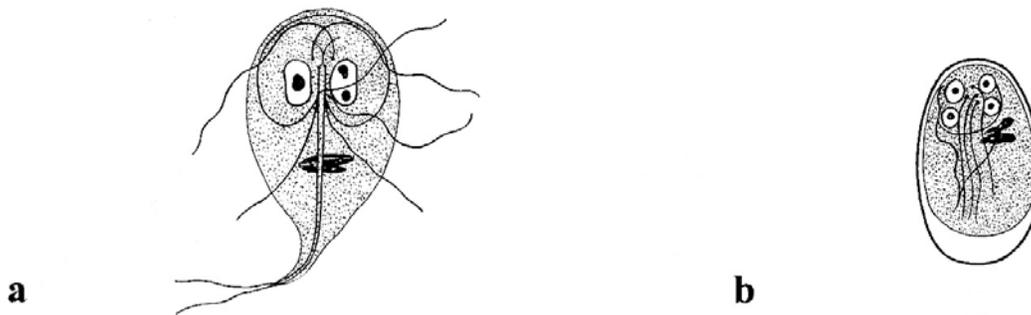
*Figure 8. Giardia spp. (x 2000) **a)** trophozoite **b)** cyst (Taken from Wenyon, C.M. (1926). Protozoology. London: Balliere, Tindall & Cox.)*

Giardia duodenalis is unlike the coccidia in that it replicates entirely asexually and prefers to clamp onto the intestinal epithelium rather than to invade it. Two forms of the parasite can be seen in faeces (Fig.8), a trophozoite which can be seen moving using eight flagella and a cyst stage containing two of these trophs. Prevalence of the cysts, found in the August 2001 faecal samples, are included in Table 4.

Table 4. Prevalence of Eimeria spp. and G. duodenalis from August 2001 faecal samples. There is a significant drop in prevalence with increasing host age for all species ($p<0.05$), except E. granulosa, whose prevalence significantly increases in yearlings before dropping in adults ($p<0.01$).

	Prevalence (%) in August 2001		
Parasite species	**Lambs (2001)** (n=50)	**Yearlings (2000)** (n=34)	**Adults(<2000)** (n=52)
Eimeria ahsata	92.0	30.8	19.2
Eimeria bakuensis	90.0	32.4	25.0
Eimeria crandallis	98.0	85.3	52.0
Eimeria faurei	48.0	11.8	2.0
Eimeria granulosa	8.0	47.1	3.1
Eimeria intricata	38.0	2.9	5.8
Eimeria marsica	44.0	29.4	5.8
Eimeria ovinoidalis	38.0	20.6	7.7
Eimeria pallida	36.0	38.2	5.8
Eimeria parva	76.0	32.4	7.7
Eimeria weybridgensis	96.0	73.5	51.9
Giardia duodenalis	38.5 (n=65)	20.0 (n=40)	1.6 (n=63)

Prevalence of *G. duodenalis* shows the typical pattern of being highest in lambs then falling to a very low prevalence in adults and this pattern is shared by most of the *Eimeria* spp. with one notable exception. *Eimeria granulosa* has highest prevalence in yearlings.

Soay sheep population density in August 2001 was very high and as a consequence there would have been increased environmental contamination of infective protozoa. In addition, sheep in poor condition would have been more susceptible to infection. The population crash in the winter and spring of 2002 would leave much better conditions for sheep by August of that year. A comparison of prevalence values for *C. parvum* between the years has been made and the results, in Table 5, show a major reduction of infection in the youngest age class.

Table 5. *Comparison of C. parvum prevalence in pre and post crash years. There was a significant drop in prevalence in lambs but not in adults (p<0.01).*

Prevalence (%) for *C. parvum*

	LAMBS	YEARLINGS	ADULTS
August 2001	34.9 (n=83)	27.8 (n=54)	20.0 (n=105)
August 2002	1.4 (n=69)	no data	13.4 (n=142)

Lambs born in 2001 would have been susceptible to high levels of oocysts in the environment and would have been less able to cope with chronic infection. This could explain the high prevalence in August 2001. Last year's cohort (2002) were born into better conditions and any infection acquired soon after birth would have probably dissipated by the time of the August catch.

Assessment of parasitic nematode diversity in Soay sheep using molecular markers

Barbara Wimmer, Barbara Craig and Josephine Pemberton

In the Soay sheep, parasitic nematodes are a major factor contributing to mortality, especially in malnourished host individuals. To date the worm burden on the island has been regularly monitored by post-mortem analysis and faecal egg counts of living hosts. But it is very time-consuming and difficult to distinguish parasitic nematode adults, and almost impossible to identify eggs down to species level. These practical difficulties mean that so far we have only tested questions about differences between sheep in overall parasite resistance, and we have not been able to investigate whether sheep differ in resistance to different species of parasite.

The development of molecular markers to distinguish the different nematode species is therefore highly desirable. Our first step towards a non-invasive method of monitoring the diversity of infection was to develop markers that would identify the species unambiguously. For marker development, single adult nematodes were morphologically identified to species level and DNA was extracted from them for analysis. The following species are present in the study population of Soay sheep on St. Kilda: *Teladorsagia circumcincta* (Tc), *Teladorsagia trifurcata* (Tt), *Teladorsagia davtiani* (Td), *Trichostrobgylus axei* (Ta) (**abomasal** parasites); *Bunostomum trigonocephalum* (Bt),

Trichostrongylus vitrinus (Tv), *Nematodirus filicollis* (Nf), *Nematodirus battus* (Nb) (parasites of the **small intestine**); *Trichuris ovis* (To), *Chabertia ovina* (Co) (parasites of the **large intestine**)*; Dictyocaulus filarial* (Df) (**lung** parasite).

