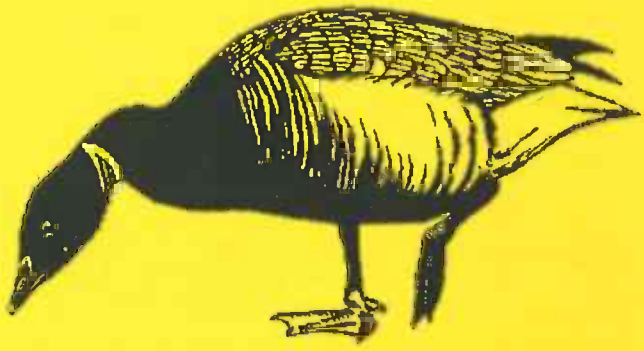
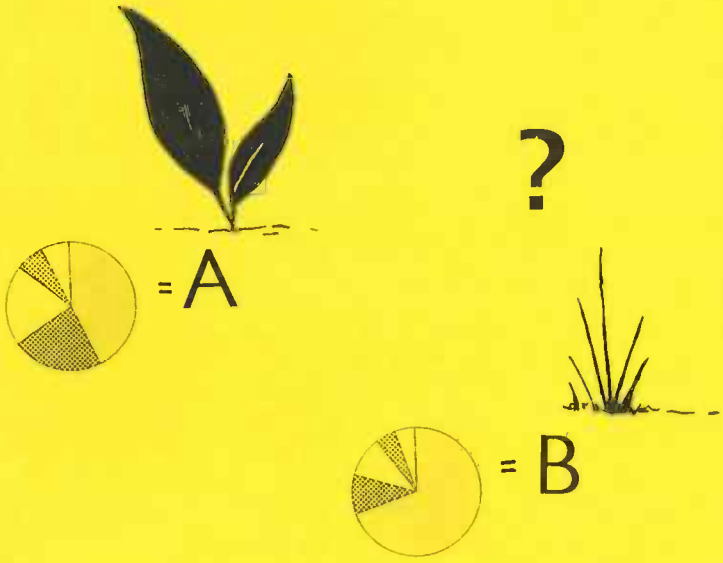
Food preference and food quality of Brent geese in a gradient of primary succession





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SUMMARY

According to classical exploitation theory, the increase in primary productivity found over the successional gradient of the salt marsh of Schiermonnikoog should result in an increased grazing pressure. However field data obtained from the salt marsh showed the highest Brent geese grazing pressure at salt marsh areas with low primary productivity. The grazing pressure was relatively low in the more productive parts later in succession. We hypothesised that this could be due to plant species replacement along the successional gradient, with, in the course of succession, abundance of by the geese less preferred species.

Therefore this study examined the relations between Brent geese and their food stock along the successional gradient with two major questions in mind. First, is there a correlation between observed grazing pressure distribution and abundance of by Brent geese preferred food plants? Second, can we explain Brent geese preference for plant species by means of a qualitative analysis of plant material?

In order to answer the first question we investigated the diet of Brent geese at three different successional stages and their preference for plant species at these areas.
We determined the availability of preferred and disfavoured plant species at the successional stages of different age and compared this with the observed grazing pressure distribution.

Our data show that grazing pressure and abundance of preferred species are correlated. The relative abundance of preferred species is highest in the youngest areas.
Brents are, in the course of succession, faced with vegetation composed of an increasing amount of disfavoured plant species. Diets however, changed only marginally, incorporating only 15% disfavoured species in the oldest successional stage.

An answer to the second question was sought by determining energy intake, soluble carbohydrates, crude protein, fibre, ash and *in vitro* digestibility of most plant species present at the salt marsh and comparing these with the observed preference.

Three clearly defined plant species clusters were found. One cluster was formed by the monocots, *Festuca rubra, Puccinellia maritima, Juncus gerardi* and *Elymus sp* which were all high in soluble carbohydrates and fibres. *Triglochin maritima,* in which the concentration protein was highest, formed a second cluster, and a third was composed of the dicots *Plantago maritima, Spergularia maritima* and *Aster tripolium,* highest in ash content.
The percentages of carbohydrates of neutral preferred species was significantly higher than the carbohydrate content of disfavoured species. There was also a tendency that preferred plants were more easy to digest.

We can conclude that the observed grazing pressure distribution correlates positively with the relative abundance of preferred forage. Data, however, indicate that it is difficult to link the observed food preference to one of the analysed food quality aspects. Future studies will have to provide more insight in the feeding strategies of Brent geese.

Contents

1 INTRODUCTION

Brent geese (*Branta bernicla bernicla*) are migratory herbivores. The salt marshes in the Wadden Sea area are their major feeding areas, both during fall and spring migration. During their spring staging period, Brent geese encounter favourable feeding conditions due to the onset of plant growth and increasing day length. Feeding and the search for food are then their predominant activities. Foraging around 14 hours a day -almost the entire daily light period- increases their total body weight by on average one third (Ebbinge *et al.*, 1975). This accumulation of large amounts of body reserves prior to migration to their Arctic breeding areas is crucial for reproductive success. Reserves are needed to bridge the time of egg laying, gosling care and moult in the Arctic, during which the geese have little possibility to feed. The condition of the birds at the moment of departure from the Wadden Sea area, correlates with their reproductive success (Ebbinge & Spaans, 1982). Geese that are heaviest after spring fattening, are most likely to return with young in the subsequent autumn (Ebbinge & Spaans, 1995).

Schiermonnikoog, one of the Wadden Sea islands, is one of the locations were this spring fattening takes place. On this island a gradient of primary succession can be found on the salt marsh since the island is gradually extending eastwards (Bakker, 1989; Olff, 1992). Most recently developed plant communities are found on the eastern part of the island. Going westwards, over a distance of about 6 kilometres, older communities are found up to plant communities of 200 years of age on the most western part of the salt marsh. The oldest parts of the salt marsh have always been cattle grazed, but the island has unexploited salt marsh parts ranging from a few years to about 50 years of age, on which this paper will focus on.

The present study, executed in spring 1995, fits in the framework of long standing plant and animal research at Schiermonnikoog carried out by the Rijksuniversiteit Groningen. During the late 70's most Brent geese could be counted at the nowadays 35-40 years old salt marsh area, in that time about 20 years of age. Some years later the prime foraging area for the geese was found at the present 25 years old salt marsh part and according to figure 1, the area with the highest grazing pressure moved to the east again. Apparently salt marsh ageing leads to less intensive use by Brent geese, since the geese are now occupying young salt marsh areas a few kilometres eastwards from the areas exploited some years ago (Bakker *et al.*, 1997; van der Wal, unpublished).

Previous studies at Schiermonnikoog have shown that above-ground living biomass increases with salt marsh age (van de Koppel *et al.*, 1996). Theoretically, above a certain level of available plant biomass, the herbivore population is expected to increase with primary production (Oksanen *et al.*, 1981, McNaugton *et al.*, 1989). At high levels of primary production, the herbivore density may level off due to predator control of herbivores (Oksanen *et al.*, 1981, Hairston *et al.*, 1960). Brent geese do no suffer from predation on the island. They are fully protected by the Dutch hunting legislation since 1950 and no natural enemies of the geese are present.

However, at Schiermonnikoog we observe highest grazing pressure at salt marshes early in successional stage, so at areas with low primary productivity. This in contrast with the low grazing pressure at the oldest study areas, where primary productivity is high. This doesn't seem compatible with the previous described "top-down" control predictions along a succession gradient. The question therefore is "why do we find the highest grazing pressure of Brent geese at salt marsh areas early in succession and low in productivity?"

On possible explanation might be food preference of Brent geese (Olff *et al.*, 1997). By vegetation succession the occurrence and dominance of plant species are altered. Brent geese may prefer certain plant species that do not occur or less frequently occur at the older salt marsh parts. Therefore we compared the diet of the geese flocks at three salt marsh parts with different stages of development. Previous studies investigated the diet of the Brent geese on the salt marsh area of intermediate age (Prop & Deerenberg, 1991), but comparative studies have not been performed.

Assuming that geese prefer plant species that appear with a greater frequency in their diet than in the environment, we examined whether or not the geese diet represented the vegetation composition at the three areas. In case geese were eating plant species in other proportions than present in the vegetation we aimed to find out if this selection for certain plant species was similar at the different successional stages. If so, we wanted to know at which successional stage of the salt marsh the preferred plant species were mainly present.

Because geese retain their food for only a short period in the alimentary tract, there is little time for digestive processes more complex than the absorption of cell contents to occur. To meet their nutritional requirements geese are supposed to harvest large quantities of relatively high quality forage every day. This has important consequences for their foraging strategy. The observed highest grazing intensity on areas with low biomass suggests that, based on the animal's perception of cost-benefit constraints, foraging at vegetation types early in succession is more profitable than in older areas. Trying to understand the underlying basis for plant selection, by analysing the nutritional value of food plants, was the second goal in this study.

There is no general agreement on which measurements define food quality best. High quality forage can be expressed by a high energy content. However, several studies indicate that simple considerations of energy maximisation can not account for food preference alone and show that nutrient constraints may have been important in food choice in these cases (Tinbergen, 1981). Others consider the absence of negative factors, like digestibility-reducing substances, a better indicator of high food quality (Bryant & Kuropat, 1980).

In this study we have chosen for a combination of previous mentioned food quality measurements. We compared qualitative aspects of twelve salt marsh species by calculating their profitability on basis of energy intake per bite. We determined protein, soluble carbohydrates, ash and fibre content, of which the latter two are both undesirable gutfilling components. To enable a palatability ranking of the plant species under investigation, we analysed *in vitro* digestibility. Measuring *in vitro* digestibility gave us also the opportunity to find out whether values obtained by the *in vitro* digestibility method, approach *vivo* digestibility values of Brent geese. Already the qualitative aspects of four salt marsh species on Schiermonnikoog were known from the work of Prop & Deerenberg (1991) were, but a comparison of more species had not yet been performed.

To get a good impression of the quality of the plant material and detect a possible change in the qualitative aspects of the food plants during the Brent geese staging period, we analysed plant material originating from March to June, but focused on the geese staging period from mid April till the end of May.

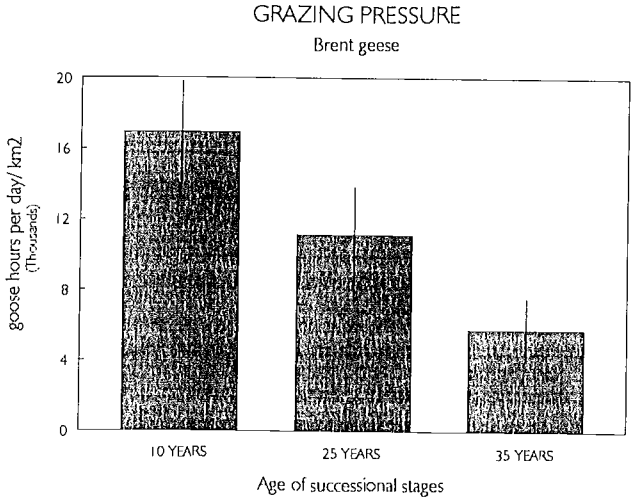


Figure 1 Brent geese grazing pressure at three successional stages

2 METHODS

2.1 Study area

This study was conducted in spring 1995 at Schiermonnikoog, one of the Frisian Wadden Sea islands in the Netherlands. The research was carried out at three locations along the successional gradient of the island: at a very young area of 10 years old, a 25 and a 35 years old salt marsh (Fig.2).

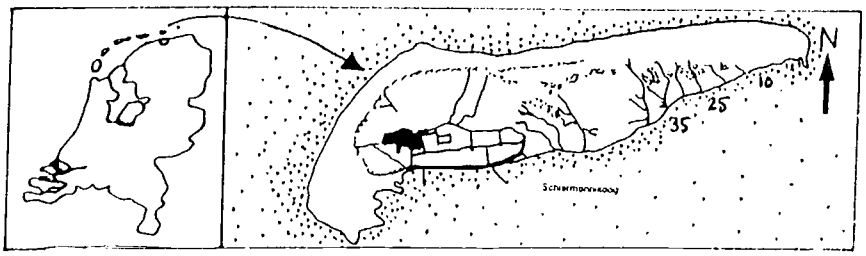


Fig. 2 The location of Schiermonnikoog in the Dutch Wadden Sea and the three study areas at the island: a 10, 25 and a 35 years old salt marsh.

Besides the succession gradient from east to west, also three major vegetational zones on elevation from south to north, can be distinguished. One zone, the higher marsh, is dominated mainly by *Festuca rubra* and inundated by sea water only during extreme high tides. A second zone is formed by the lower parts of the salt marsh covered with *Limonium vulgare* and *Puccinellia maritima.* The third is a transitional zone, where slight height diferences cause a mosaic pattern of small islands, separated by little gullies were many species can be found. These three vegetational zones are persistent along the successional gradient. The research presented here focuses on the transitional "island-zone" of the 10, 25 and 35 years old salt marsh parts.

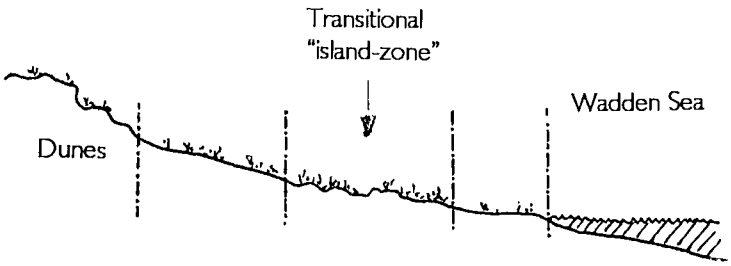


Fig. 3 Location of the transitional "island- zone" at the salt marsh of Schiermonnikoog

From the second week of April onwards, spring staging Brent geese grazed the salt marsh at the eastern part of the island. Approximately 3500 Brent geese spent this spring on the salt marshes of the island. By the 26th of May almost all Brent geese left the island for migration to their Arctic breeding grounds. During their spring staging period at Schiermonnikoog, foraging geese groups were frequently observed from dawn to dusk at the three successional stages. During these observations from towers the total number of geese, and their distribution over the study area was recorded every 30 minutes.

2.2 Statistical analysis

Overall differences among the three successional areas under investigation (Fig. 5,7,8) and data of the mean values of the chemical analysis with respect to neutral, negative and positive selected for plant species (Table 2) were analysed with Kruskal-Wallis tests and if necessary followed by Mann-Whitney tests. A Kruskal-Wallis test was also used to analyse the distribution of preferred and disfavoured plant species in KJ/bite and KJ/gram (Table 5,6). ANOVA with Tukey-contrasts was used to analyse for differences between percentages crude protein, soluble carbohydrates, NDF and *in vitro* digestibility of the salt marsh species under investigation (Fig. 10,11,12,13). Percentages were arc sine-transformed before testing.

2.3 Diet composition

Since geese have a relatively inefficient digestion, many of the cell wall structures of digested plants will stay intact. Specific characteristics of the epidermis like size, form and position of the cells and stomata, the structure of the edge of the leaf and the presence of hairs, if any, will therefore remain recognisable and enable us to describe the diet by examining droppings.

Since analysing diet is very time consuming, we were forced to restrict ourselves to material of one zone: the transitional "island-zone" of the 10, 25 and 35 years old salt marsh. Droppings used for the microscopical faecal analysis were collected (if possible) after each daily observation period during the season (Table 1). Observants sampled mixed samples of fresh droppings, taking care that only droppings originating from the transitional "island-zone" were collected. Geese retain their food for only a short period in the alimentary tract. Therefore droppings originating from the "island-zone" could be identified by using continuous recordings of the distribution of the geese from the observation tower and by allowing a throughput time of about 1½ hours. The droppings were oven-dried at 70° C for about 48 hours, before microscopical examination took place.

Table 1. Sampling days material diet analysis

Age of salt marsh	Date material diet analyses (May 1995)	N
10 years	01-07-09-16-20-23-25	7
25 years	10-11-18-19-20-24	6
35 years	08-11-15	3

Analysis of the diet based on the surface area of epidermal fragments rather than on the frequency of plant fragments is recommended, as it gives a better impression of the quantitative intake of the various plant species (Stewart, 1967). In this study the line intersect method was used which accounts for differences in fragmentation size between species (Seber & Pemberton, 1979). The line intersect method consists of measuring the lengths of all cuticles that intercept the line of the ocular-micrometer, irrespective of the shape and the orientation of the cuticles. Summation of the lengths of a particular species and dividing it by the total length of all species in the sample gives the proportion of that species in the diet. We did not adjust for differences in the ratio of mass versus leaf area.

From a homogenised sample of ten droppings, all of the same date and location, a randomly taken subsample was put on a microscopic slide. The material was as uniformly distributed as possible, to prevent particles to overlap each other. One hundred fragments, present on several examined vertical and horizontal lines, were identified on each microscopic slide. After about eighty identifications per sample, the proportion of a species stabilised (Fig. 4). A hundred identifications per sample were executed for analysis.

Drawings and photographs of most food species, as well as a reference collection with plant material of all plant species present in the study area, were available. Observants trained each other in recognising epidermal structures using object-glasses of the reference collection on which plant names were covered. An identification key was made to standardise the examination of the faecal samples (Appendix 2).

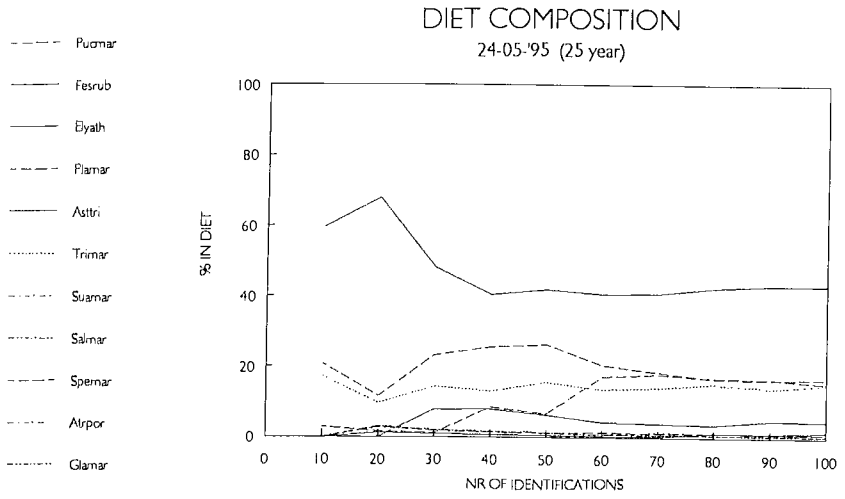


Fig. 4 The number of identifications per sample. At hundred determinations per sample the proportion of a species is stabilised.