We have tested several genomic regions for their suitability as species markers. All sequences obtained were checked against published sequences to verify the species. Of the regions tested, the so-called ITS-2 region (part of the ribosomal DNA) proved to be most suitable, being able to distinguish between closely related species, except for the three species of *Teladorsagia* which are hereafter grouped together as Tc. Therefore the ITS-2 region was chosen to develop species-specific primers for PCR identification of nematode species. Each pair of primers was designed to produce a DNA band of distinct size on an electrophoresis gel. The primers were tested for their specificity, and no non-specific amplification was observed between the species.

As the ultimate aim of the study is to use DNA from eggs or larvae from faecal samples, it is also important to check for inhibitory effects of a mix of DNA samples of all the species on the success of the PCR. All possible species combinations were tested against each other and no such effect was observed.

To test if the primers also work on different developmental stages of parasitic nematodes (e.g. eggs and larvae), they were used to identify species from a bulk DNA extraction of a larval culture and an egg harvest from a pooled faecal sample taken from several individual sheep on St. Kilda in August 2002. An amplification product from the larval DNA extraction was obtained for several (Co, Df, Ta, Tc, Tv), but not all species (no product for Bt, Nb, Nf, To). As the species composition of this sample was unknown, it is not clear whether this was due to failure to amplify or simply due to the absence of certain species. The sample had been taken from healthy animals and the missing species are usually of very low prevalence in the Soays on St. Kilda. Hence it is very likely that these species were not represented in the sample. Also it is known that not all parasitic nematode species survive culturing equally well. But all the species showing a positive signal in the larval culture should also be found in an egg harvest of the same faecal sample, so the same procedure was undertaken for a bulk DNA extraction of an egg harvest of the same sample. An amplification product was obtained for all the species found in the larval culture experiment. Additionally there was a positive signal for Bt and Nb. So both results were in accordance with each other with the exception of Bt and Nb, which might have been lost through culturing.

A final test for the reliability of this technique is to compare the species composition of adult worms identified morphologically in post-mortem samples and the molecular method applied to an egg harvest of a faecal sample of the same animal. A sample was taken from a healthy ram that was killed in a fight in the rut. The egg count was very low (100 eggs/g faeces). Adults of the following nematode species were found in the post-mortem sample: Tc, Ta, Bt, Nf and Co. The species identified by using the molecular markers and the faecal samples were identical with the exception of Nf, which could not be detected in the faecal sample. This shows that the molecular method using faecal samples can be used for species detection, but at this stage has its limitations in detecting very low infection rates.

Our next two aims with this project are to make the species-specific tests quantitative and to clarify the species status of the *Teladorsagia* species. There is increasing evidence that the three named species of *Teladorsagia* found on St. Kilda are in fact one species that has a polymorphic phenotype; we will test this using microsatellite analyses on individuals of different morphs.

The effects of parasite removal on over-winter survival in Soay sheep (2001-2).

Owen Jones & Jill Pilkington.

Gastrointestinal (GI) parasites are known to be able to function as top predators via their negative effect on survival. As such, they can potentially influence population dynamics. In order to establish if and how such effects operate, experimental manipulation of parasite burden is necessary. If parasites have a detrimental effect on survival, then individuals that have been treated to remove the parasites would be more likely to survive.

The main parasites of Soay sheep are GI parasites of the same kind that infect domestic sheep. They can be removed with the application of a bolus releasing the anthelmintic drug albendazole over a 3-month period.

Survival data were analysed for 52 2-year old sheep (26 males, 26 females) involved in a 2001 experiment that was originally designed to look at the effects of parasitism on foraging behaviour (see elsewhere in this report). For this analysis, the treatment groups are defined as (1) receiving an anthelmintic bolus (AH+) and (2) not receiving an anthelmintic bolus (AH-).

Survival was monitored over the winter and into spring. 32 out of the 52 sheep died over the winter and for 28 of these, the day of death was determined. For the remaining 4, the month of death was known, and for the purposes of the analyses, death date was recorded as the 15th of the month. In all cases, status (alive/dead) in August was known.

Table 6. Over-winter survival data for 2-year old sheep involved in the foraging behaviour experiment of 2001. The numerator is the number of survivors while the denominator is the number of subjects in each treatment class.

	SEX	
TREATMENT	FEMALES	MALES
AH+ (parasites absent)	5/9	4/11
AH- (parasites present)	8/17	3/15

Initially, the data were analysed using a generalised linear model with a binary response (status in August: 1=alive, 0=dead) and a binomial error structure. A maximal model was fitted including sex, weight, treatment and initial parasite burden as explanatory variables

(plus interactions). Then non-significant terms were removed by deletion, highest order terms first.

This approach revealed that none of the explanatory variables accounted for a significant amount of the survival probability (p>0.05) – all the sheep were equally likely to die.

To examine the timing of the deaths, a Cox proportional hazards survival model was fitted. It was clear from this model that treatment did not have a significant effect on survivorship (χ^2= 0.3 on 1 d.f., p=0.614). There were, however differences in survivorship between the sexes (χ^2= 6 on 1 d.f., p=0.041) (see Fig.9). There were no significant effects of weight for either sex (Male: z=-0.910, p=0.36; Females:z=-0.767, p=0.44) or treatment class (AH+: z=-0.187, p=0.85l; AH-1.036, p=0.30).

The results of these analyses suggest that, when considered as a binary response, parasite burden, sex and summer weight did not play major roles in influencing over-winter survival in the 2001-2. Furthermore, when the response was the time of death, as analysed by survival analysis, only sex was revealed as an important factor.
This is most likely due to the differences in behaviour during the November mating season (rut). At this time, while females continue to spend most of their time feeding, males spend a large proportion of their time searching for, and defending, consorts at the expense of feeding. Thus they enter winter in a relatively poor condition compared to females and their meagre fat reserves are used up before those of females.

Pathogens such as GI parasites tend to cause chronic rather than acute infections. They do not cause death directly but they affect condition and it is and other reductions in fat reserves that lead to death (see next report). Because of this, it is often easier to detect effects on measures of condition rather than on survival rates. This has been demonstrated by recent work on the Svalbard reindeer project which, despite not demonstrating an effect of parasites on survival, has shown significant effects on the condition and fecundity in the year following treatment, with treated animals being in better condition and having a higher fecundity.

Unfortunately, our sample size was low, with only 8 survivors with weights measured in the summer of 2002 it is not surprising that an effect of treatment on body condition in the following year was not detected (t=-0.4453, d.f.=5, p=0.672).

Previous studies have examined over-winter survival in 1988/89, 1991/92 and 1995/96. The strength of the effect of parasite removal differed from year to year with some years not having a demonstrable effect.

Work is currently in progress to examine why there is an effect in some years and not others. Possible explanations involve the age of the host, weather severity and food availability. Perhaps the parasites only affect survival in non-prime animals, when the weather is particularly severe or when food availability is low? Or maybe, in some years the forage quality is so low that even parasite free animals are likely to die from starvation? Effects on condition indices and fecundity will also be examined.
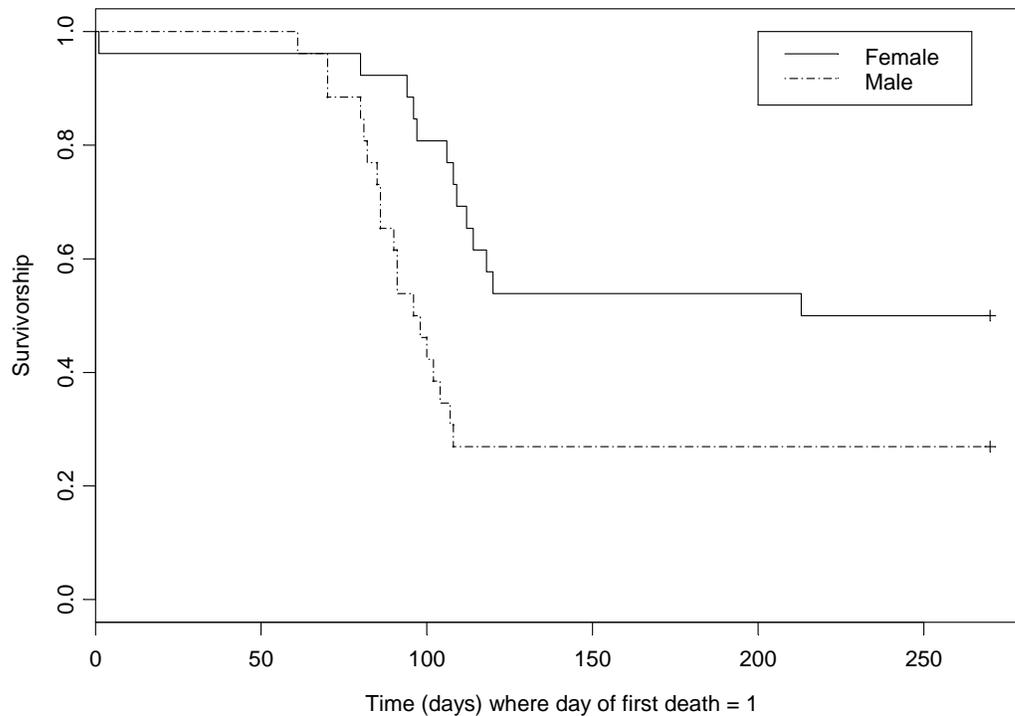
Figure 9. *Survivorship curves for 2-year old females and males over the winter of 2001-2. Survivorship indicates the percentage of the animals that are still alive.*

Foraging strategy and parasite burden of Soay sheep.

Owen Jones, Jill Pilkington, Bob Mayes & Roslyn Anderson.

Parasites have a range of both physiological and behavioural effects on their hosts. For the gastrointestinal (GI) parasites of sheep, the main physiological effects are increased endogenous protein loss and mucoprotein secretion and damage to the gut tissue, often accompanied by blood loss and anaemia. A common behavioural effect is a reduction in voluntary food intake (parasite induced anorexia or PIA) of 30-60%.

Traditionally, this has been viewed as a pathological response to parasitism with no functional basis. However, the immune response is costly and time-consuming to operate, and it is likely that a behavioural mechanism has evolved to carry out a similar role by (1) avoiding food items that are sources of parasites, (2) consuming items to make the intestinal environment less hospitable or (3) selecting foods with anti-parasitic compounds.