In general a magnification of 100x was used to examine fragments. Particles with less than five cells were considered to have insufficient characteristics for determination and were not used for quantitative assessment of plant species in the diet. Besides this there was a non-identifiable epidermal structure which had characteristics of both *Festuca rubra* and *Puccinellia maritima*. Photographs of the so called *Festuca*[2] epidermal structure have been made in order to enable future determination (Appendix 5).

To quantify the amount of food available for geese at the three areas, plant species abundance was estimated, just after the geese had left the island. On each study area, twenty randomly selected patches of one 1 m^2, were recorded, estimating plant cover in percentages.

In this report we assumed that neutral feeding occurs when foods were found in the same proportion in droppings as they were encountered in the field. Preference is shown for species that appear in the diet with a greater frequency than in the environment. Species appearing less frequently in the diet than in the set of available foods are said to be disfavoured (Crawley, 1983).

An often used definition of "preference ratio" is the proportion of food in the diet divided by the proportion of the food in the habitat. The difficulty with this ratio lies in the accurate estimation of the availability of food items but also in a good estimate of the availability of all other potential food (Crawley, 1983). An example of this can be found in Appendix 8. This problem, mainly caused by working with percentages, occurs especially when non food-species are abundant, which was the case on the 35 years old salt marsh. Therefore we decided to distinguish three groups of species. If plants were above the line % in diet=% cover (See Fig. 6) and twice the standard deviation did not overlap this line a plant species was said to be positively selected for. The same arguments were used to label plants beneath the line as disfavoured species. The remaining plants were classified neutral.

2.4 Food quality

From early March until mid April plant material was collected every two weeks. During the Brent geese staging period at Schiermonnikoog, from mid April until the end of May, samples were collected every five days. A schedule with location and date of collection, can be found in Appendix 6. Nearly all material originated from the "island-zone" at the 25 years old salt marsh. Food plants samples were collected by hand, taking care to sample only those parts that would have been selected by the geese. The material collected was washed thoroughly and oven-dried at 70° C for 24 hours, grounded by a 1 mm sieve and stored in jars of glass.

Chemical qualitative analyses included *in vitro* digestibility, crude protein, soluble carbohydrates, ash and cell wall components. The twelve species being analysed were: *Aster tripolium- Atriplex portulacoides- Elymus sp- Enteromorpha sp- Festuca rubra - Juncus gerardi- Limonium vulgare- Plantago maritima- Puccinellia maritima-Spartina anglica- Spergularia maritima- Triglochin maritima.*

Potential digestibility of plant material was determined by an *in vitro* procedure (Tilley and Terry, 1963) where samples were incubated in rumen fluid from a fistelated cow for 6 hours. This incubation period of 6 instead of 48 hours appeared most appropriate to simulate the intention of degradation of plant cell walls by geese *in vivo* (Soldaat & Slager, 1985; Prop & Vullink, 1992). Dietary crude protein was determined by the Kjeldahl procedure (Kjeldahl-nitrogen times 6.25). For the procedures followed to determine *in vitro* digestibility, cell wall components (Neutral Detergent Fibre) and soluble carbohydrates is referred to Appendix 10 and 12. In Appendix 11 adjustments to the normal Tilly & Terry *in vitro* method are elucidated.

The bite sizes, necessary for the energy calculations per bite, of *Festuca rubra, Puccinellia maritima* and *Plantago maritima,* were taken from literature (Prop & Deerenberg, 1991). Bite sizes of *Elymus sp* and *Juncus gerardi* were assumed to be same as the bite size of *Festuca rubra.*

Field experiments and a feeding trial have been carried out, in order to define bite sizes of the other plant species compared at the qualitative analysis. In order to define the bite size of *Spergularia maritima*, captive Barnacle geese in an exclosure at the field station on Schermonnikoog were allowed to graze on salt marsh sods with inconspicuously marked plants. The bill of Barnacle and Brent geese are of a similar size. A few marked *Spergularia* plants at the youngest study area were, supplementary to the feeding trail, examined to record the length of each leaf, before and after the visit of a Brent geese flock. Field experiments on *Triglochin maritima* at the same study areas carried out by M. Egas (1995), revealed the bite sizes of Brent geese on this species. *Limonium vulgare, Aster tripolium* and *Atriplex portulacoides* at the salt marsh showed grazing marks by which the bite size could be reconstructed, using the outline of the leave. In order to estimate *Enteromorpha* bite size, a Brent goose scull was used to take bites of the same size as the Brent geese did, after some practice on *Limonium vulgare* and *Aster tripolium* leaves.

3 RESULTS

Diet

3.1.1 Diet composition

The main components of the Brent geese diet were *Puccinellia maritima, Festuca rubra, Triglochin maritima, Atriplex portulacoides* and *Festuca*[2]. Diet composition of geese differed among salt marshes of increasing age (Fig.5). Significant differences between the three marshes were found for the percentage *of Puccinellia maritima, Festuca rubra, Triglochin maritima, Atriplex portulacoides* and *Artemisia maritima* (Kruskal-Wallis, Mann-Whitney, $p<0.05$, $p<0.01$). In every sample on average 5.5% (SE=0.61) of the total measured length of encountered particles could not be recognised.

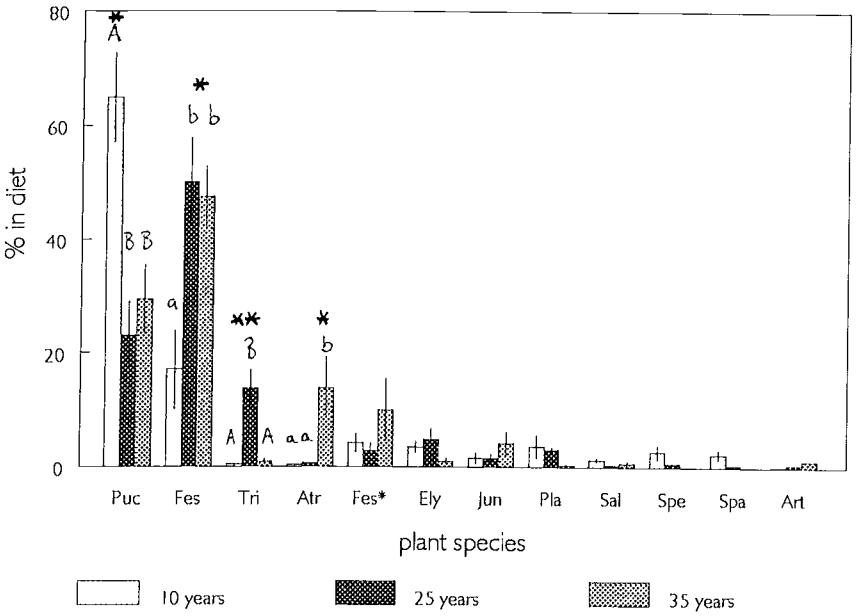
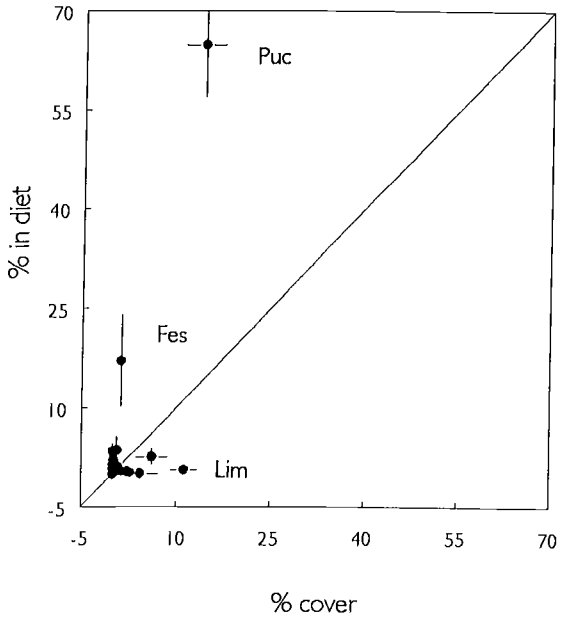


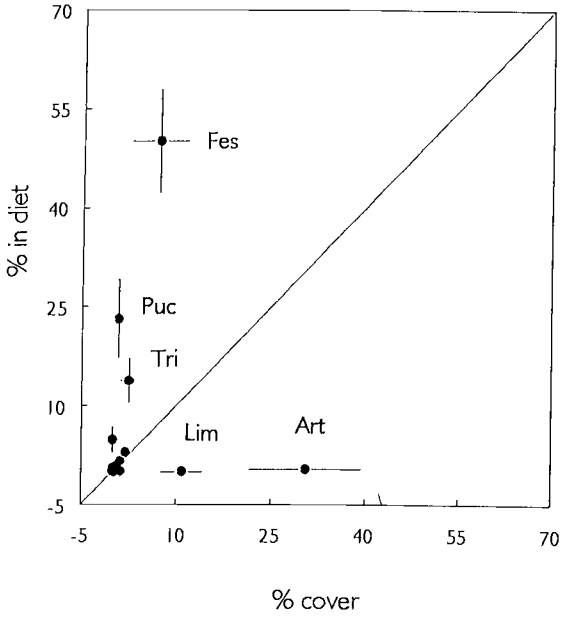
Fig. 5 Comparison of the diet composition of Brent geese for the 10, 25 and 35 years old salt marsh areas. Bars are mean ± S.E. Levels of significance are indicated with: (p<0.05) and ** (p<0.01). For the used abbreviations of plant names see Appendix 7.*

In the following three graphs the percentage of plant species found in the diet is plotted against the percentage cover. A plant species on the diagonal line (y=x) would indicated that this plant is eaten as much as it is encountered by the geese. Preference is shown for plant species above and disfavourance for those below the line, when twice the standard deviation does not intersect the diagonal line (indicated by plant name abbreviation in graphs).

10 years



25 years



35 years

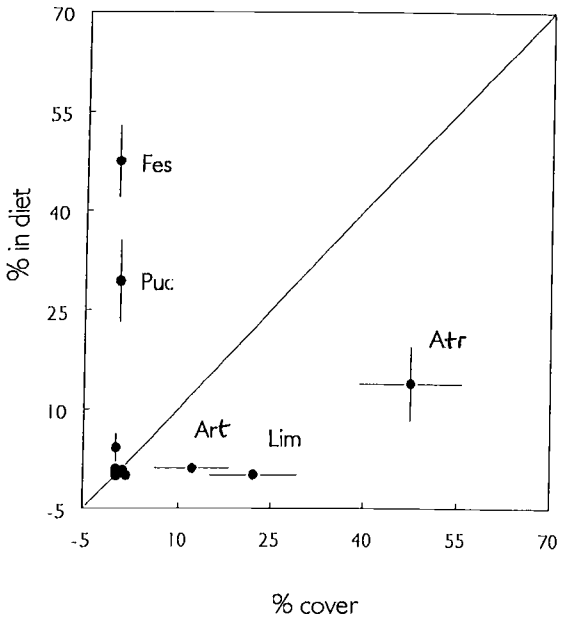


Fig. 6 Brent geese diet for the 10, 25 and 35 years old salt marsh in relation to the estimated plant cover. The line y=x is shown as a guide for preference determination. Vertical and horizontal bars show standard errors. See appendix 7 for the used abbreviations of plant names.

In each of the three study areas *Puccinellia maritima* and *Festuca rubra* were found in high percentages in the diet despite the fact that they covered only a small percentage of the area. *Limonium vulgare,* on the contrary, was less common in the diet than expected on basis of its abundance in the field. At the 10 years old salt marsh there was virtually no *Triglochin maritima* available, but at the 25 years old salt marsh were this plant species is more abundant, it was preferred by Brent geese. *Atriplex portulacoides* and *Artemisia maritima,* increasing in abundance at the 25 and 35 year old successional area, were mainly excluded from the geese diet.

On basis of these data, three groups of plant species were distinguished and grouped according to preference by the geese.

positive	neutral	negative
Festuca rubra	*Armeria maritima*	*Artemisia maritima*
Puccinellia maritima	*Aster tripolium*	*Atriplex portulacoides*
Triglochin maritima	*Elymus sp*	*Limonium vulgare*
	Enteromorpha	
	Glaux maritima	
	Juncus gerardi	
	Plantago maritima	
	Salicornia sp	
	Spergularia maritima	
	Spartina anglica	
	Suaeda maritima	

Total abundance in percentage of the three previously labelled "positive, neutral and negative" geese plant clusters, plotted for the 10, 25 and 35 years old salt marsh is shown in figure 7. By comparing the three areas one can see that the relative abundance of preferred plant species is the highest in the youngest areas and significantly lower at the 35 years old salt marsh. In the oldest area, the vegetation is virtually entirely composed of species disfavoured by the geese. With increasing age of the salt marsh, the cover of plant species disfavoured by the Brent geese is changing significantly from an average cover of 16% to 82% (Kruskal-Wallis, Mann-Whitney, $p<0.01$).

Since no dead material or bare soil are presented in the graph it is obvious that the percentage of total plant cover is increasing with increasing age of the salt marsh. At the youngest successional stage less than 50% of the area is covered by plants. At the 25 and 35 years old salt marsh this amounts to 59% and 86%, respectively.

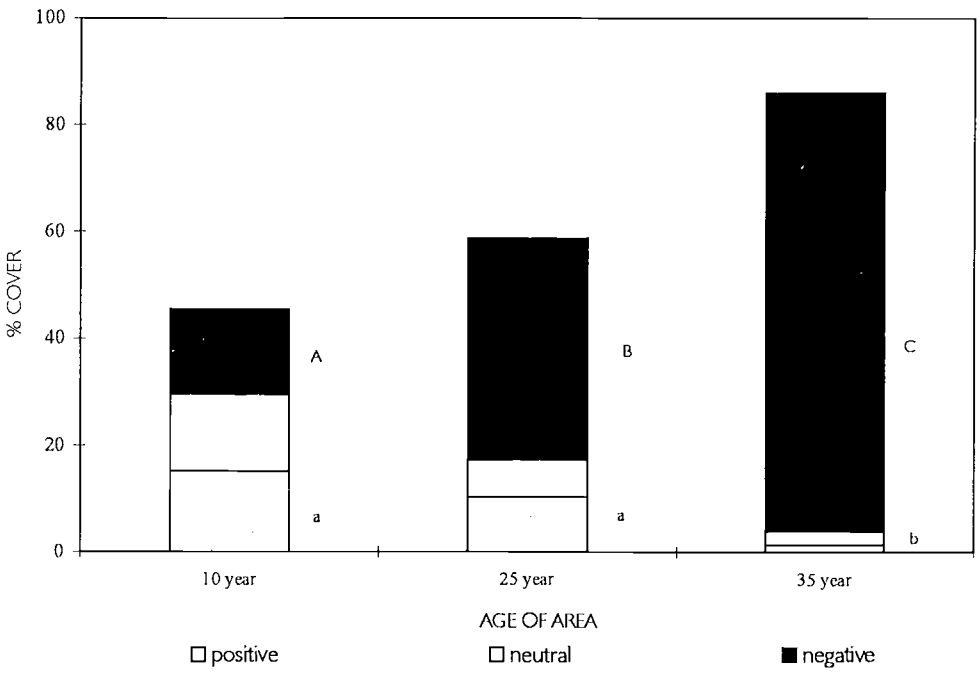


Fig. 7 Cover of plant species that Brent geese selected for positively, neutrally or negatively at the 10, 25 and 35 years old salt marsh. Bars with different letters differ with among areas ($p<0.01$).

The percentage of preferred plant species in the geese diet did not differ significantly among the three salt marsh areas compared. A large proportion of the diet of geese foraging at the 35 years old salt marsh area consist of preferred plant species despite the low cover estimates of preferred plant species recorded in the field. The proportion of disfavoured plants in the diet was nonetheless the highest in the oldest area (Kruskal-Wallis, Mann-Whitney, $p<0.05$).

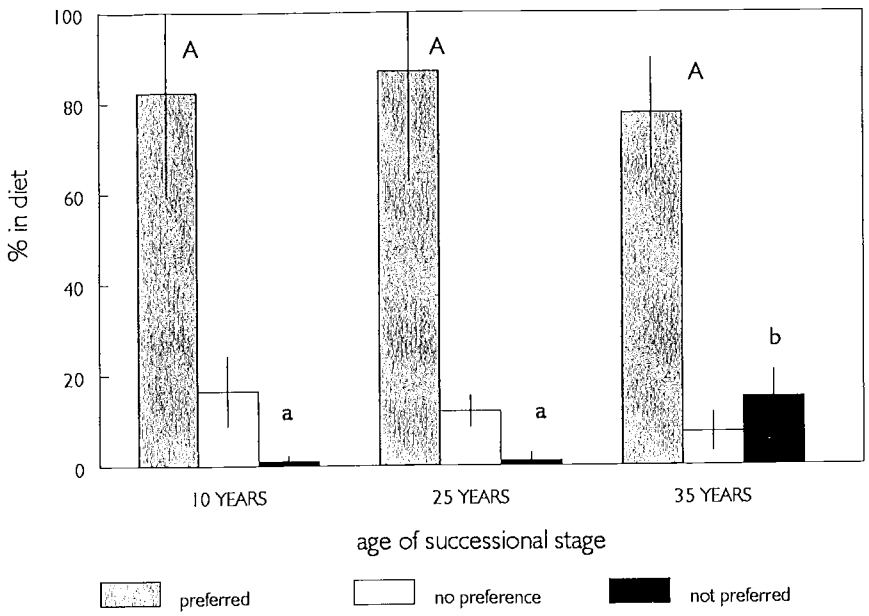


Fig. 8 Plant species, separated in three groups, found in the diet of Brent geese at the 10, 25 and 35 years old salt marsh. Bars with different letters differ among areas ($p<0.05$).

3.1.2 Discussion

We can conclude that over the course of succession, Brent geese are faced with vegetation composed of an increasing amount of disfavoured plant species. Diets however, changed only marginally, incorporating only 15 % disfavoured species in the oldest successional stage. It seems logical that maintenance of a diet composed of favourite food plants in areas with a low availability of these favourites is costly, but one could also argue that considering the low grazing pressure in these areas, geese are less vulnerable to depletion of their preferred forage and spent less energy in interactions with other geese. The interplay between disfavoured food on offer and possibly less competition for food, remains to be quantified. The number of Brent in the oldest area however, is significantly lower, indicating low preference for later successional marsh stages.