As intake rate is inversely proportional to selectivity, anorexia may be a manifestation of this increased selectivity. However, although intake rate declines, compensatory selection for food of a higher nutritional value means that nutrient intake can remain unchanged.

A study was carried out in summer 2001 to determine whether the Soay sheep population exhibit PIA and, if they do, to ascertain whether selectivity is also altered. 26 female and 26 male sheep were captured and assigned to one of 3 treatment groups: (1) treated with

15

two slow-release capsules, one releasing an anthelmintic to eliminate parasites and another releasing an alkane to allow the estimation of intake rate (see below), (2) treated with the alkane only and, (3) the control (no treatments).

The alkane capsule was used to estimate intake rate based on the comparison of a naturally occurring odd-chained C_{33} alkane in faeces and ingested herbage, with the dosed even-chained C_{32} alkane in faeces[1]. Diet composition was estimated by exploiting the differences in patterns of naturally occurring alkane components between plant species and relating them mathematically to the patterns observed in the faeces[1].

Faecal samples were collected from the animals and analysed by gas chromatography. Intake rates and diet composition estimates were then calculated and standard statistical techniques were used to analyse the data. Sex was not included as a covariate because it was confounded with weight.

Overall, intake rate increased with body weight (slope=23.25±7.95, intercept=173.51±191.11; $F_{1,31}$=8.55, p=0.006) and the mean over both sexes was 723gDM/day (range: 458-1346 gDM/day). These estimates correspond fairly well to expectations for animals of this size range (15-31 kg).

The minimum adequate model included weight ($F_{1,29}$=10.04,p=0.003), hind leg length ($F_{1,29}$=0.38, p=0.543) and the interaction between them ($F_{1,29}$=7.01, p=0.012). For large sheep, intake rate increases with weight. As size decreases, the slope decreases, until it becomes negative for smaller animals (Fig.10, left).

Diet composition (Fig.10, right) was not influenced by any of the explanatory variables, thus providing no evidence of any parasite induced changes in selectivity.

Theoretical work has shown that pathogens such as GI parasites can potentially influence herbivore population dynamics by increasing herbivore mortality rates. Additionally, mathematical models indicate that PIA could have also have a significant effect.

Nevertheless, this study found no evidence for any significant PIA in Soay sheep. Power analysis suggests that, given the sample sizes used, and the estimated variance, we would have been able to detect a 30% difference in intake rate between the treatment groups. So if there is an effect, then it is certainly smaller than 30%.

However, this work was carried out in the summer, when the sheep are approaching peak physical condition, and parasites are known to have a greater effect on hosts that are in poor condition and immuno-compromised. We would hypothesise that if the experiment were to be repeated in the winter, the treatment effect would be larger and therefore easier to detect. Unfortunately, large-scale experiments like this are logistically difficult at the best of times and, notwithstanding the animal welfare aspect, would be inadvisable during the winter.
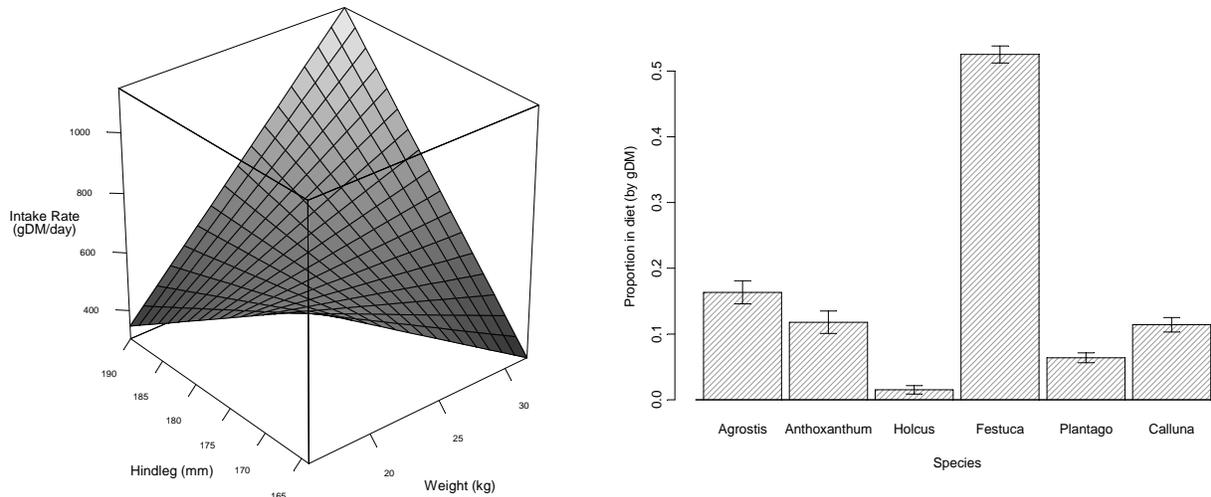
Figure 10. (Left) *The interacting effects of hind leg length and weight on intake rate*
*($F_{1,29}=7.01$, $p=0.012$). **(Right)** The botanical composition of Soay sheep diet (by gDM),*
error bars represent ±1SEM.

Reference: 1. Dove, H. and Mayes, R.W. (1996) *Journal of Nutrition* 126, 13-26

The impact of parasitism on ewe reproduction and suckling behaviour.

Louisa Tempest & Ken Wilson.