The ranking of plant species according to preference, with *Puccinellia maritima, Festuca rubra* and *Triglochin maritima* being preferred and *Limonium vulgare, Atriplex portulacoides* and *Artemisia maritima* being disfavoured forage is also reported from other studies. Brent geese foraging at the Norfolk coast during spring, selected for *Puccinellia maritima* and *Triglochin maritima*, together with *Aster tripolium* and avoided *Limonium vulgare* (Summers, 1993). *Puccinellia* was found to be the staple food of Brent in Terschellinger marshes (Ebbinge, 1980) and Danish marshes (Madsen, 1989). In the diet composition of Brent at Schiermonnikoog established by Prop en Deerenberg (1991) *Puccinellia maritima* was the principal food species together with *Festuca rubra, Triglochin* and *Plantago maritima.* The latter two were less commonly consumed.

From field experiences and literature data (Prop & Deerenberg, 1991; Summers, 1993) *Aster tripolium, Plantago maritima* and *Spergularia maritima* were also expected to belong to the "positively selected" group, but there was not enough evidence to conclude this from our own results. Probably the percentage of their presence in the diet was too low to classify them in one of our three classes. We consider it unlikely that this is due to differential digestion of plant species, considering the outcome of the experiment of Summers (1993). He gave, in order to test for differential digestion, a mixture of *Triglochin maritima* and *Lolium perenne,* to a captive Brent. The same percentage of remains in the droppings as the percentages fed to the goose were found after adjusting for differences in the ratio of mass versus leaf area.

In general, true feeding preferences can only be determined under the strictest controlled experimental conditions, when all differences in availability between the different foods are eliminated. These so called "cafeteria trails" offer nonetheless various interpretation problems. The comparative examination of geese diet and food preference at three different successional stages of salt marsh development is hard to study in an experimental set-up. We therefore studied free ranging geese. Consequently the interpretations of the results are hampered by the inextricably intertwined effects of availability and preference.

As a measure of food availability we quantified the percentage cover per plant species immediately after the departure of the geese. The results might be improved by the estimation of the biomass of available food items instead of percentage cover of the species. Measuring biomass at various times intervals during the spring staging period and not afterwards, would be more accurate too. Both improvements involve several practical difficulties which were not possible to overcome in the time budget of this research.

The diet analysis performed to determine the food preference of geese flocks imposed two restrictions. The geese observations at the 35 years old salt marsh were hampered by the dense vegetation present. This resulted in low sample size for the dropping analyses at this area since little material was available. Secondly, by a very time consuming diet-analyses, we were forced to choose between the replication of the analysis with material of few data points or analyse material originating from several time intervals during the geese staging period without replication. The latter was considered to be best, since more variation in diet composition was found between succession stadia then within material of different dates originating from one of the successional areas.

Notwithstanding the items discussed above, this study has demonstrated that grazing pressure and the abundance of preferred plant species are correlated. The relative abundance of preferred plant species is highest in the youngest areas. In the oldest area, the vegetation is virtually only composed of species that hardly occurred in the diet of the geese.

Food quality

3.2.1 Qualitative analysis

Ash

Remarkable variation was found analysing ash content of various salt marsh species (Fig. 9). This was an important reason to present the results of the qualitative analysis as percentage of dry weight and not as percentages of ash free dry weight (see discussion).

In general, most dicotyledonous species contained a high percentage of ash whereas monocots did not. In some plant species like *Plantago maritima* almost a quarter, or in *Spergularia maritima* and *Atriplex portulaciodes* even a greater percentage, consisted of ash only. The high percentage of ash in algae could be due to the fact that cleaning them from sand particles was very difficult, but that will hold for the geese as well.

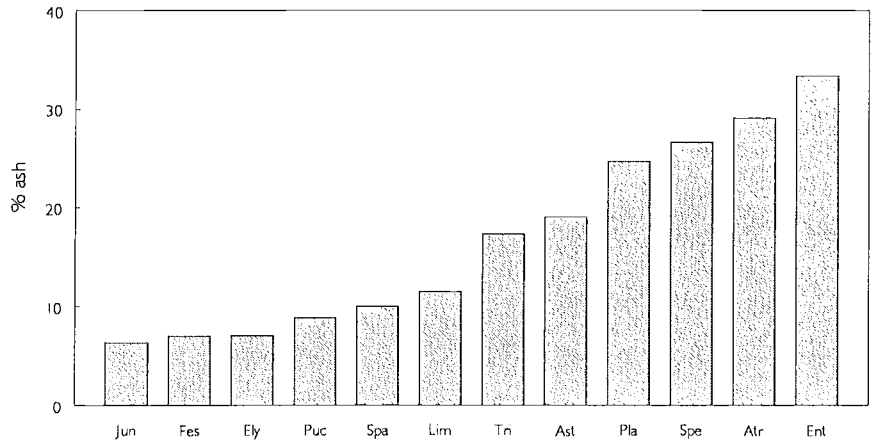


Fig. 9 Ash content in % of the dry matter. All were based on duplo samples of 19/06/95. Species ranked according to increase in ash percentage.

Soluble carbohydrates

Large differences in soluble carbohydrate content were found (Fig 10). The plants with the significantly highest content in their leaf tips were grasses and grass-like species (Tukey, $F=15.73$, $p<0.001$). Low in soluble carbohydrate content were dicotyledonous species. The *Enteromorpha* soluble carbohydrate content lies in between those two groups.

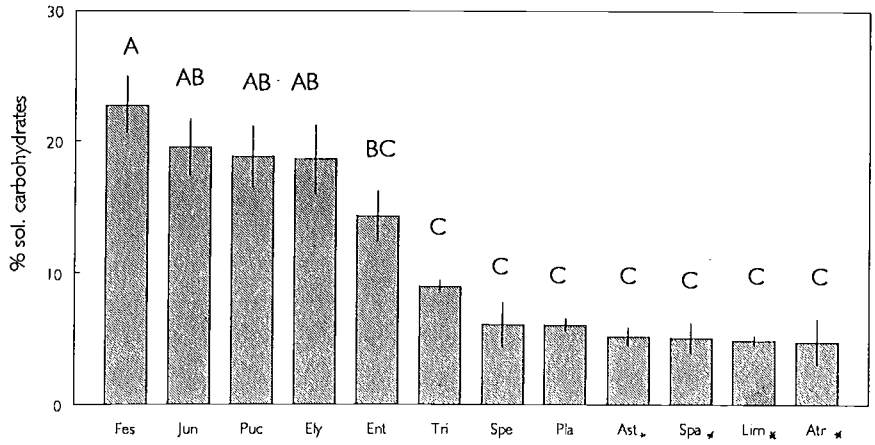


Fig. 10 Soluble carbohydrate content in % of the dry matter. Means (± SE) were based on 5 replicates except those indicated with an asterisk which had only 3 or 4. Significant differences are indicated with different capitals (p<0.001).*

Cell wall components

Spartina anglica together with the other grasses and grass-like species were significantly higher in fibre content than the dicots and *Enteromorpha* (Tukey, F=33.38, p<0.0001). The lowest percentage of cell wall components, 13 %, was found for *Triglochin maritima,* whereas *Spartina anglica* consisted for 63 % of cell wall components on dry weight basis (Fig. 11).

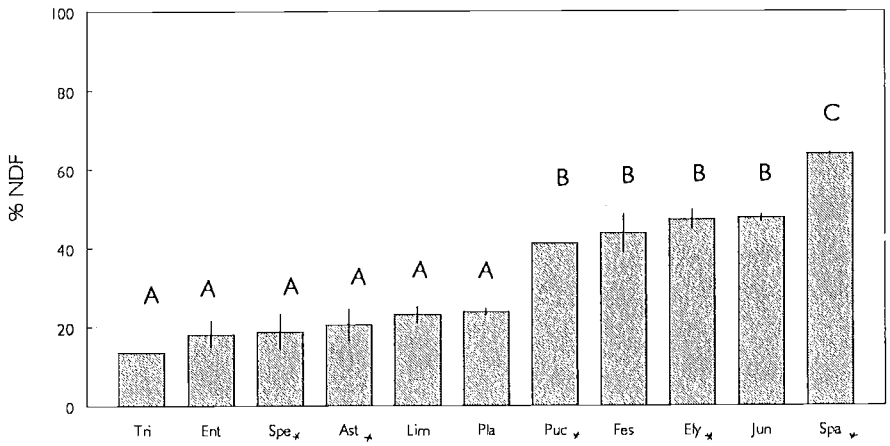


*Fig. 11 Cell wall components (NDF) in % of the dry matter. Means were based on 3 replicates except those with an asteriks * which had 1 or 2. Vertical bars show mean with S.E. Significant differences are indicated with capitals (p<0.0001).*

Crude protein

Protein concentrations on dry matter basis differed considerably, with the highest concentration being 3 times higher than the lowest concentration (Fig. 12). *Triglochin maritima* had the highest protein content of all salt marsh species under investigation and Enteromorpha the lowest (Tukey, F=70.44, p<0.0001)

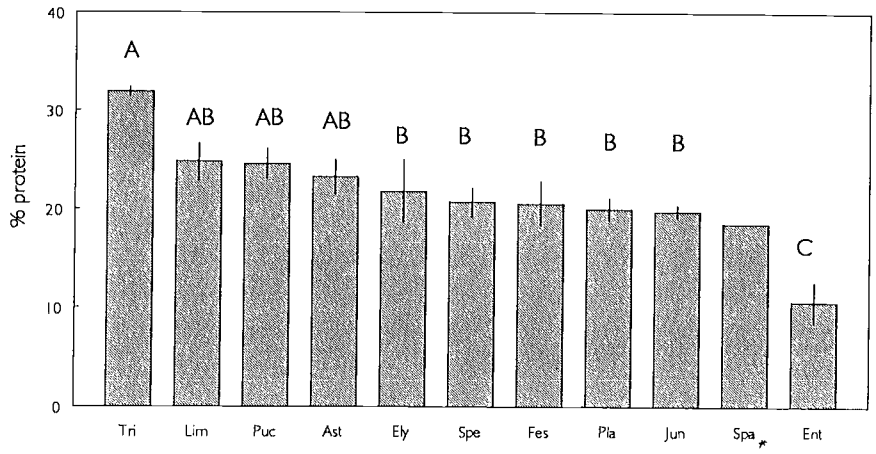


Fig. 12 Protein content in % of the dry matter. Means (± SE) were based on 5 replicates except those indicated with an asteriks which had only 3-1. Significant difference are indicated with capitals (p<0.0001)*

In vitro digestibility

There are no large differences in the *in vitro* digestibility percentages between the plant species under investigation. Of *Juncus gerardi, Spartina anglica* and *Limonium vulgare* no more than 43, 39 and 35% respectively, was digested (Tukey, F=18.95, p<0.001).

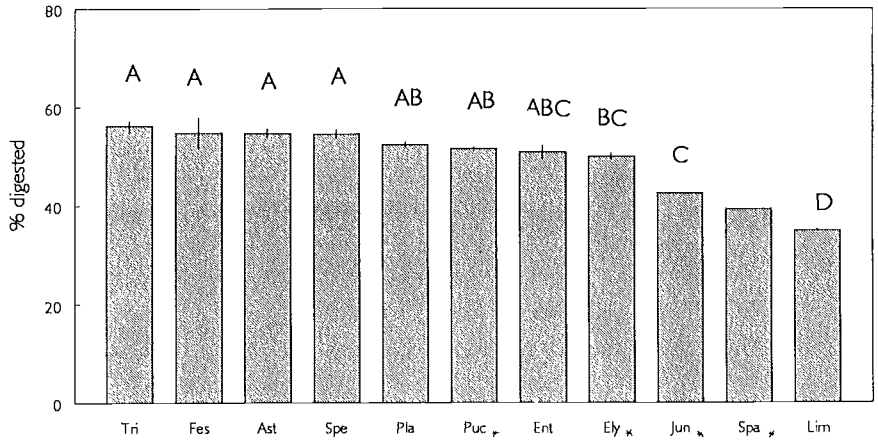


*Fig. 13 In vitro digestibility in % of the dry matter. Means were based on 3 replicates, except those indicated with an asteriks * which had 1-2. Significant difference are indicated with capitals. (p<0.001)*

Combined features

In figure 14 the previously presented date are summarised. Each "kite" represents one of the compared food species of the Brent geese. The percentage fibre, crude protein, ash and soluble carbohydrates are plotted on the top, right, bottom and left side respectively. Four clusters can be distinguished. At first the grasses (except *Spartina*) and grass-like species have a characteristic form recognisable at a high percentage fibre and about equal percentages protein and soluble carbohydrates. The only plant species with more than 30% protein is *Triglochin maritima,* being group number two. The third corresponding features are those of *Plantago maritima, Spergularia maritima* and *Aster tripolium* with a higher percentage ash and a lower percentage fibre than the first group mentioned and, besides this, a higher level of protein than soluble carbohydrates. *Limonium vulgare* has a lower ash percentage and is included in rest group number four, together with *Spartina anglica, Enteromopha* and *Atriplex portulacoides.* Unfortunately the NDF content of the latter was not measured.

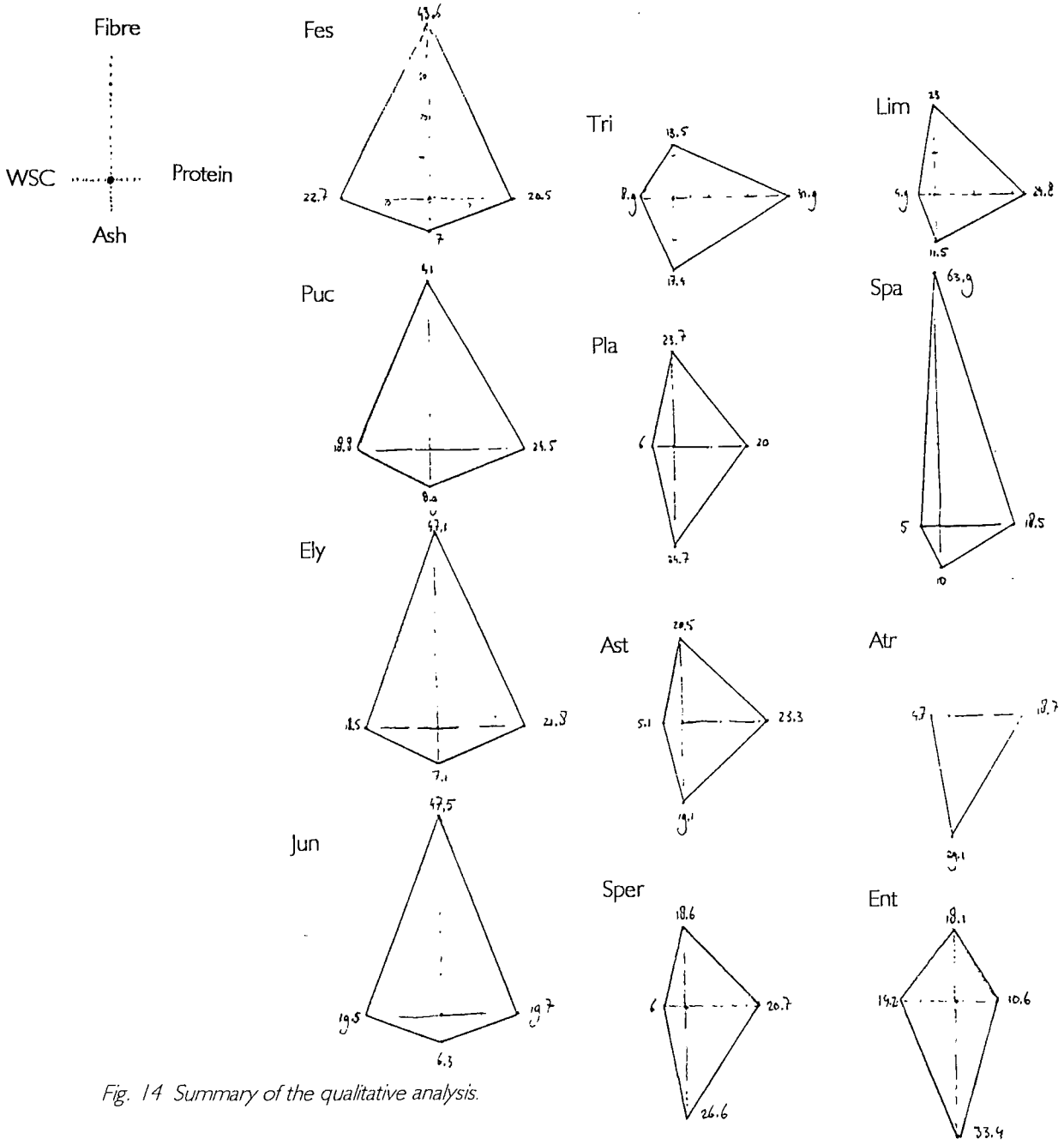


Fig. 14 Summary of the qualitative analysis.

The plant material analysed here originated from the geese staging period (April to May). A summary of the qualitative analyses per plant species from March to June can be found in Appendix 9.

Comparison with preference

Analysis of the mean chemical values of neutral, positively and negatively selected plant species showed a significant difference between the water soluble carbohydrate content of the neutral and negatively selected plant species (Table 2). An indication of difference was observed between the *in vitro* digestibility values of the neutral and preferred plant species.

*Table 2 Mean values chemical analysis of "negatively, neutral of positively selected for" plant species tested. For the plant species division into three groups see pag 10. The 'negatively selected for' group here, exist of Limonium vulgare and Atriplex portulacoides (2) or Limonium vulgare(1) alone if other data were not available.. Symbol * indicates a significant difference (p< 0.05).*

Plant species selection by Brent	Ash	(n)	NDF	(n)	WSC	(n)	Protein	(n)	*In vitro* digestibility	
Negative	20.3 %	(2)	23.0 %	(1)	4.79 %	(2)	3.47 %	(2)	34.9 %	(1)
Neutral	18.1 %	(7)	34.2 %	(7)	10.6 %	(7)	3.07 %	(7)	48.8 %	(7)
Positive	11.0 %	(3)	32.7 %	(3)	16.8 %	(3)	4.10 %	(3)	54.6 %	(3)
p value	0.41		0.85		0.04		0.24		0.06	

There was no correlation between the water soluble carbohydrates content and the *in vitro* digestibility.

3.2.2 Energy

Besides quality aspects, the amount of material gained per foraging action is an important part of the 'benefits' animals can obtain. In table 3 the average mass per length of eleven plant leaf tips are summarised, ranked from high to low. (See Appendix 18 for data from March to June)

Table 3. Average dry weight per length of the different plant species in May. In 10^{-1} mg / mm, except for those with a shaded background: 10^{-1}*mg / mm²*

Plant	Spa	Pla	Spe	Tri	Ely	Jun	Fes	Puc	Lim	Atr	Ast
Mean	2.29	1.85	1.73	1.14	0.83	0.72	0.40	0.40	1.17	0.84	0.60
SE	0.19	0.07	0.50	0.03	0.02	0.03	0.01	0.03	0.3	0.02	0.05

In the next table the average bite weight of the Brent geese per plant species for the period of May is summarised.

Table 4 Average bite weight for the period of May in mg/bite.

species	Lim	Ent	Atr	Pla	Ast	Spa	Spe	Tri	Ely	Jun	Fes	Puc
b.weight	10.4	7.51	3.38	3.22	2.33	2.06	1.80	1.60	1.16	1.01	0.58	0.36
source	1	1	1	3	1	1	1	2	1	1	3	3

Source mm/bite 1. This study 2. Egas, 1995 3. Prop & Deerenberg, 1991

When combining the information of the qualitative analysis with the previous data one can calculate the energy gain per bite after digestion. See Appendix 13 for information of the used method.

Table 5 Energy gain per bite

Plant species	Kjoule /bite	preference
Limonium vulgare	49.78	disfavoured
Enteromorpha	31.25	
Plantago maritima	14.25	
Spartina anglica	11.66	
Atriplex portulacoides	10.87	disfavoured
Aster tripolium	10.61	
Triglochin maritima	9.12	preferred
Elymus sp	8.88	
Spergularia maritima	7.74	
Juncus gerardi	7.01	
Festuca rubra	4.15	preferred
Puccinellia maritima	2.57	preferred

There was no significant difference between the spreading of preferred and disfavoured plant species.

In table 6 the percentages fibre, sugars, protein, fat and ash of each food plant are listed. The contribution of each cell component, based on 1 gram plant material, is given. Of each component the digestion by Brent and the energy gain through this digestion is calculated. Of each food species the total energy gain by digesting 1 gram plant material is marked in bold. In the last column the contributions of cell wall, soluble carbohydrates, crude protein and fat to the total energy digested by the geese from their plant food, can be found. See Appendix 14 for more information about the calculation method followed.

The plant species are ranked from high to low energy content per gram plant material, with at the start *Festuca rubra, Puccinellia maritima* and *Triglochin maritima*, plant species preferred by the Brent geese. Yet no significant difference between the place of preferred and disfavoured plant species was found.

Remarkable is that the cell components, fibre, sugars, protein, fat and ash, of the grasses and glasslike species sum up to approximately 100%, while for the dicotyledons and other plant species on the contrary, a component seems underestimated or missing.

Table 6 Contributions of cell wall, sol. carbohydrates, protein and fat to the total energy digested by the geese from ther plant food.

Species		Perc	gram	Digested (gram)	KJoule	Perc
Festuca	NDF	43,6	0,44	0,13	1,67	23,03
	WSC	22,7	0,23	0,16	2,74	37,76
	Protein	20,5	0,21	0,13	2,28	31,46
	Lipid	5	0,05	0,01	0,56	7,75
	Ash	7	0,07			
	Total	98,8	0,99		7,25	100
Pucccinella	NDF	41	0,41	0,12	1,57	22,03
	WSC	18,8	0,19	0,13	2,27	31,82
	Protein	24,5	0,25	0,15	2,73	38,26
	Lipid	5	0,05	0,01	0,56	7,89
	Ash	8,9	0,09			
	Total	98,2	0,98		7,12	100
Elymus	NDF	47,1	0,47	0,14	1,80	25,68
	WSC	18,5	0,19	0,13	2,23	31,77
	Protein	21,8	0,22	0,14	2,43	34,54
	Lipid	5	0,05	0,01	0,56	8,00
	Ash	7,1	0,07			
	Total	99,5	1,00		7,02	100
Junger	NDF	47,5	0,48	0,14	1,82	26,27
	WSC	19,5	0,20	0,13	2,35	33,96
	Protein	19,7	0,20	0,12	2,19	31,66
	Lipid	5	0,05	0,01	0,56	8,12
	Ash	6,3	0,06			
	Total	98	0,98		6,92	100
Triglochin	NDF	13,5	0,14	0,04	0,52	9,07
	WSC	8,9	0,09	0,06	1,07	18,82
	Protein	31,9	0,32	0,20	3,55	62,25
	Lipid	5	0,05	0,01	0,56	9,86
	Ash	17,4	0,17			
	Total	76,7	0,77		5,70	100
Spartina	NDF	63,9	0,64	0,19	2,45	43,15
	WSC	5	0,05	0,03	0,60	10,63
	Protein	18,5	0,19	0,12	2,06	36,30
	Lipid	5	0,05	0,01	0,56	9,91
	Ash	10	0,1			
	Total	102,4	1,02		5,67	100
Limonium	NDF	23	0,23	0,07	0,88	18,37
	WSC	4,9	0,05	0,03	0,59	12,33
	Protein	24,8	0,25	0,16	2,76	57,57
	Lipid	5	0,05	0,01	0,56	11,73
	Ash	11,5	0,12			
	Total	69,2	0,69		4,79	100

Aster	NDF	20,5	0,21	0,06	0,78	17,23
	WSC	5,1	0,05	0,03	0,61	13,50
	Protein	23,3	0,23	0,15	2,59	56,92
	Lipid	5	0,05	0,01	0,56	12,34
	Ash	19,1	0,19			
	Total	73	0,73		4,55	100
Plantago	NDF	23,7	0,24	0,07	0,91	20,54
	WSC	6	0,06	0,04	0,72	16,37
	Protein	20	0,20	0,13	2,23	50,37
	Lipid	5	0,05	0,01	0,56	12,72
	Ash	24,7	0,25			
	Total	79,4	0,79		4,42	100
Spergularia	NDF	18,6	0,19	0,05	0,71	16,56
	WSC	6	0,06	0,04	0,72	16,82
	Protein	20,7	0,21	0,13	2,30	53,55
	Lipid	5	0,05	0,01	0,56	13,07
	Ash	26,6	0,27			
	Total	76,9	0,77		4,30	100
Atriplex	NDF					
	WSC	4,7	0,05	0,03	0,57	
	Protein	18,7	0,19	0,12	2,08	
	Lipid	5	0,05	0,01	0,56	
	Ash	29,1	0,29			
	Total	57,5	0,58			
Enteromor	NDF	18,1	0,18	0,05	0,69	16,71
	WSC	14,2	0,14	0,10	1,71	41,29
	Protein	10,6	0,11	0,07	1,18	28,44
	Lipid	5	0,05	0,01	0,56	13,55
	Ash	33,4	0,33			
	Total	81,3	0,81		4,15	100

- no lipid analysis was carried out and a constant value of 5% for all plant species was assumed
- Schmidt-Nielsen, 1975: lipid 39,5 KJ/g , protein 17,8 KJ/g , sol. carbohydrates 17,6 KJ/g
- Hungate, 1966: NDF 13,2 KJ/g (* taken from J.Prop & T. Vulink, 1992)*
- R.Buchsbaum, 1986: AD lipid 28,5%, protein 62,5%, sol.carbohydrates 68,5%, NDF 29%

3.2.3 Discussion

The analysis of soluble carbohydrates, NDF, protein and ash content of the plant species under investigation showed four clusters. The monocotyledonous *Festuca rubra, Puccinellia maritima, Juncus gerardi* and *Elymus sp* were all high in sugars and fibres. *Triglochin maritima,* in which the concentration protein was highest. A third cluster with dicotyledonous *Plantago maritima, Spergularia maritima* and *Aster tripolium,* highest in ash content and a forth, rest cluster, formed by *Limonium vulgare, Spartina anglica, Enteromorpha* and *Atriplex portulacoides* with several characteristic features.

Cell components individually

Ash

The ash percentage of the species analysed varied highly. This was an important reason to present the results of the qualitative analysis as percentage of dry weight and not as percentages of ash free dry weight. Correcting for ash, which is often found in literature, would not only overlook an undesirable gutfilling component (sometimes a quarter or even more of the plant material) but also give a wrong quality impression. This is because percentages of other plant components like protein and carbohydrates change for the better when a correction for ash is carried out (see also Appendix 19).

The measurements of the ash content fit well with other studies in the same season. In general most dicotyledonous species contained a higher ash percentage whereas monocots did not. Normally an increase in ash percentage is expected when plant material is growing older, so in autumn, which could explain some deviating percentages found in other studies (see Appendix 15).

Soluble carbohydrate

The leaf tips of grasses and grasslike species had significantly higher soluble carbohydrate content than those of the dicots. Our results are in good agreement with those of Briens & Larher (1982), who demonstrated a low content of inorganic ions and a high content of sugars in the leaves of monocotyledons contradictory to dicotyledons species that had a high content of inorganic ions and a low content of sugars in their leaves. In our study *Spartina anglica* formed an exception on this. It might be that *Spartina* and also the dicots, allocate soluble carbohydrates to their below ground storage organs.

The measurements of the soluble carbohydrate content seem to be somewhat low compared to other studies. This could be due to a not corresponding analysing period (summer versus autumn) but might also be caused by the anthrone method (Allen 1989) used in this research. The anthrone method should measure all carbohydrates including starch but might not be sound in measuring the latter (Bakker, 1997).

Crude protein

Crude protein concentration was highest for *Triglochin maritima.* Compared with ash and soluble carbohydrate analyses less variation was found in protein content among the species

under investigation. The measurements of the crude protein content fit well with other studies in the same season (see Appendix 17).

Digestibility

Juncus gerardi, Spartina anglica and *Limonium vulgare* were found difficult to digest in comparison to the other salt marsh species. Only around 40 % of these species could be digested compared with ± 55% digestibility of the other species.

As stated in the introduction we analysed digestibility with two questions in mind. We wanted to know in what proportion the plant species under investigation related to each other. Secondly is we could, using the *in vitro* method, approach the vivo digestibility values of the salt marsh species of which some are know from literature. With an incubation period of 6 hours (for arguments see appendix 11) we expected to be able to answer both questions. Unfortunately this incubation time did not gave us the expected variation in digestibility values. To detect larger differences a shorter incubation period would be advisable, which might also help to approach the true *in vivo* values which are lower than the values we found.

NDF

The concentration NDF was the highest for *Spartina anglica* and other grasses and grass-like species. The dicots were significantly lower in NDF.

When the proportion of cell wall in plant tissues increases, during maturation for example, the digestibility will be reduced. Expecting this inverse correlation and noting the strong variation among the plant species NDF content, strengthened our opinion that, to approach in *vivo values,* the *in vitro* incubation time should be reduced.

Energy per bite or gram

To get an impression of attainable energy intake we calculated the energy gain per bite. Field observations on wild Brent geese indicate that it is possible to incorporate more than one leaf in a single bite (Prop & Deerenberg, 1991; Van der Wal, pers com). Besides this there is also variation in geese bite rate per plant species. In assessing which species is more profitable for the geese to forage on, attainable energy intake rate will prove to be important (see general discussion). Future studies will have to provide more information on this subject. In this report we focused on plant interior quality, therefore the energy gain per gram digested plant material is given.

Festuca rubra, Puccinellia maritima and *Triglochin maritima,* plant species preferred by the Brent geese had a high energy content per gram material. Yet no significant difference between the place of preferred and disfavoured food species was found.

Besides this, the calculated energy per gram plant material showed us that the cell components, fibre, sugars, protein, fat and ash of the grasses and grasslike species sum up to approximately 100%, while for the dicots and other species a component seems underestimated or missing.

4 GENERAL DISCUSSION

On the eastern part of Schiermonnikoog a gradient of primary succession can be found on the salt marsh since the island is gradually extending eastwards (Bakker, 1989; Olff, 1992). According to classical exploitation theory, the increase in primary productivity found over the successional gradient (van de Koppel *et al.*, 1996), should result in an increased grazing pressure. On Schiermonnikoog, however, we observed that recently developed plant communities are intensively used by Brent geese. The grazing pressure was relatively low in more productive parts later in succession.

This study has demonstrated that grazing pressure and the abundance of preferred plant species are correlated. The relative abundance of preferred plant species is highest in the youngest areas. In the oldest area, the vegetation is virtually only composed of species that hardly occurred in the diet of the geese. Diets however, changed only marginally, incorporating only 15 % disfavoured species in the oldest successional stage.

The ranking of plant species according to preference, with *Puccinellia maritima, Festuca rubra* and *Triglochin maritima* being preferred and *Limonium vulgare, Atriplex portulacoides* and *Artemisia maritima* being disfavoured forage is also reported from other studies. Brent geese foraging at the Norfolk coast during spring, selected for *Puccinellia maritima* and *Triglochin maritima*, together with *Aster tripolium* and avoided *Limonium vulgare* (Summers, 1993). *Puccinellia* was found to be the staple food of Brent in Terschellinger marshes (Ebbinge, 1980) and Danish marshes (Madsen, 1989). In the diet composition of Brent at Schiermonnikoog established by Prop en Deerenberg (1991) *Puccinellia maritima* was the principal food species together with *Festuca rubra, Triglochin* and *Plantago maritima.* The latter two were less commonly consumed.

In the introduction we mentioned three possible considerations to unravel the cause of observed food preference. These are energy maximisation, nutrient constraints and the absence of negative factors, like digestibility-reducing substances.

The accumulation of sufficient body reserves prior to their flight to the high-Arctic breeding grounds is highly important for Brent. Not only for migration to the high-Arctic itself, but also for survival and reproduction in an environment still inhospitable at the time of arrival. Maximising energy intake to build up body reserves can be approached on two scales.

One is the efficiency by with forage can be ingested. This is determined by distribution and appearance of the species in the field. Handling time and bite rate, together with mass and volume per bite, are aspects necessary to evaluate here. The energy intake rate will level off, when the accessibility of leaf tips is hindered, for example, by the presence of dead material and amount of structural components of a plant species (van der Wal *et al.*, in press).

The second level to evaluate energy maximisation concerns the plant interior quality, on which we focused in this report. We found that the percentage carbohydrates of disfavoured plant species was significantly lower than the carbohydrate content of neutral preferred species. Secondly, a trend was visible indicating that preference was shown for highly digestible plant species. This corresponds with data of Boudewijn (1984) that showed that Brent switched from feeding on *Poa protensis* and *Lolium perenne* to *Puccinellia maritima* when the digestibility of *Puccinellia* was higher than that of the pasture grasses.

Our data suggest that in terms of energy maximisation, foraging on disfavoured plant species is less profitable. The observed differences, however, did not came to significant expression in calculated digestible energy per gram of a plant species.

A combination of attainable intake rate together with interior plant quality is probably very important in assessing which species is more profitable for a geese to forage on. *Puccinellia maritima* for example can be preferred because of its high soluble carbohydrates content and also because Brent geese can easily feed uninterruptedly on the closed sward of the grass.

Several studies indicate that considerations of energy intake can not account for food preference alone and stress the importance of nutrient constraints in food choices. Mattson (1980) argued that protein is in limiting supply for non-rumant herbivores due to its relative shortage in plants. The crude protein content of the, in this report compared salt marsh species, varied little. Except for the by the Brent preferred *Triglochin maritima*, which had a much higher crude protein concentration.

Other studies suggest that herbivores base their feeding preference on minimising the concentrations of toxins, repellents and digestibility reducing substances in their diets. All the animals in the study of Bryand and Kuropat (1980) for example ranked their foods on neither energy or nutrient content. Instead their food preference was strongly negatively correlated with terpenes and phenolic resins. In this report we compared ash and fibre, two undesirable gut filling components. In relation to the food preference of the geese there was no significant difference between preferred and disfavoured plants.

We have to conclude that it is difficult to tie down the observed food preferences to one of the analysed factors. This might not be surprising since the animal will receive stimuli from the various attributes of a plant and make a comparison of its favourable and unfavourable characteristics with that of an other species. It can, for example, be the high percentage of crude protein which makes foraging on *Triglochin maritima* attractive even though its ash percentage is high. We compared characteristics of the preferred species with disfavoured species. It also counts that the reasons for a high preference ranking of one species may be quite different from those for another species.

We can conclude that the observed grazing pressure distribution correlates positively with the relative abundance of preferred forage. Data, however, indicate that it is difficult to link the observed food preference to one of the analysed food quality aspects. Future studies will have to provide more insight in the feeding strategies of Brent geese.

ACKNOWLEDGEMENTS

The acknowledgements are among the things of a report I mostly read first. Behind all graphs, tables and text, there's a whole world hidden. The acknowledgements mostly reveal a tip of the veil of the accomplishment of the presented report : the hard labour, fun and discussions before the final daft was there. It is a pleasure to finally being able to thank the many people that made a contribution to the research that is presented here. Its accomplishment would never have succeeded without their help.

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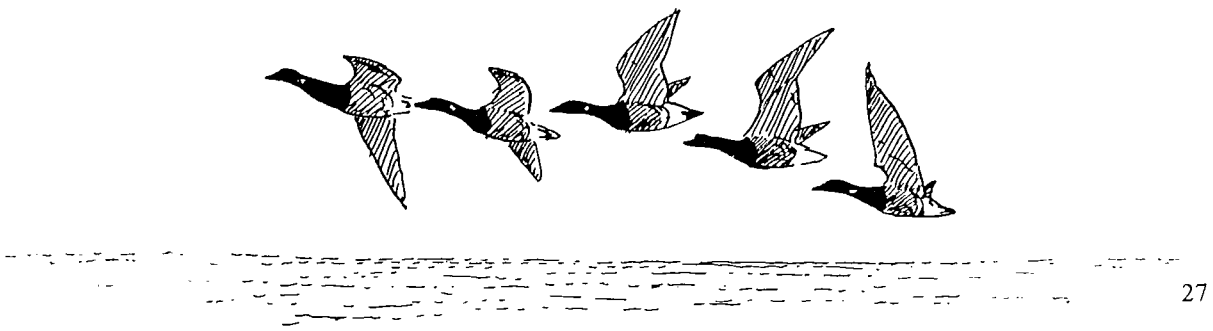
And last but certainly not least the Brent geese themselves! My respect for their performance has grown every day, especially when I was sampling leave tips.. Luckily I did not have to live on the food I sampled. Finishing this report, I have far more questions on these fascinating birds than at the beginning... Will there be a next time?