Previous studies on St Kilda have shown that animals with higher parasite burdens have a
higher probability of dying overwinter (Gulland et al., 1993). It has also been shown that
ewes with higher parasite burdens tend to have lower conception and weaning rates (K.
Wilson, unpublished analyses). These results suggest that parasites impact on both ewe
survival and fecundity. However, it is only by experimentally manipulating parasite load
that we can determine the true impact of parasitism on the fitness of ewes, because low
survival/fecundity and high parasitism might both be caused by a third factor, such as
poor body condition. In a previous experiment, animals that were chemically relieved
from their parasite burdens, using anthelmintic boluses, had higher over-winter survival
than control animals which had their parasite loads intact (Gulland et al., 1993).
However, the impact of parasitism on ewe reproductive success has not yet been
adequately addressed, and this is the ultimate aim of our study.

As part of an experiment to examine the effects of parasites on Soay sheep feeding
behaviour, 9 ewes and 11 rams were administered with an anthelmintic bolus during the
August Catch of 2001, when all of the experimental animals were 2 years old (see
preceding reports. Initially, we planned to examine the effects of parasites on ewe
reproductive success by comparing the lambing rates and suckling behaviours of the
bolussed ewes with a group of control ewes of similar age and weight in the spring of
2002. However, as a result of the high level of mortality that occurred over the winter of

2001/2002, sample size was reduced and the planned experiment was abandoned. Instead, we conducted a pilot study to determine the best experimental design for a future bolussing experiment comparing the suckling behaviours of bolussed and control ewes (this experiment is planned for August 2003, if the necessary permissions are granted).

We conducted behavioural watches on a small group of adult ewes, each of which had a single lamb and was classified as either heavy or light (based on their body weights in the previous summer). The behavioural studies comprised a series 90 minute-long watches of six mother-lamb dyads (3 in each weight category) over a six week period between 15th April and 25th May 2003. Each dyad was watched on average 5-6 times for the first three weeks of the lamb's life. Three main lamb behaviours we considered: time spent *sucking* (defined by direct contact with teat and thrusting action, often accompanied by tail-wagging); time spent *nuzzling* (no contact with teat, but some apparent "searching" behaviour under the ewe's belly and around the teats); and time spent *grazing* (where the lamb appears to nibble at the grass, although it is unclear whether they are eating vegetation). These behaviours are mutually exclusive and are part of a series of recorded behaviours that also included *rest, play, move* and *other* activities. We predicted that ewes in poorer body condition (i.e. lighter body weight or with higher parasite load) would be able to invest less in their lambs, in terms of milk provision, hence necessitating lambs attempting to suck for longer. We also predicted that lambs would seek to make up for their shortfall in nutrition by grazing at higher rates (though it is believed that lambs cannot digest grass until they are about eight weeks old; Reale et al., 1999)

The proportion of time the lambs were engaged in each of the three behaviours varied with age (Fig.11). The proportion of time lambs spent sucking tended to decrease during the first three weeks of life, from about 2½ minutes per hour during the first week, to approximately 1½ minutes per hour by the third week (generalised linear model on arc-sine transformed proportion of time: $F_{1,30} = 3.23$, $p = 0.08$). Similarly, nuzzling behaviour declined with age, from 45 seconds per hour in the first week to just 5 seconds per hour in the third week ($F_{1,30} = 17.33$, $p = 0.0002$). Grazing behaviour, on the other hand, increased from 11½ minutes per hour to 28 minutes over the same period of time ($F_{1,30} = 5.62$, $p = 0.024$). This is to be expected as the lamb becomes less reliant on milk, and begins to include other food sources in its diet. These patterns are in line with earlier observations of suckling behaviour on St. Kilda (Roberston et al., 1992).

Although our analysis detected significant temporal variation in lamb behaviour, we found no significant differences in the proportions of time spent sucking, nuzzling or grazing between the lambs of heavy and light ewes ($p > 0.1$). However, there was a trend for the lambs of light ewes to spend more time sucking than heavy lambs. If we assume that the magnitude of the difference in sucking behaviour between lambs born to heavy and light ewes is comparable to that of bolussed and non-bolussed ewes, then a power analysis suggests that in order to achieve 80% power we would need to compare the behaviours of approximately 22 bolussed and non-bolussed mother-lamb dyads.

As a pilot study, this work suggests that compromised ewes in poor condition may have to suckle lambs for longer. To investigate if a similar pattern occurs in ewes constrained by parasitism, we hope to conduct another anthelmintic bolussing experiment this summer. We predict that ewes relieved of their parasite burden before the winter, will be in a better condition and will be better able to invest in their lambs, plus recover more