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APPENDICES

1. Brent geese grazing pressure at three successional stages: calculation figure 1
2. Identification key epidermal structures
3. Photographs, drawings and notes epidermal structures
4. Diet composition
5. Photographs *Festuca*[2]
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12. Plant species cover at the 10, 25 and 35 years old salt marsh
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16. Comparing WSC values
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Appendix 1

Brent geese grazing pressure at three successional stages
Calculating figure 1 page 2:

Average goose hours per day
(day of 14 hours)

10 years	= 666.23	SD = 412.78	n=13
25 years	= 843.58	SD = 1107.83	n=28
35 years	= 1000.45	SD = 1038.53	n=11

surface area of transitional "island -zone" on map

10 years	= 252 squares	$=3.9375 * 10^{-2}$ km^2
25 years	= 486 squares	$=7.59375 * 10^{-2}$ km^2
35 years	= 1108 squares	$=1.7312 * 10^{-1}$ km^2

4 cm	=500 m
1 cm	=125 m
1 cm^2	=15625 m^2
1 square	=156.25 m^2
	=156.25 * 10-6 km^2

Average goose hours per day/ km^2

10 years	= 16920.12	SD = 10483.3	SE = 2907.5
25 years	= 11108.87	SD = 14588.7	SE = 2757.01
35 years	= 5778.772	SD = 5998.9	SE = 1808.74

Appendix 2

DE SLEUTEL

1 - Huidmondjes 2
- Geen huidmondjes 19

2 - Haltervormig 3
- Boontjes 8

3 - Celstructuur rechthoekig, vaak geribbelde cellen 4
- Celstructuur opgeblazen, cellen glad 7

4 - Tussencellen vierkant = *Puccinellia maritima* (oz)
Verdere kenmerken: randcellen bol, dakpansgewijs gerangschikt. Humo's geconcentreerd per rij van cellen. Haltervormige huidmondjes (zonder ellips?) NB. Tussencellen hoeven niet vierkant te zijn, maar er zijn altijd vierkante aanwezig
- Tussencellen nooit vierkant 5

5 - Huidmondjes in rijen, ononderbroken door cellen. Ellips om haltertjes = *Elymus athericus* (oz)
- Niet in ononderbroken lijnen 6

6 - Onderscheid bedenken tussen *Spartina* en de bovenkant van *Elymus athericus*. *Elymus athericus* dakpancellen als *Festuca*, maar dan spits

7 - Tussencellen aanwezig, haren uit tussencellen. Huidmondjes smal en lang = *Festuca rubra* (bz)
- Tussencellen niet aanwezig, geen haren maar wel korte stekels(?), huidmondjes verspreid = *Puccinellia maritima* (bz)
-Huidmondjes haltervormig Ribbels in nerf (zie foto) = *Agrostis*

8 - Dubbele boontjes 9
- Geen dubbele boontjes 10

9 - Regelmaat, huidmondjes in rijen, gescheiden door rijen met lange smalle cellen. Cellen tussen huidmondjes
vierkantig. Boontjes soms bruin gekleurd. Evenwijdige nerven overeenkomstig met rijen smalle cellen = *Triglochin maritima*
- Minder regelmaat; geen duidelijke indeling in rijen, cellen tussen de huidmondjes niet duidelijk vierkantig = *Armeria maritima*

10 - Huidmondjes op scheiding van twee cellen. Lijken in een grote cel te liggen omdat de scheiding van de twee cellen moeilijk zichtbaar is = *Plantago maritima*.
Verder: veel huidmondjes regelmatig verspreid
- Niet 11
- Steeltjes aanwezig of cellen op steeltjes (Atriplex + Glaux)

zo →

en niet zo.

[nieuwe- Steeltjes, verder kunnen cellen gegolfd zijn, dikkere celwanden = *Glaux maritima*
10] - Steeltjes of met een cel erop, verder cellen hoekig. Geen dikke celwanden onregelmatige structuur = *Atriplex portulacoides*

maar ook zo

11 - Celwanden geribbeld als de grassen, maar alleen aan de rand smalle rechthoekige cellen soort van dubbele
boontjes=*Juncus gerardi*
- Niet 12

12 - Cellen gegolfd 13
- Cellen hoekig, tot afgerond 15

13 - Cellen licht gegolfd =*Artemisia maritima*
- Cellen sterk gegolfd 14

14 - Huidmondjes groot en dik en in ander vlak itt *Spergularia maritima.* Cellen met dikke dubbele celwanden. Steeltjes! = *Glaux maritima*
- Huidmondjes klein, cellen op rijen (?) Ook steeltjes (?) =*Spergularia maritima*

15 - Cellen rond huidmondjes kleiner. Rommelige structuur, cellen variabel, cellen geconcentreerd rond huidmondjes =*Limonium vulgare*
- Niet 16

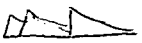
16 - Huidmondjes een beetje kleiner dan de omliggende cellen =*Aster tripolium.* Huidmondjes enigzins bovenop? bijna bolrond, veel huidmondjes
- Huidmondjes duidelijk een stuk kleiner dan de omliggende cellen 17

17 - Cellen op steeltjes =*Atriplex portulacoides*
- Niet 18

18 - Huidmondjes onregelmatig van grootte. Veel huidmondjes, langwerpige boontjes ingeklemd. Grote hoekige cellen = *Sueda maritima*
- Huidmondjes regelmatig van grootte, cellen minder hoekig, veel huidmondjes =*Salicornia europaea*

19 - Celstructuur lange rechthoekige cellen, geribbeld, met smalle korte tussencellen =*Festuca rubra (oz)*
- Niet 20

20 - Weefsel bestaand uit heel veel kleine celletjes =*Enteromorpha*
- Niet? Tja,..

Definities:
humo's =huidmondjes
dubbele boontjes= 
bz= bovenzijde
oz=onderzijde
dakpancellen als *Festuca* maar dan spits= 
steeltjes=  

Appendix 3

Tekeningen, foto's en beschrijvingen van epidermale structuur van:

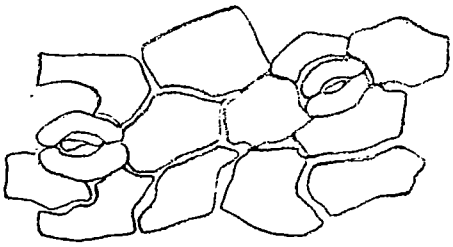
Ammophila arenaria
Armeria maritima
Artemisia maritima
Aster tripolium
Atriplex portulacoides
Elymus farctus
Entromopha sp
Festuca rubra
Fucus sp
Glaux maritima
Juncus gerardi
Limonium vulgare
Monostroma sp
Plantago maritima
Puccinellia maritima
Salicornia oliostachya
Spartina townsendii
Spergularia maritima
Suaeda maritima
Triglochin maritima
Ulva lactuca
Zostera sp

Met dank aan

Charlotte Deerenberg
Martijn Egas
Dijkstra & Dijksta- De Vlieger
Summers et al., 1993

Aster tripolium

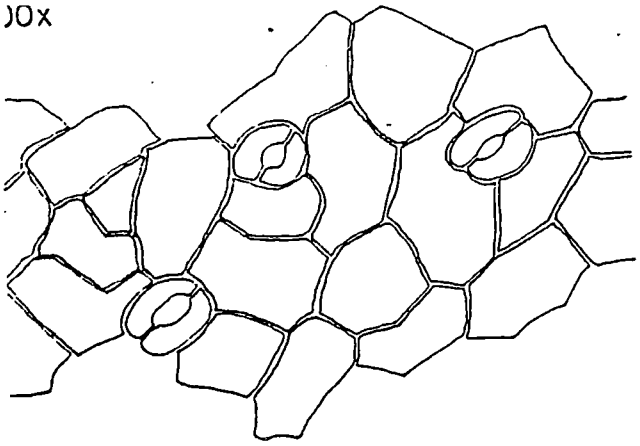
(zie ook: Dijkstra & Dykstra-De Vlieger)



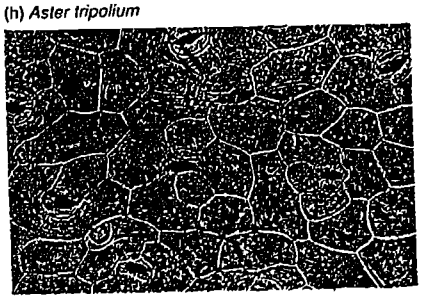
vrij grote, onregelmatige cellen
bijna bolronde huidmondjes
– enigzins bovenopliggend.

boven- en onderzijde ± gelijk

)0x

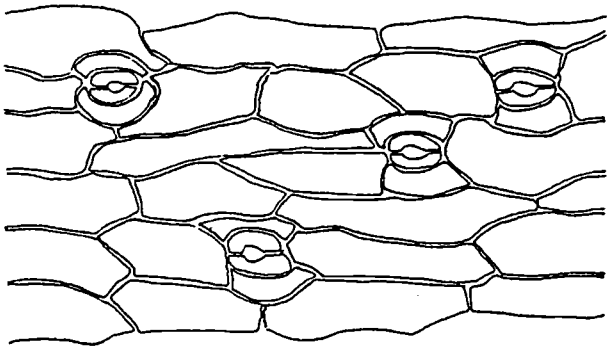


(h) *Aster tripolium*

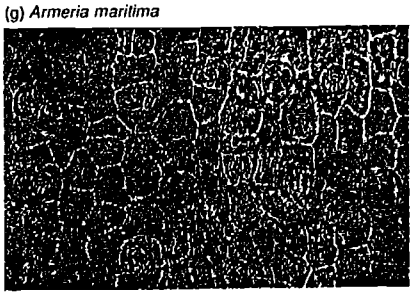


hoekige grote cellen
humo's ± tussen andere cellen in
bijna bolrond
± even groot – iets kleiner dan overige cellen
enigzins boven opliggend

Armeria maritima
200x



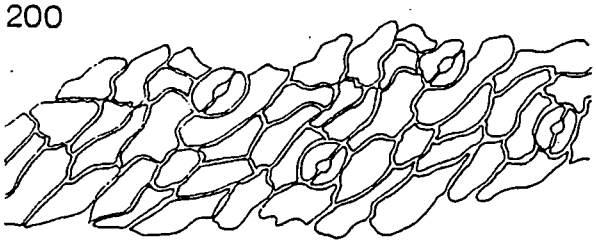
(g) *Armeria maritima*



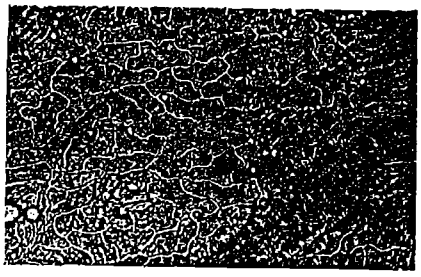
Armeria maritima (Plate IIg)

Stomata surrounded by two curved cells as for *Triglochin maritima* but cells not arranged in regular rows. Circular non-stomatal structure present as for *Limonium vulgare*.

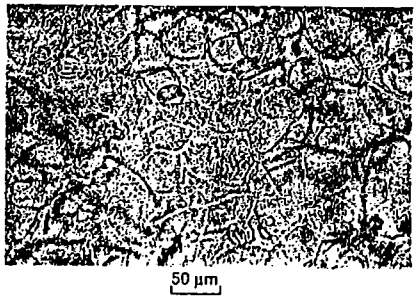
Artemisia maritima
200



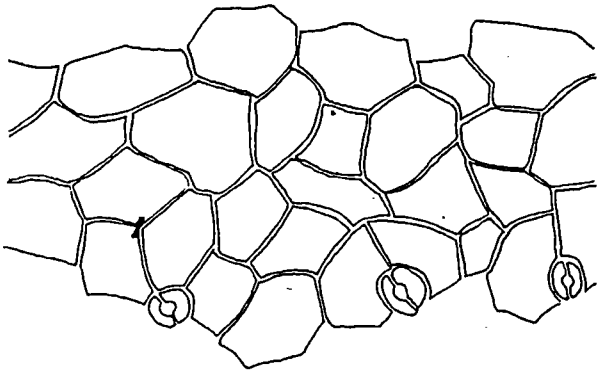
(d) *Artemisia maritima*



(h) *Halimione portulacoides*



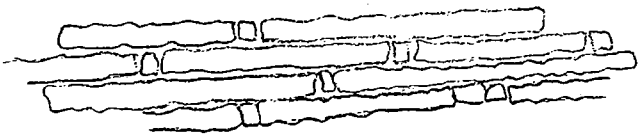
Halimione portulacoides
200x



grote ronde schetsmatige
cellen Hang effect

Festuca rubra

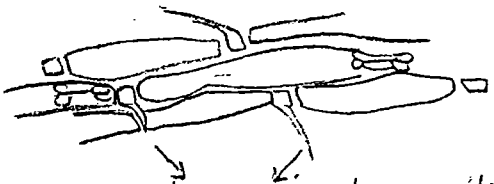
onderzijde (zie ook: Dijkstra & Dijkstra-De Vlieger)



geen huidmondjes

lange, smalle cellen
fijn gekarteld
soms twee tussencellen naast elkaar

bovenzijde

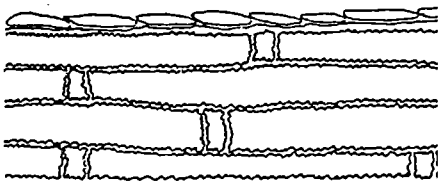


haren, uit tussencellen

opgeblazen, ± gladde cellen
huidmondjes ! smaller, langer dan bij Puccinellia

smalle tussencellen
humo's aan bovenzijde ! met gladde bollige cellen
lange haren mn. langs nerven bovenzijde
cellen scherp rechthoekig
soms dubbele tussencellen
vaak opvallend grote tussencellen
bv op nerven

Festuca rubra
200x



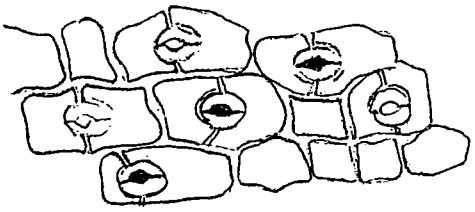
Festuca rubra

Abaxial surface—cells bigger than for *Puccinellia maritima*—80–400 × 15–20 μm. Thickening of walls present but more finely folded than *P. maritima*. Silica bodies present, often in twos or threes. Mostly shorter (in direction of leaf axis) than for *P. maritima*. No stomata. Cells on leaf edge more flattened, giving a smoother edge.

Adaxial surface—cells without lumps, but whole cell bulges outwards and sideways from the narrow end walls adjoining the silica bodies. Cells arranged so that in adjacent rows the narrow ends of the cells are partly obscured by the bulging of the cells on either side. Short hairs grow from

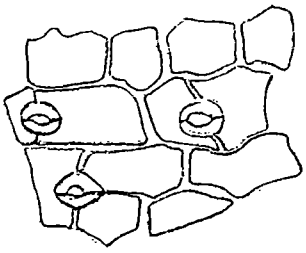
Plantago maritima

onderzijde

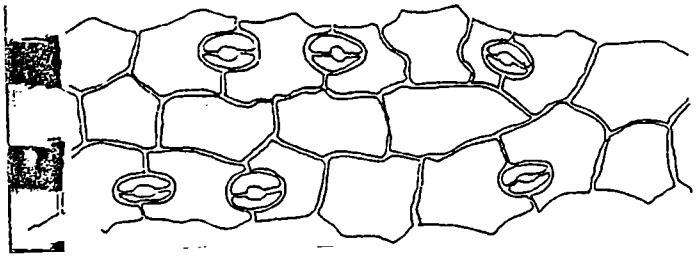


huidmondjes altijd met twee uitlopers
— op scheiding van twee cellen (?)
veel huidmondjes
cellen ± op rijen

bovenzijde



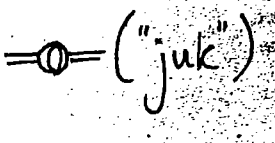
cellen wij onregelmatig



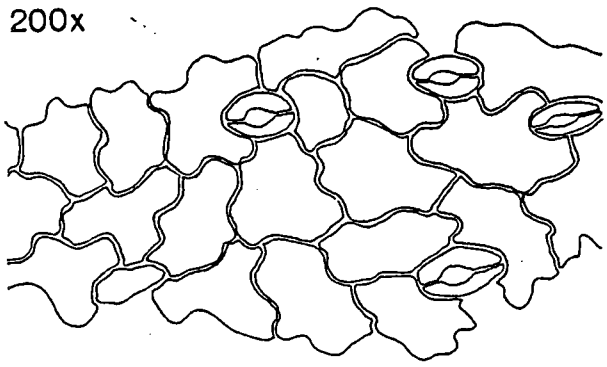
(e) *Plantago maritima*

Stomata surrounded by 2 or 3 cells only. Cell junctions perpendicular to stomatal axis.

humos klein binnen de cel
op scheiding van twee cellen
humos boven en onder

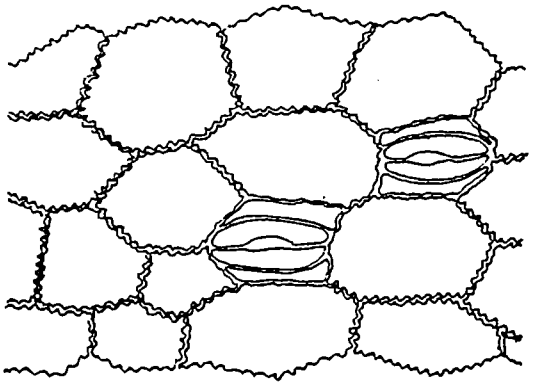


Glaux maritima
200x



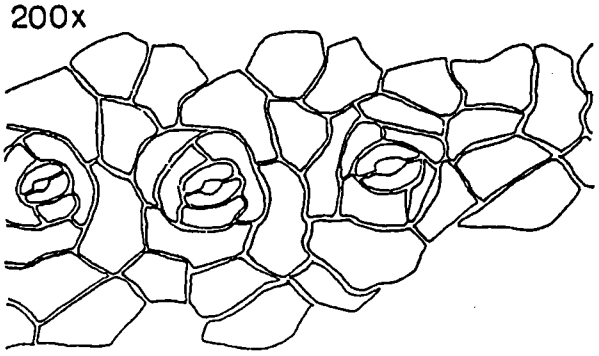
onregelmatige cellen
humos tussen andere cellen
humo's groter en dikker
dan Spergularia
dikke, dubbele celwanden
humo's boven + onder,
boven boven op liggend.