quickly from lambing (gestation, parturition, and lactation), in terms of their body condition and the characteristic peri-parturient rise in faecal egg counts. As we plan to conduct this experiment in a non-crash year, when there are fewer constraints on breeding ewes, ewe survival will be higher and the effects of parasitism on reproductive costs may be more apparent.
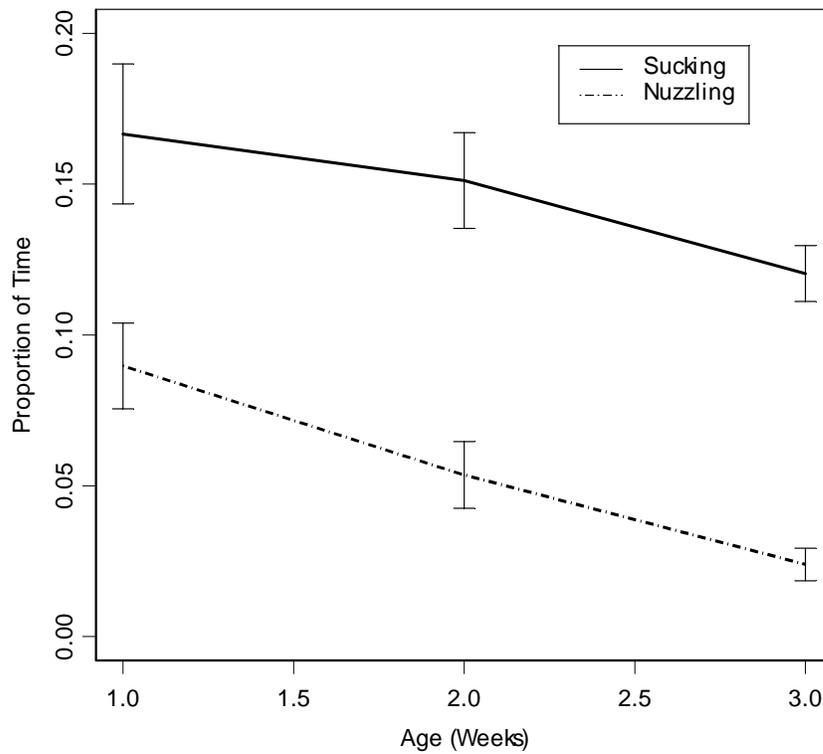

Figure 11. *The proportion of time the lambs of heavy and light ewes spent sucking and nuzzling (see text for definitions) during the three weeks after parturition. Times have been arc-sine transformed before analysing as a GLM in Splus.*

Soay sheep database.

Ian Stevenson.

All core data collected by the Soay Sheep Project are now incorporated into a relational database management system. This has been implemented in Access 2000, which is ideally suited for a non-networked, small-scale database that must be available to all project members without specialist software.

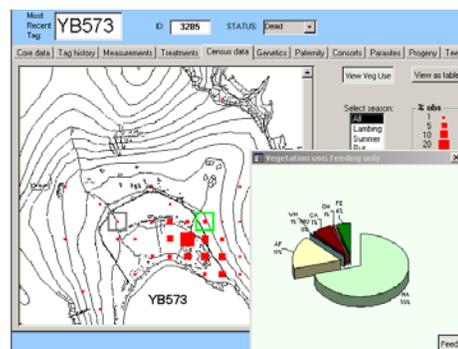There are two main advantages to this approach:
i)   errors in the data can be minimised; and
ii) there is huge flexibility in manipulating data so that information from very different sources can be combined.

Errors are minimised principally through rigorous checking and cross-referencing of data as they are entered from the keyboard or imported from loggers and field computers. Moreover, since any piece of information is only stored in a single location, there is no scope for multiple versions of the data to exist, each subtlety different and none guaranteed completely up-to-date (the 'Excel effect').
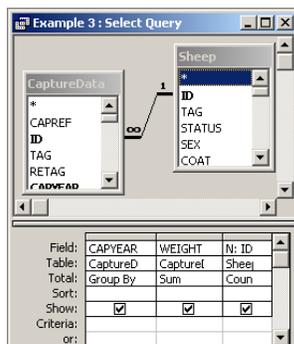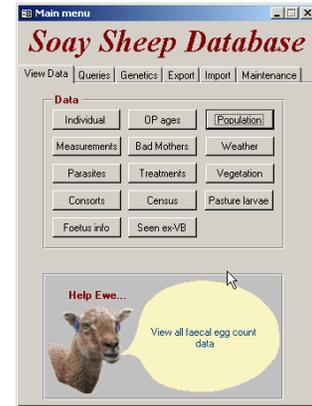
Having ensured accurate data, the next requirement is to generate datasets for analysis. For those without extensive experience of Structured Query Language, and who also wish to retain their will-to-live, Access provides a very useful graphical interface for linking data together. Time spent investigating this will be repaid handsomely, as most of the basic manipulation required to generate a dataset for analysis can be achieved in this way.

The database can also play a part in the analysis process. Graphing features can allow patterns in the data to be displayed and identified, and the database can integrate directly with GenStat or S-Plus. Both these packages can use the queries created and stored in the database as the source of the data for an analysis. This can be done either through the menu systems or with a single command line. In this way, the proliferation of dodgy datasets can be eliminated totally since all data remain in the database, without the requirement for any intermediate Excel files.

Learning to use Access as more than just a glorified spreadsheet takes time, but it bears repeating that time invested in this way will pay dividends in the long term.

20

Soay sheep: Population Dynamics and Selection on St. Kilda
Edited by T.H. Clutton-Brock and J.M. Pemberton

The edited book about the Soay sheep project is currently in production with Cambridge University Press and due to be published around September 2003. Outline details are as follows:

Chapter 1: **Individuals and Populations.**
T.H. Clutton-Brock and J.M. Pemberton

Chapter 2: **The sheep of St. Kilda.**
T.H. Clutton-Brock, J.M. Pemberton, T.N. Coulson, I.R. Stevenson and A.D.C. MacColl.

Chapter 3: **Population dynamics in Soay sheep.**
T.H. Cutton-Brock, B.T.Grenfell, T.N. Coulson, A.D.C. MacColl, A.W. Illius, M.C. Forchhammer, K. Wilson, J. Lindstrom, M.J. Crawley and S.D. Albon.