Juncus gerardii
200x



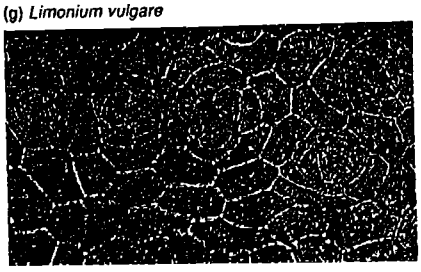
humo's in de cel
bovenzijde rand - smalle
regelmatige rechthoekige cellen
midden - grote cellen
onderzijde : regelm. vierk. - langu
cellen. humo's!

Limonium vulgare
200x



ronde humo's, 2 "boontjes"
zwarte opening, rondom
humo's kring (2-3-4) v.
begeleidende cellen
Cellen variabel, rommelige
structuur.

(g) *Limonium vulgare*

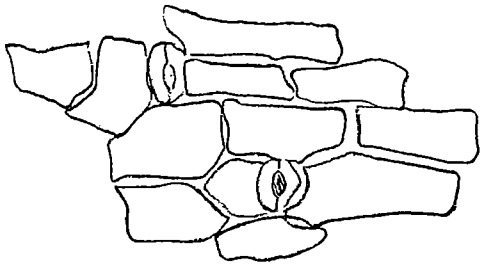


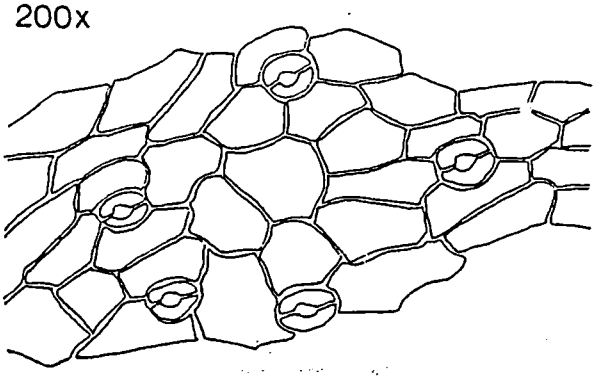
Salicornia oligostachya

iemplantje

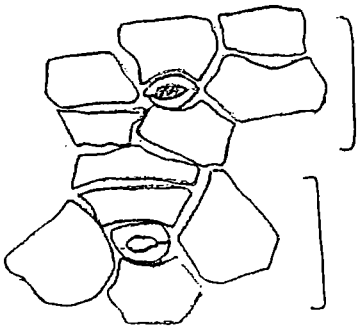
Salicornia europaea
200x

kiemblad





1e blad



kenmerkende celstructuur rond huidmondjes

(h) *Salicornia europaea* agg.

wortel

cellen onregelmatig hoekig
kleiner en voller (minder ijl)
dan Suaeda. Humo's tussen
andere cellen in. Ovaal,
vaak zwarte opening
meestal cellenpatroon
verder onregelmatig

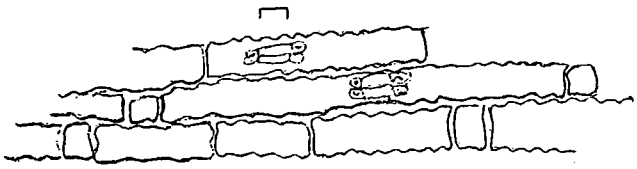
één nerf

"wollig" uiteinde of in punt uitlopend met wortelharen
(vgl. Suaeda!)

Puccinellia maritima

onderzijde

middendeel bovenopliggend



huidmondjes geconcentreerd per rij van cellen

huidmondjes lijken ± rond (door bijcellen ?)

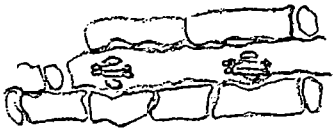
(zie ook Dijkstra & Dijkstra-De Vlieger)

NB. randcellen dik, dakpansgewijs

knobbel

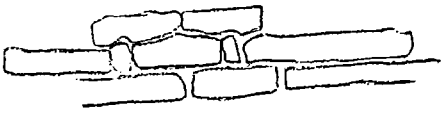
1e blad

legere cellen minder gekarteld



cellen lijken nog niet volledig gestrekt

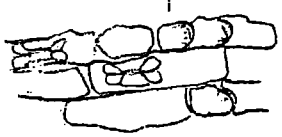
3e blad



cellen smal, nauwelijks gekarteld (glad)

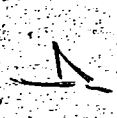
bovenzijde

knobbels, alle naar één kant



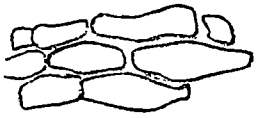
huidmondjes ± verspre

korte stekels aanwez



cellen rommelig, niet geribbeld

3e blad



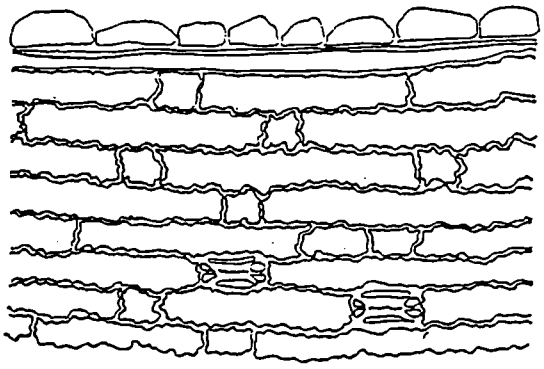
enigzins opgeblazen cellen
geen (duidelijke ?) knobbels
cellen wisselend van grootte: niet altijd duidelijk verschil tussen cel

Puccinellia : ± vierkante tussencellen
humo's bovenop liggend, boven en onder (zie fest!)
randcellen dik, één zijde is dikker (dakpannen)
hoeken meer afgerond
soms knobbels op cellen, alle naar één kant
boven gladdere, rondere cellen (rommeliger)

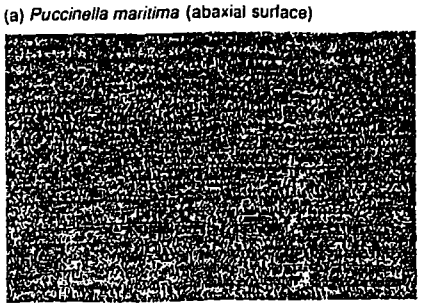
v. a voorjaar - geribbelde cellen
v. a najaar - gladde cellen
→ onderscheid mogelijk tussen jong en oud blad

± tot mei : glad
mei : geribbeld
najaar : oud + geribbeld
jong 1e/2e - glad

Puccinellia maritima
200x



(a) *Puccinella maritima* (abaxial surface)



Puccinella maritima (adaxial surface)

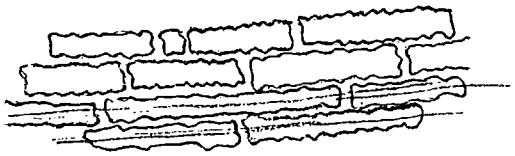
Adaxial and abaxial surfaces differ. Morphology varies according to growth state.

Abaxial surface—old leaves: cell walls thickened and folded, silica bodies present between cells. Stomata present but not very dense. Cells on leaf margins rounded giving corrugated edge. Cell sizes 60–220 × 10–15 μm. Young leaves: cell walls thin, not folded. Silica bodies few or absent. If present they occur near leaf margins. Stomata present.

Adaxial surface—many cells with circular lumps. Cell walls often thickened and folded, but not

Spartina townsendii

onderzijde

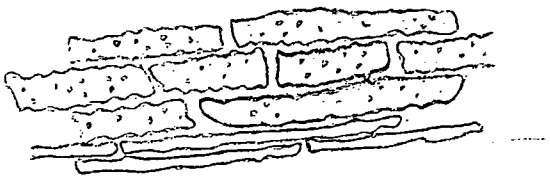


~~geen~~ weinig huidmondjes

vgl. Puccinellia en Festuca
weinig tussencellen
veel nerven – lange smalle cellen
overigens wat brede, vrij korte cellen

steeltjes in banen
op plaats tussencel
in die banen ook de huidmondjes

bovenzijde



huidmondjes aanwezig
onduidelijk – moeilijk zichtbaar

gegolfde celwanden
alle cellen met "belletjes" – zoutkristallen ?
nerven = smalle lange cellen

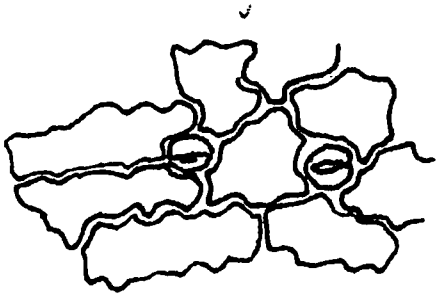
Spartina anglica

Only the abaxial surface is characteristic. Cells covered in evenly spaced silica nodules appearing as small bright spots. Otherwise cells very like *P. maritima*. Cell size 80–250 μm long.

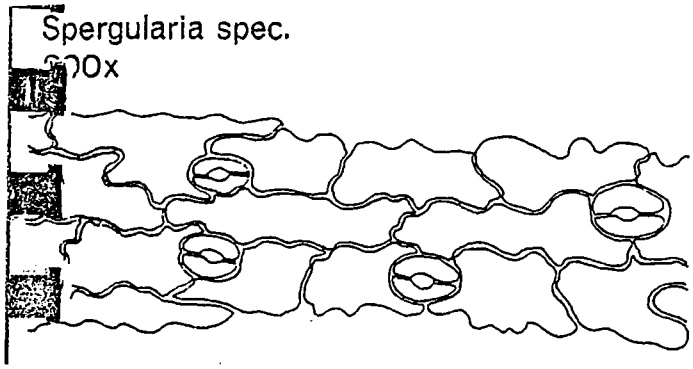
Spergularia maritima

variërend tussen
deze uitersten

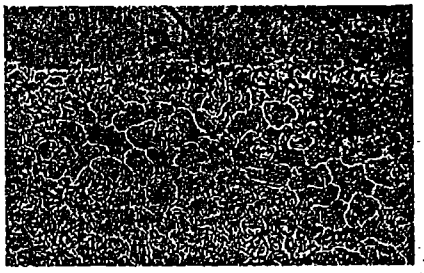
meest gegolfde celwanden
± rijen



huidmondjes vrij klein



(d) *Spergularia media*



Spergularia media & *Artemisia maritima* (Plate IId)

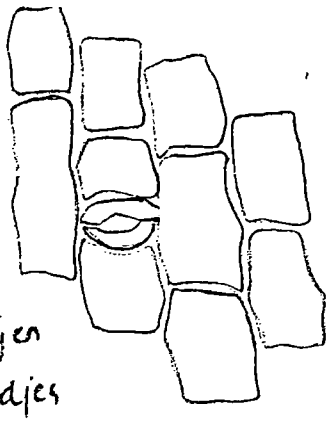
Cells have jigsaw piece appearance due to wavy edges, more so in *Spergularia*.

cellen ± op rijen
grof gekronkeld ("puzzelstukjes") → ± glad
humo's op scheiding van cellen
humo's boven + onder, klein  of 

Suaeda maritima

kiemplant

kiemblad

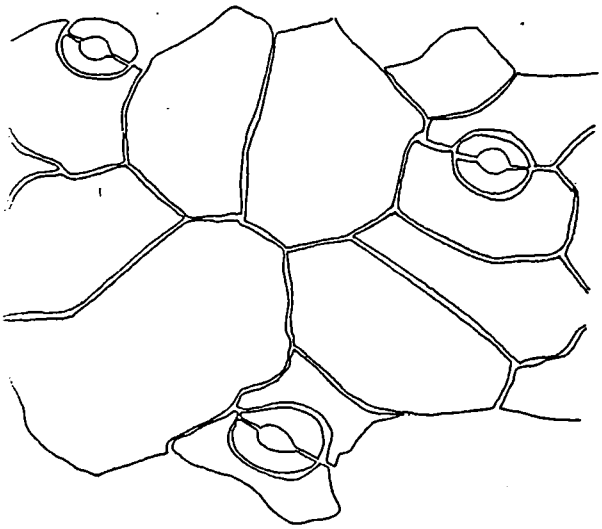


grote cellen, rijen
grote huidmondjes

wortel

langwerpige, gladde cellen
(vgl. Salicornia!)

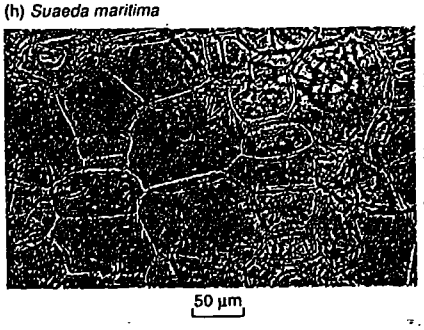
Sueda maritima
200x



← knobbeltjes

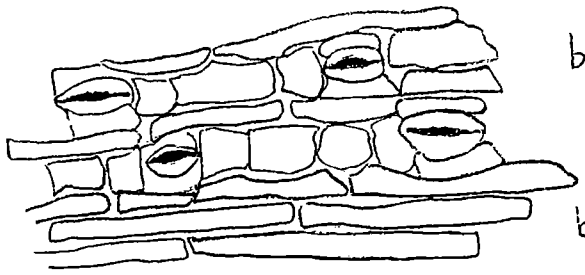
eén nerf (vrij breed)

(h) *Suaeda maritima*



- grote hoekige ce
- rel. kleine eni
langwerpige
(langwerpig) zei

Triglochin maritima



baan met
1-3 rijen huidmondjes

baan van lange, smalle cellen

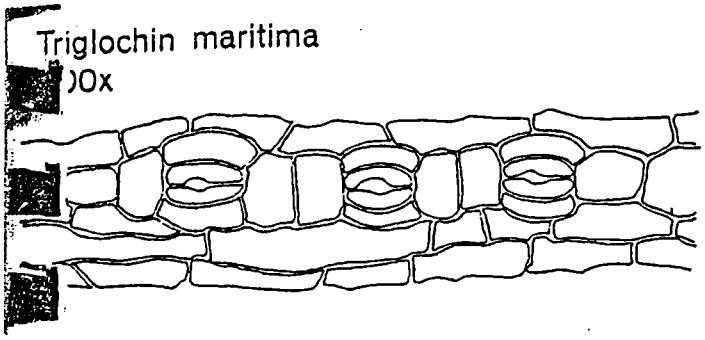
boven- en onderzijde gelijk

kelkbladen

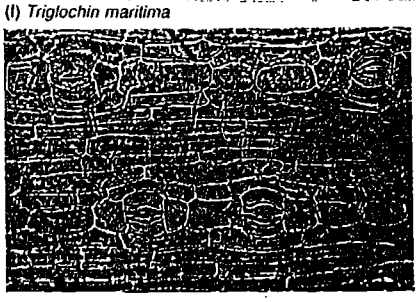
zaadjes

geen duidelijk herkenbare structuur

vruchtepidermis
vgl. kelkbladen
cellen niet / nauwelijks te onderscheiden



(I) *Triglochin maritima*

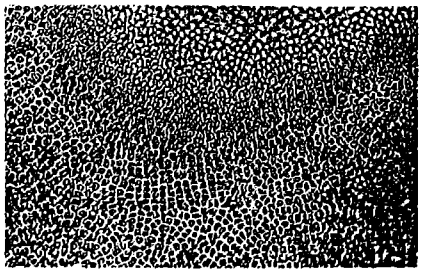


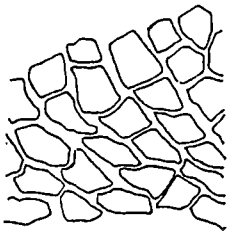
Triglochin maritima (Plate IIf)

Stomata and elongated cells arranged in regular rows. Two curved subsidiary cells surround the stomata, lying parallel to the guard cells. A single row of cells stretches between stomata.

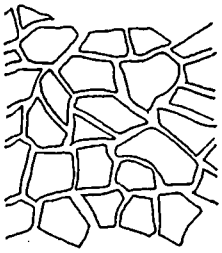
huma's in banen van vierkantige hoekige cellen

(c) *Enteromorpha* sp.





Enteromorpha spec.
400x

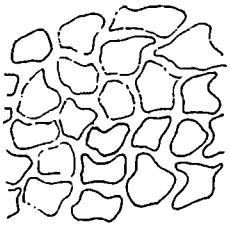


Ceramium rubrum
400x

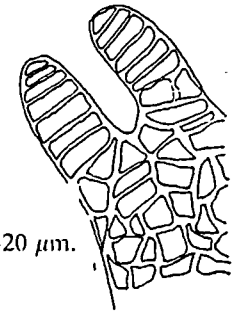
Enteromorpha sp. filamentous

Some cells arranged in rows. Cells slightly more rounded than *Zostera noltii*. Filaments narrow.
Cell size 5–15 μm.

Cells often in rows and most square or rectangular. Lamina broad. Cells sometimes arranged radially around a central cell. Size 8–20 μm.



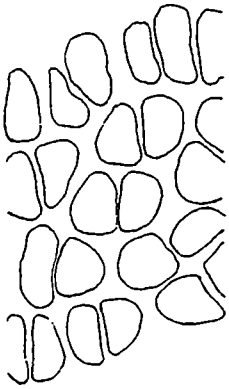
Ulva lactuca
400x



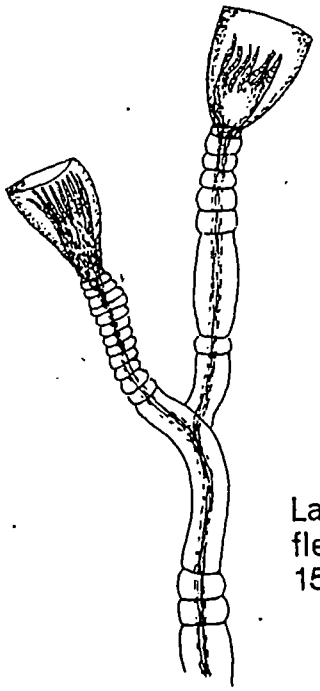
groeitop
Ceramium rubrum
400x

Ulva lactuca

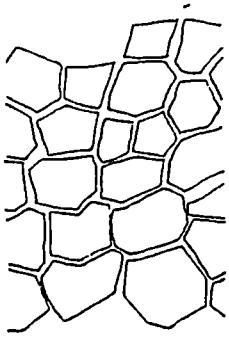
Cells not arranged in rows and no stomata. Cells irregularly shaped 10–20 μm.