Chapter 4: **Vegetation and sheep population dynamics.**
M.J. Crawley, S.D. Albon, D. R. Bazely, J. Milner, J.G. Pilkington and A.L. Tuke.

Chapter 5: **Parasites and their impact.**
K. Wilson, B.T. Grenfell, J.G. Pilkington, H.E.G. Boyd and F.M.D. Gulland.

Chapter 6: **Mating patterns and male breeding success.**
J.M. Pemberton, D.W. Coltman, J.A. Smith and D.R. Bancroft.

Chapter 7: **Selection on phenotype.**
J.Milner, S.D. Albon, L.E.B. Kruuk and J.M. Pemberton.

Chapter 8: **Molecular genetic variation and selection on genotype.**
J.M. Pemberton, D.W. Coltman, D.R. Bancroft, J.A. Smith and S. Paterson.

Chapter 9: **Adaptive reproductive strategies.**
I.R. Stevenson, P. Marrow, B.T. Preston, J.M. Pemberton and K. Wilson.

Chapter 10: **The causes and consequences of instability.**
T.H. Clutton-Brock

PUBLICATIONS

Coltman, D.W., Pilkington, J.G., and Pemberton, J.M. Fine-scale genetic structure in a free-living ungulate population. Molecular Ecology 12: 733-742.

Coulson, T. & Hudson, E.J. (2003) When is the birth rate the key factor associated with population dynamics? P114-130 in Reproductive Science and Integrated Conservation eds. Holt, W., Pickjard, A., Rodger, J. and Wildt, D. Cambridge University Press, Cambridge.

Lindström J., Coulson,T., Kruuk, L., Forchhammer, M.C., Coltman, D.W. and Clutton-Brock, T.H. Sex ratio variation in Soay sheep. Behavioral Ecology and Sociobiology 53: 25-30.

Marshall, T.C., Coltman, D.W., Pemberton, J.M., Guinness, F.E., Spalton, J.A., Slate, J., Smith, J.A., Pilkington, J.G. and Clutton-Brock, T.H. Estimating the prevalence of inbreeding from pedigrees. Proceedings of the Royal Society of London, B. 269: 1533-1539.

Preston, B.T., Stevenson, I.R., Pemberton, J.M., Coltman, D.W. & Wilson, K. (2003) Overt and covert competition in a promiscuous mammal: the importance of weaponry and testes size to male reproductive success. Proceedings of the Royal Society, Series B. 270: 633-640.

ACKNOWLEDGEMENTS

APPENDIX A: PERSONNEL CHANGES & SCHEDULE OF WORK

Personnel Changes

In 2002 Barbara Wimmer joined the project on a 2 year Marie Curie fellowship on parasite diversity and Nathalie Charbonnel joined on a 1 year Lavoisier fellowship on population genetics. Dan Nussey started his NERC PhD studentship on maternal effects in red deer and Soay sheep.

Schedule of work on St Kilda

Winter - Spring

Jill Pilkington, Barbara Craig, Josephine Pemberton, Owen Jones, Louisa Tempest and one volunteer monitored mortality from February 1st until May 10th. During this period, detailed data were collected on individual sheep found dead, and samples were taken for genetic and parasitological study.

From mid March until May 10th, Jill Pilkington, and three volunteers carried out ten population censuses and tagged and sampled lambs for ongoing genetic studies. 171 lambs were born to 155 ewes; these figures include 16 sets of twins (5 ewes held both lambs, 5 lost one twin). 101 lambs (56 male and 45 female) were caught and tagged; a further 70 lambs died before any tagging attempt.

Louisa Tempest stayed on till the 28th of May to continue with suckling watches and Mick Crawley returned to the island from the 3rd till the 5th of July to complete a grass flowering survey.

Summer

Jill Pilkington, Louisa Tempest and one volunteer returned to Hirta on July 16th to carry out ten population censuses, conduct mortality searches (yielding 5 tagged and 1 untagged dead animals), and prepare for the main catch-up of study area sheep. The catch-up took place from August 10th - 22nd, was led by Josephine Pemberton, and conducted by a team of 11 other project members and volunteers. 237 sheep were caught and processed, of which 84 were lambs (47 males and 37 females), 10 were yearlings (1 male and 9 females), 18 were adult males, and 125 were adult females. All animals were weighed and measured to monitor growth, and sampled for parasite and genetic analyses. 16 sheep were retagged because of damaged or missing tags. 22 previously untagged sheep (17 lambs, 2 yearlings and 3 adults) were caught and processed. Jill Pilkington, Owen Jones and one volunteer remained on Hirta until 3rd September to complete parasite counts and vegetation monitoring.

Autumn

From October 18th to December 6th Jill Pilkington, Owen Jones, Louisa Tempest and three volunteers monitored the mating period, carrying out focal watches, capturing and processing 19 incoming tups and carrying out ten population censuses. Three dead sheep was found. Faecal samples were also collected for parasitic egg counts.

CIRCULATION LIST - *(Please advise J.Pilkington of any changes or additions)*

Prof. S. Albon	CEH, Hill of Brathens, Banchory, Kincardineshire, AB31 4BY.
Dr. W. Amos	Dept. Zoology, Cambridge Univ., Downing St., CB2 3EJ.
Dr. H. Armstrong	Forest Research, Northern Research Station, Roslin, Midlothian,EH25 9SY.
Dr. S. Bain	St Kilda, c/o QinetiQ, Balivanich, Benbecula, HS7 5LA.
Dr. D. Bancroft	GPC AG, Lochhamer Str. 29D-82152, Munich, Germany.
Mr. R. Balharry	Loch-na-Leoba, Old Glen Road, Newtonmore, Invernesshire.
Mr. A. Bennett	NTS, Balanain House, 40 Huntley St., Inverness, IV3 5HR.
Dr. P. Burman	NTS, 28 Charlotte Square, Edinburgh, EH2 4DU.
Dr. K. Byrne	ICAPB, Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Dr. N. Charbonnel	CBGP, Campus de Baillarguet, 34 988 Montferrier s/Lex cedex.
Prof. T. Clutton-Brock	Dept. Zoology, Cambridge Univ., Downing St., CB2 3EJ.
Mr. D. Clark	St. Kilda, c/o QinetiQ, Benbecula, HS7 5LA.
Dr. D. Coltman	Dept. Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
Dr. T. Coulson	Dept. Zoology, Cambridge Univ., Downing St., CB2 3EJ.
Miss B. Craig	ICAPB, Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Prof. M. Crawley	Dept. Biology, Imperial College, Silwood Park, Ascot, SL5 7PY.
Mr. R. Elliot	NTS, Balanain House, 40 Huntley St., Inverness, IV3 5HR.
Dr. J. Fenton	NTS, Balanain House, 40 Huntley St., Inverness, IV3 5HR.
Dr. M. Forchammer	Dept.of Landscape Ecology, NERI, Kalo, DK-8410 Ronde, Denmark.
Prof. I. Gordon	Macaulay Institute, Craigiebuckler, Aberdeen, AB15 8QH.
Dr. B. Grenfell	Dept. Zoology, Cambridge Univ., Downing St., CB2 3EJ.
Dr. F. Gulland	TMMC, Marin Headlands, Sausalito, CA 94965, USA.
Dr. J. Harden	NTS, Balanain House, 40 Huntley St., Inverness, IV3 5HR.
Dr. A. Hester	Macaulay Institute, Craigiebuckler, Aberdeen, AB15 8QH.
Prof. A. Illius	ICAPB, Edinburgh Univ., West Mains Rd, Edinburgh, EH9 3JT.
Mr. O. Jones	Dept. Biology, Imperial College, Silwood Park, Ascot, SL5 7PY.
Dr. L. Kruuk	ICAPB, Edinburgh Univ., West Mains Rd, Edinburgh, EH9 3JT.
Prof. I. Kyriazakis	SAC, Bush Estate, Penicuik, Midlothian, EH26 0PW.
Dr. G. Lincoln	MRC Centre for Rep. Biol., 37 Chalmers St., EH3 9EW.
Dr. J. Lindstrom	IBLS, Univ. of Glasgow, Glasgow, G12 8QQ.
Mr. J. Love	SNH, Stilligarry, South Uist, HS8 5RS.
Dr. R Luxmoore	NTS, 28 Charlotte Square, Edinburgh, EH2 4DU.
Dr. A. MacColl	Dept. of Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
Mr. A. Macdonald	Advisory Services, SNH, Anderson Place, Edinburgh, EH6 5NP.
Mr. D. MacLennan	SNH, 17 Frances St., Stornoway. Lewis, Outer Hebrides.
Mr A. Malo	Dept. De Ecologia Evolutiva, CSIC, 28006 Madrid, Spain.
Dr. T. Marshall	23A Oaklands Grove, London W12 0JD
Mr. A. McRae	ICAPB, Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Dr. J. Milner	SAC, Kirkton Farm, Crianlarich, Perthshire, FK20 8RU.
Prof. B. Morgan	Inst. Maths.& Stats., Univ. Kent., Canterbury, Kent, CT2 7NF.
Mr. S. Murray	c/o SNH, Stilligarry, South Uist, HS8 5RS.
Mr. D. Nussey	ICAPB, Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Dr. A. Overall	ICAPB, Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Dr. S. Paterson	IBS, Stirling Univ., Stirling, FK9 4LA.
Dr. J. Pemberton	ICAPB, Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Mrs J. Pilkington	Fell House, New Alston, Haydon Bridge, Hexham, NE47 6HR.
Dr. B. Preston	Leahurst Veterinary Field Station, Neston, South Wirral, CH64 7TE
Mr. A. Robinson	SNH, 17 Frances St., Stornoway. Lewis, Outer Hebrides.
Dr. J. Slate	Dept. of Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
Dr. J. Smith	Gowanlea, Fintry Rd, Kippen, FK8 3HR.
Dr. I. Stevenson	Sunadal Data Solutions, 8 West Annandale St, Edinburgh, EH7 4JU.
Dr. G. Tavecchia	Dept. Zoology, Cambridge Univ., Downing St., CB2 3EJ.
Miss L. Tempest	IBS, Stirling Univ., Stirling, FK9 4LA.
Dr. S. Thomas	ICAPB, Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Mr. R. Turner	NTS, 28 Charlotte Square, Edinburgh, EH2 4DU.
Dr. P. Visscher	ICAPB, Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Dr. J. Watson	SNH, Fraser Darling House, 9 Culduthel Road, IV2 4AG.
Dr. K. Wilson	IBS, Stirling Univ., Stirling, FK9 4LA.
Dr. B. Wimmer	ICAPB, Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.