Monostroma spec.
400x

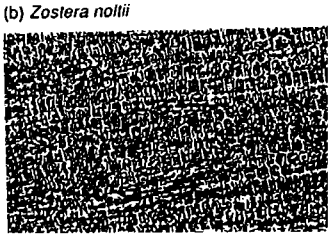


Laomedea
flexuosa Alder
15x



Fucus spec.
400x

(b) *Zostera noltii*



Zostera angustifolia

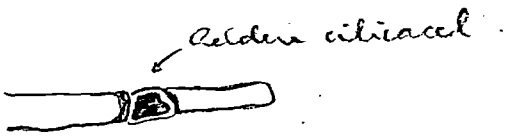
Cells square or often rectangular, long axis mostly perpendicular to leaf axis. Cells arranged in rows. Cells of inner tissues much larger. Cell size 10–25 μm. No stomata.

Zostera noltii (Plate IIb)

Cells square than *Enteromorpha* sp., and arranged in rows. Cell size 10–20 μm. No stomata.

HELM (AMMOPHILA ARENARIA)

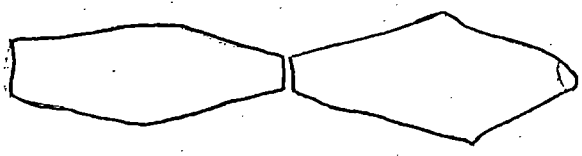
BLAD: Kleine blokkige cellen, door veel 4-kante tussencellen.



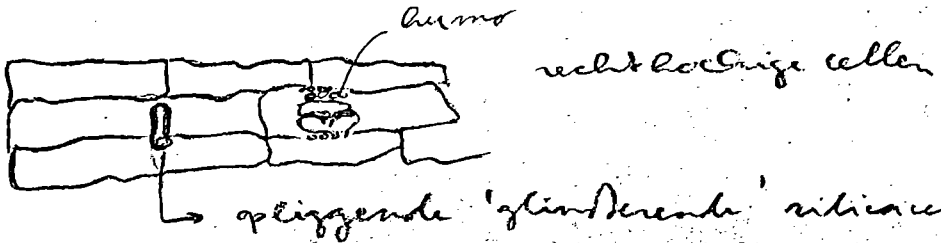
BIESTARWEGRAS (ELYMUS FARCTUS)

BLAD. VARIERENDE lang dun tapstoelopend

dik, scherp



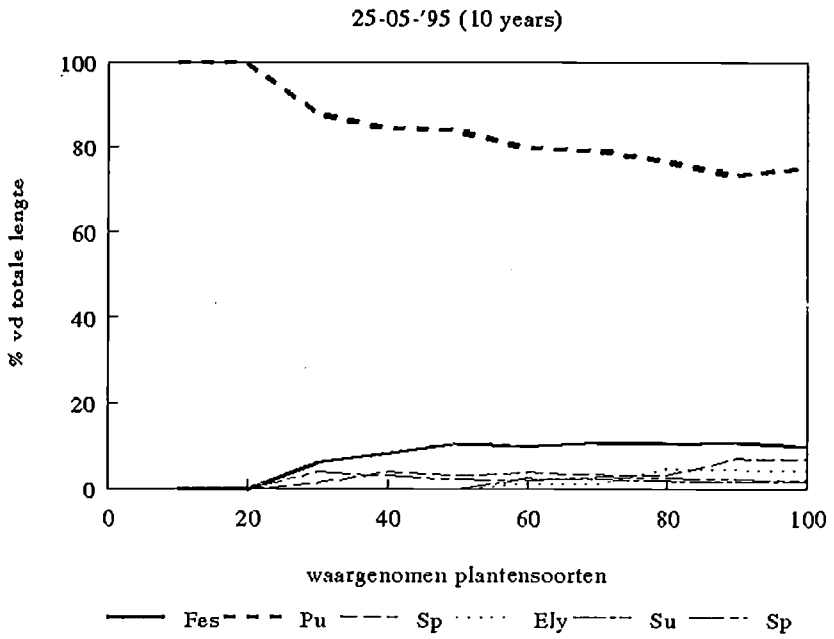
onder



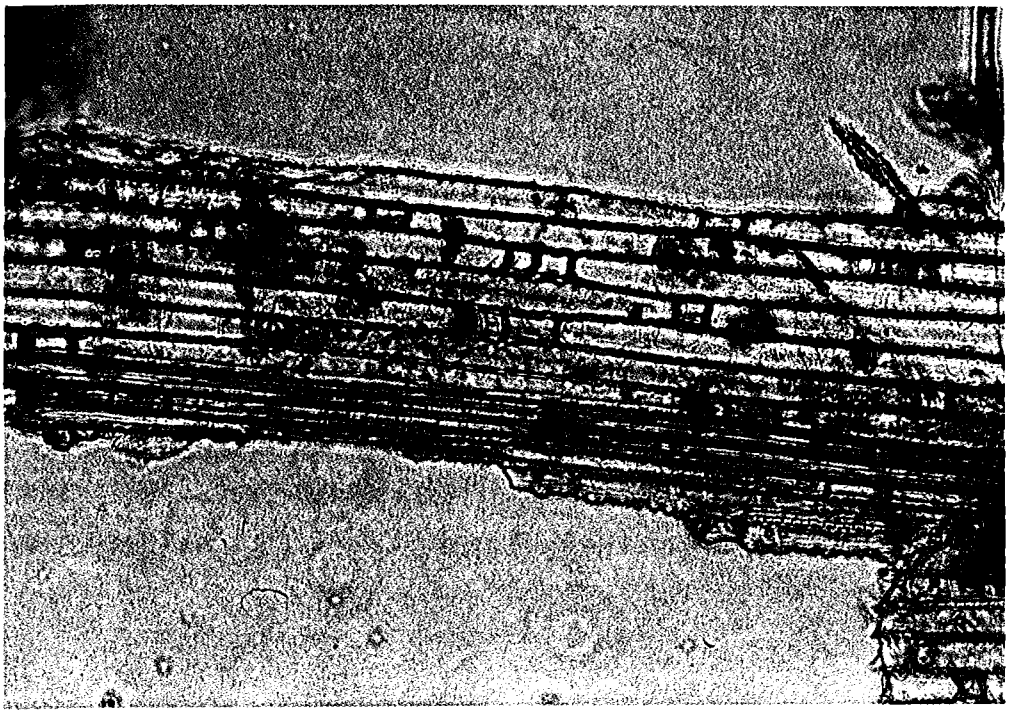
Appendix 4

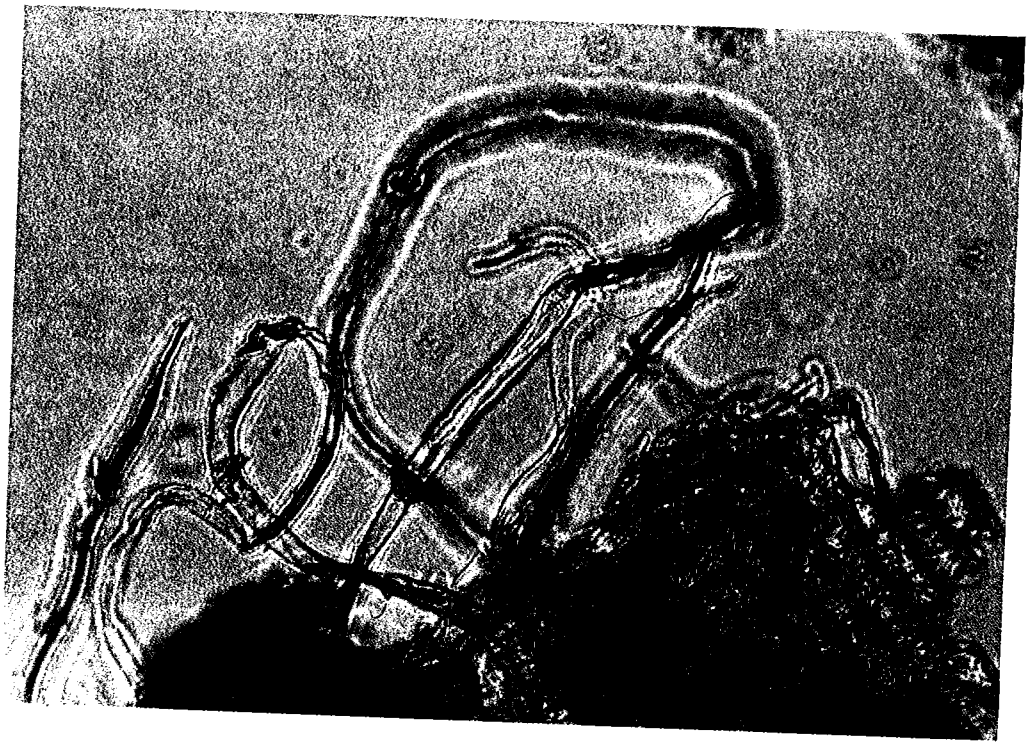
Diet analysis

Hundred identifications of a dropping sample take from the 10 years old salt marsh



Appendix 5





Appendix 6

	Fesrub	Pucmar	Trimar	Elysp	Spemar	Plamar	Junger	Entero	Limvul	Asttri	Atrpro	Spaang	Suamar
March													
02	T5-DE			T5-D									
08		T5-D											
13			T2-E				pad						
21		T0-E	T2-D		T0-E	T0-E							
22	T5			T5									
24				3e sl									
April													
06		T2											
14	OBK							T0-DE					
24		T2-D	3e sl-DE										
26	T2	T2--B	T2	T2-E	T0-DE	T2-DE	T2-DE	T2-E					
May													
01	T2-BDE	T2-BD				T2-BDE	T2-BDE	T2ABDE	T2-ABDE				
02			T2-BE	T2-BDE									
03		T2-CE	T2		T0								
04													
05													
06	T2-C	T2	T2	T2	T2-BDE	T2-A	T2	T2		T2-BE			
07													
08													
09													
10													
11	T2-ABDE		T2	T2-BDE	T2-A	T2	T2-ABDE	T2-BE	T2-ABD	T2-A			
12													
13													
14													
15													
16	T2-A		T2-ABDE	T2-A	T2-BDE	T2-ABDE	T2-A	T2-ADE	T2	T2-BD			T2
17													
18													
19													
20													
21	T2-ABDE	T2-BDE	T2-ABDE	T2-BDE	T2-BE	T2-B	T2-B	T2-AB	T2-ABDE	T2-BE	T2		
22													
23													
24													
25													
26	T2	T2-AE	T2-A	T2-A	T2-AD	T2-ADE	T2-ADE	T2-D	T2-DE	T2-DE	T2-E	T2-BE	

27		T2-A											
June													
01													
02		T2								T2-D	T2-CE	T2-CE	
18	T2-CDE			T2-CDE									
19		T2-CDE	T2-CDE		T2-CDE	T2-CDE	T2-CDE		T2-CDE	T2-ACE	T2-DE	T2-ADE	
20								T2-CDE					

Explanation of the symbols used:

Successional stage were plant material was collected:
T0=3 year
T1=10 year
T2=25 year
T3=35 year
T5=100 yea

Material of this date was used for the chemical analysis of:
A=Cell wall components
B=In vitro digestability
C=Dry weight and ash
D=Protein
E=Soluble carbohydrates

Appendix 7

Abbreviations of plant names:

Arm	Armeria maritima
Art	Artemisia maritima
Ast	Aster tripolium
Atr	Atriplex portulacoides
Ely	Elymus sp
Ent	Enteromorpha
Fes	Festuca rubra
Fes^2	Festuca^2 (see methods)
Gla	Glaux maritima
Jun	Juncus gerardi
Lim	Limonium vulgare
Pla	Plantago maritima
Puc	Puccinelia maritima
Sal	Salicornia sp
Spa	Spartina anglica
Spe	Spergularia maritima
Sua	Suaeda maritima
Tri	Triglochin maritima

Appendix 8

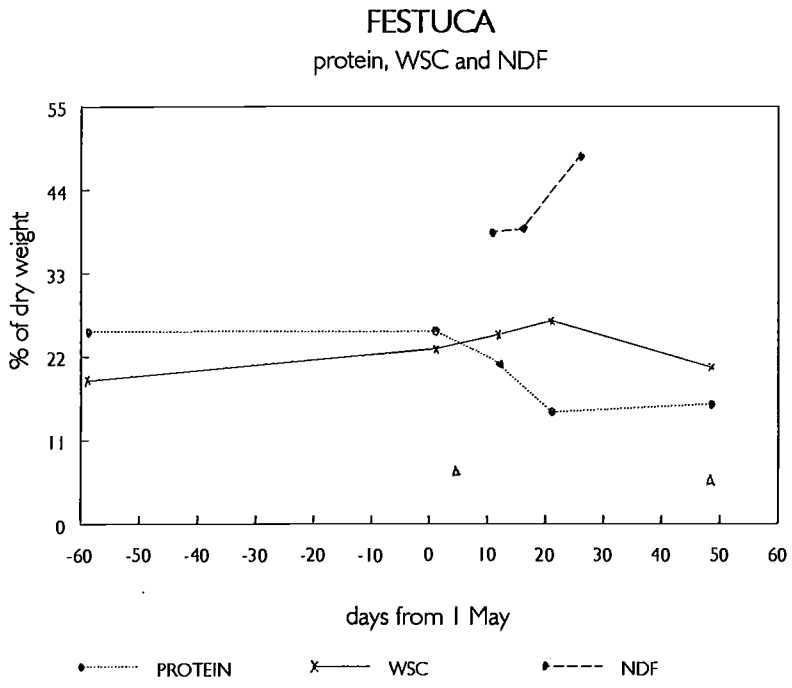
Possible difficulties when using "preference ratio" :

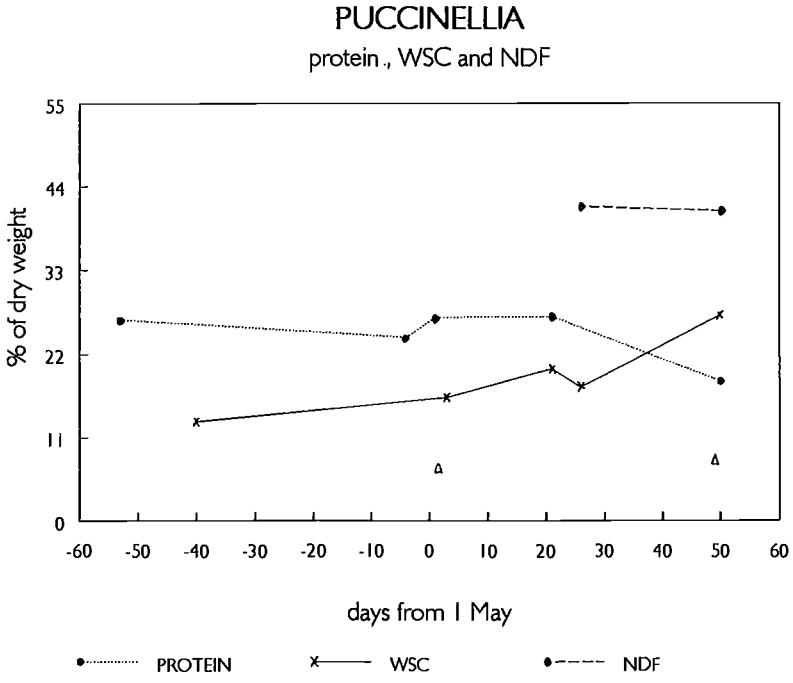
Species	A	B	C
percentage habitat	1	9	90
percentage diet	5	95	trace

When species C is included as available food, then A and B are both strongly preferred. Ratio 5.0 and 10.6 However when C is omitted from the analysis, the habitat contains 10% species A and 90% B; B is still slightly preferred, but A is quite strongly avoided. Ratios of 1.1 and 0.5 respectively.

Crawley, 1983

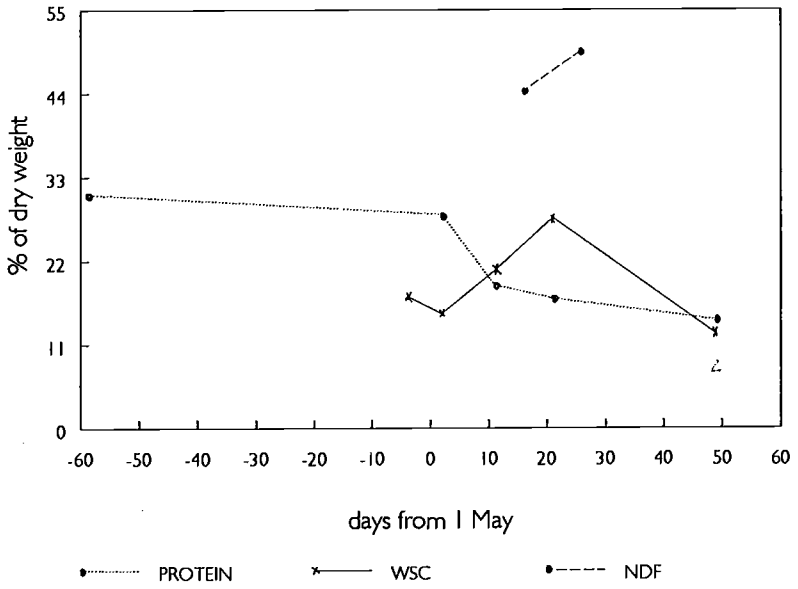
Appendix 9


FESTUCA
protein, WSC and NDF
% of dry weight
55
44
33
22
11
0
-60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60
days from 1 May
PROTEIN
WSC
NDF


PUCCINELLIA
protein., WSC and NDF
% of dry weight
55
44
33
22
11
0
-60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60
days from 1 May
PROTEIN
WSC
NDF

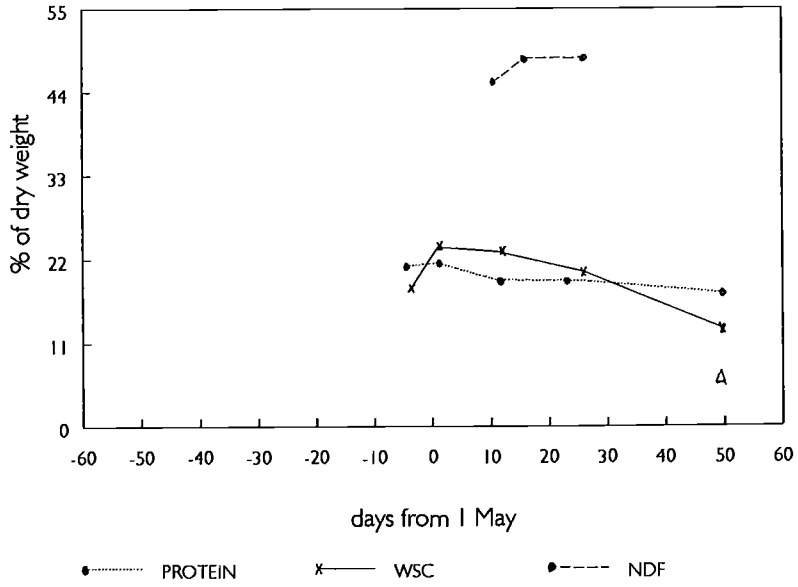
ELYMUS

protein, WSC and NDF



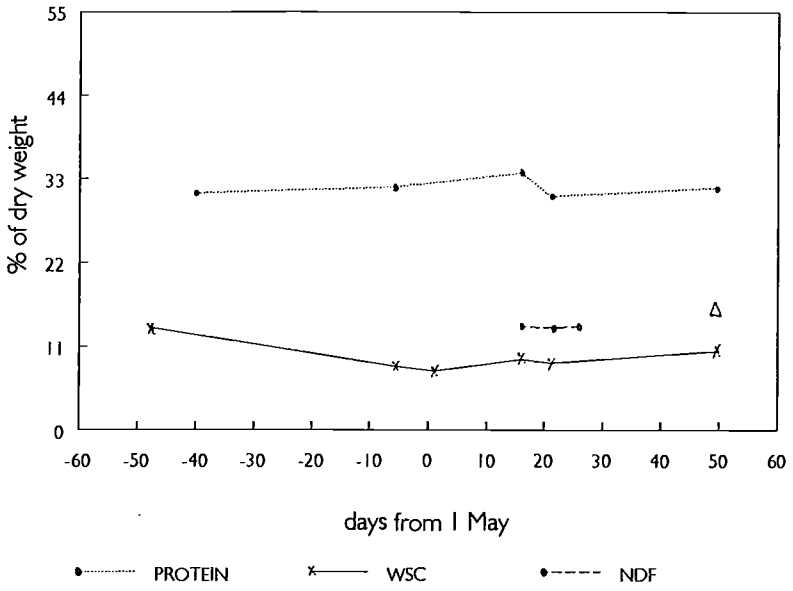
JUNCUS

protein, WSC and NDF



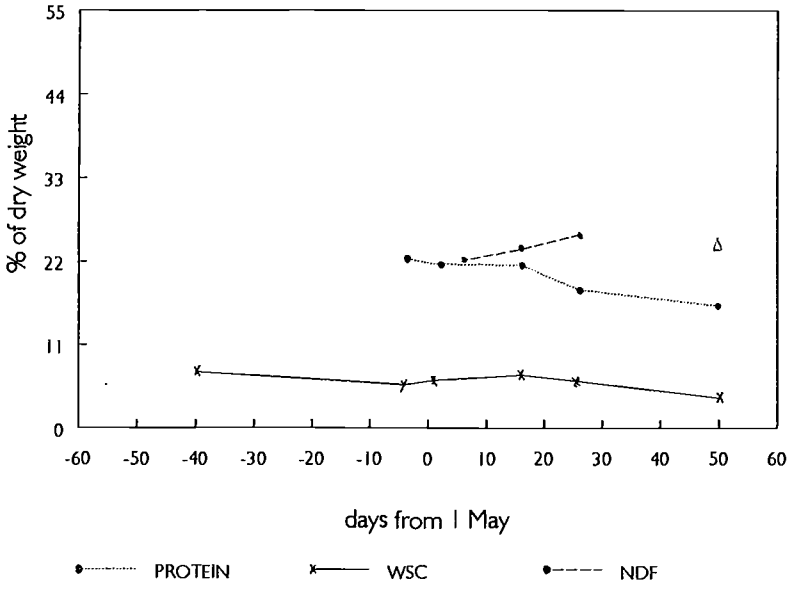
TRIGLOCHIN

protein, WSC and NDF



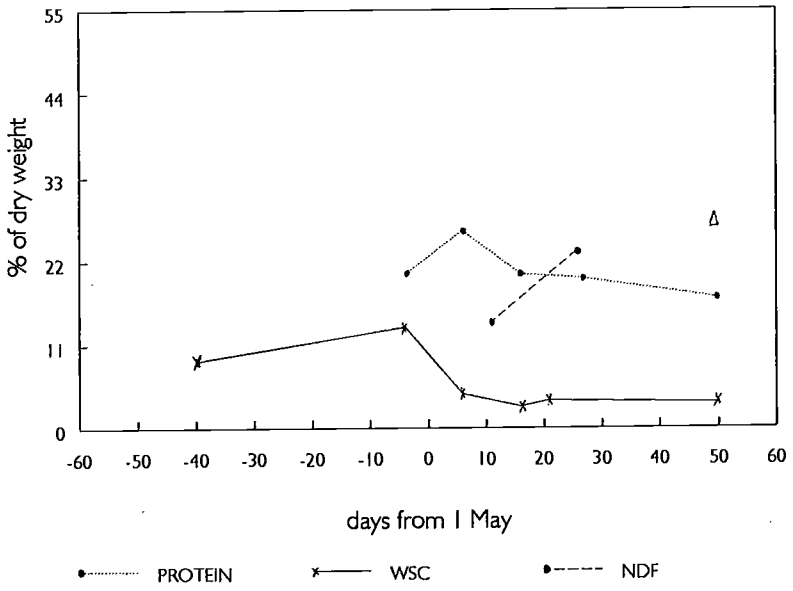
PLANTAGO

protein, WSC and NDF



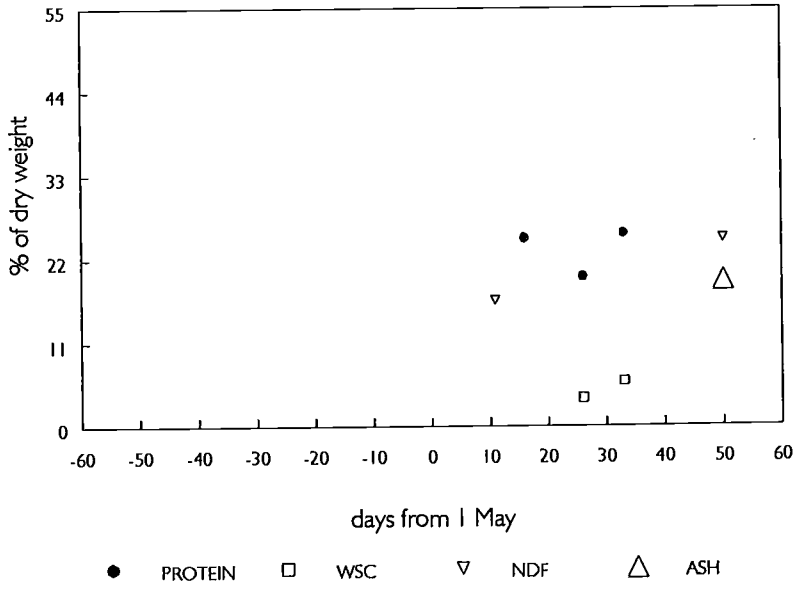
SPERGULARIA

protein, WSC and NDF



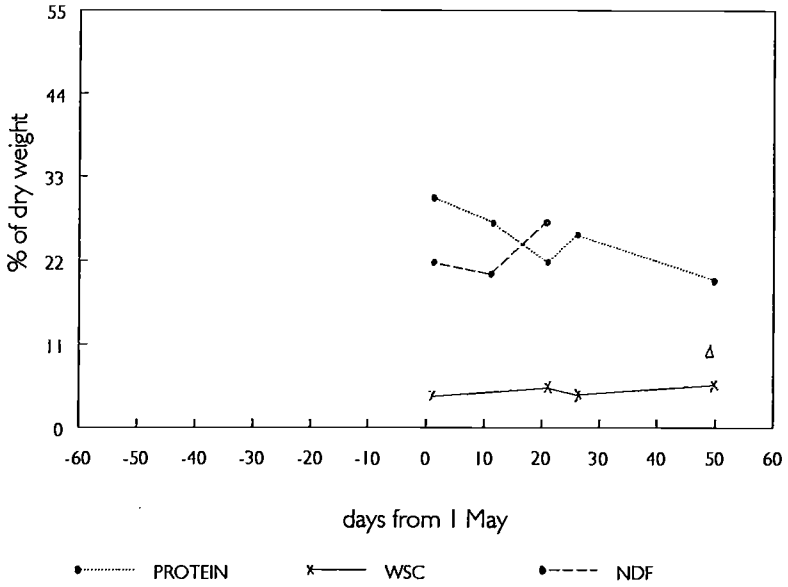
ASTER

protein, WSC and NDF



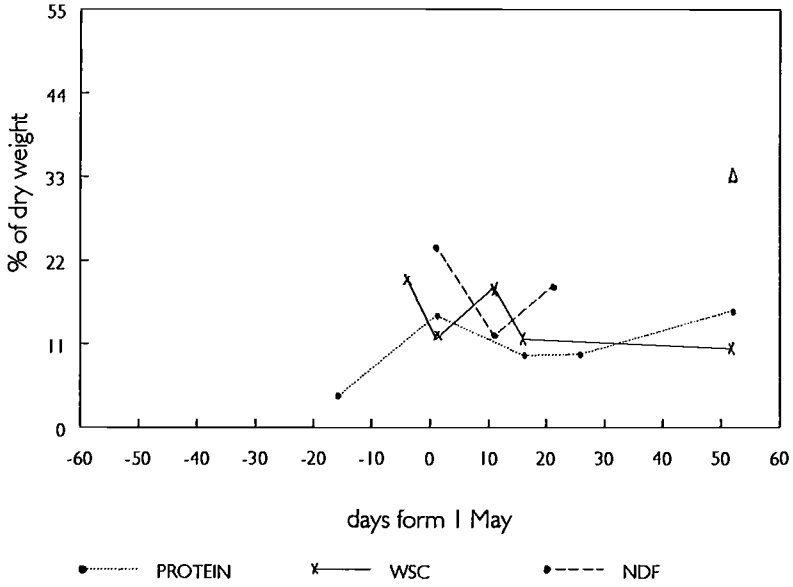
LIMONIUM

protein, WSC and NDF



ENTEROMORPHA

protein, WSC and NDF



Appendix 10

Oplosbare Koolhydraten / Water Soluble Carbohydrates

VOORBEREIDINGEN:
*De glucose-basis oplossing kan 1of meer dagen voor de bepalingsdag aangemaakt worden. Het plantenmateriaal moet goed vermalen zijn tot 0.5 mm en moet worden voor gedroogd op 70° C. Zorg ervoor dat dit maalsel goed gemengd is !
*Gemalen plantenmateriaal ? uur in stoof: ½ uur in exicator
*In het waterbad kunnen 24 monsters staan. Dus 11 monsters in duplo + blanco in duplo.

Maak een glucose-basisoplossing: (1ml ≡ 0.25 mg glucose)
*droog de benodigde D-glucose bij 70° C
*los 0.250 gram (gedoogde) D-glucose op in 1 liter gedest. water.
*Deze glucose oplossing blijft enkele weken goed.

Maak Anthrone reagent:
*Nodig oa bril, handschoenen, zuurstofkast, bak met ijs en magnetische roerplaat.
*Voeg voorzichtig 760 ml geconsentreedrd H_2SO_4 toe aan 330 ml gedest water in een "kookfles" en hou dit mengsel koel tijdens het mengen.
*Voeg 1 gram Anthrone en 1 gram Thioura toe. Gebruik hiervoor een magnetische roerplaat.
* Breng dit mengsel over in een donkere fles en laat deze oplossing twee uur staan bij 1° C alvorens het te gebruiken. (Mengsel kan na bepaling enige tijd in koelkast bewaard worden)
*Het Anthrone reagents moet stro-kleurig of lichtgeel zijn. Als het donkerder wordt, met een groene tint, heeft oxidatie plaatsgevonden en moet de oplossing weggegooid worden

Maak een reeks glucose-standaardoplossing:
*pipetteer 0, 10, 20, 30 & 40 ml glucose-basisoplossing in (5 verschillende) 100 ml maatkolven. Vul deze aan met gedest. water.
*Maak deze ijkvloeistoffen dagelijks aan!

EXTRACTIE
*Weeg 100 mg gedroogd plantenmateriaal nauwkeurig af en breng het in een 100 ml maatkolf.
*Voeg 60 ml gedest water toe.
*Plaats deze maatkolf in een waterbad met trechter en knikker en laat deze zachtjes koken gedurende 2 uur. Beweeg af en toe deze oplossing *voorzichtig*.
*Vul de inhoud van de maatkolf aan met gedest water tot 60 ml.
*Laat de vloeistof enigzins afkoelen en filter de vloeistof door een filtreerpapier (Whattman 44) in een 100 ml maatkolf.
*Spoel het filtreerpapier met lauw water en vul de maatkolf aan met demi -water tot 100 ml.
*Laat een water blanco dezelfde behandeling ondergaan
*Dit extract kan niet over de nacht bewaard worden en moet bereid worden vlak voor de kleurontwikkelings bepaling!

KLEURONTWIKKELING:
*Pipetteer 2 ml van elke standaardoplossing in reageerbuizen.
*Pipetteer 2 ml van elk extract of water blanco in reageerbuizen.

Vanaf dit punt moeten standaardoplossingen en monsteroplossingen gelijk behandeld worden.

*Voeg snel 10 ml Anthrone reagents toe en mix zeer goed (vortex) terwijl de buis wordt gekoeld door stromend water (of in een waterbad).
*Plaats de reageerbuizen in een beker kokend water, in een verduisterde zuurstof kast (of verduister de beker met aluminiumfolie) en laat de oplossing 10 minuten koken.
*Plaats de buizen in koud water en laat ze afkoelen, liefst in het donker. (Kan in spoelbak met deksel)
*Meet lichtabsorptie op 625 nm met gedestileerd water als referentie.

BEREKENING:
Maak ijkcurve mbv de standaard oplossingen en gebruik deze om de hoeveelheid glucose te bepalen. Trek de gemiddelde waarden van de blancos ervan af (mits noodzakelijk)

Bepaling van het drogestofgehalte.

Om de verschillende componenten van het plantenmateriaal te kunnen geven als percentage van het 100 % droge materiaal moet men van elk monster het drogestofgehalte kennen.
Voorverwarmde porseleinen schaaltjes worden leeg gewogen.Het monster, welke van te voren zeer goed is gehomogeniseerd, wordt in de kroes gebracht en na minimaal 8 uur drogen bij 103 °C wordt het kroesje met gedroogd meteriaal weer gewogen.

Benodigdheden:
- -porseleinen schaaltjes
- -monsterlepel
- -analyseformulier
- -kroezentang
- -balans
- -droogstoof

Werkwijze:
- -Neem schone porseleinen schaaltjes en noteer de nummers op het analyseformulier. Plaats de schaaltjes gedurende 1 uur in de droogstoof bij 103 °C. Onderin de droogstoof moeten ± 8 schaaltjes staan om de balans op te warmen.
- -Om de balans op te warmen wordt een schaaltje op de balans geplaatst gedurende 10 à 20 seconden. Na het verwijderen van het kroesje wordt het nulpunt opnieuw ingesteld. Dit wordt zo lang herhaald totdat het nulpunt niet meer veranderd (ca. 5 maal).
- -Weeg de lege schaaltjes één voor één en noteer de gewichten (leeg gew.). Controleer regelmatig of het nulpunt niet verandert, zonodig bijstellen.
  De stoof blijft aanstaan tijdens het wegen.
  De deur steeds voorzichtig openen en sluiten.
  Probeer in een constant tempo te wegen.
- -Weeg 1,5 - 2 gram monster af in het schaaltje en druk op "print". Toets de monsterkode in op de balans en druk weer op "print". Plaats de schaaltjes in dezelfde volgorde terug op het blad. Voor een duplo-bepaling moet elk monster 2 maal worden afgewogen (niet achterelkaar maar in een andere serie).
- -De schaaltjes met monster worden in de droogstoof gezet en na tenminste 8 uur drogen bij 103 °C ('s nachts) op bovenstaande manier teruggewogen (droog gew.).
- -De schaaltjes met monster bewaren voor de asbepaling.

Berekening:

$$\%drogestof = \frac{droog\ gew. - leeg\ gew.}{inweeg\ gew.} * 100\%$$

Alle gewichten zijn in grammen.
Het verschil tussen de duplo's mag niet groter zijn dan 0,2 % absoluut.

Bepaling van het asgehalte.

Het monster wordt gegloeid bij 550 °C. Bij deze temperatuur wordt alle organische stof verbrand. De gasvormige produkten die ontstaan worden afgevoerd. In het schaaltje blijven alleen de mineralen (as) en (indien aanwezig) zand over.

Benodigdheden:
- -porseleinen schaaltjes (indien mogelijk schaaltjes met monster van de drogestof bepaling).
- -moffeloven
- -droogstoof
- -balans
- -kroezentang (lang)
- -hittebestendige handschoenen

Werkwijze:
- Plaats de kroezen (max. 45) op volgorde in de moffeloven, op de metalen slede. Sluit de deur en zet de oven aan.
- Laat de schaaltjes gedurende 3 uur verassen bij 550 °C. (let op het opwarmen van de moffel is niet bij deze tijd inbegrepen).
- Zet vervolgens de schaaltjes op volgorde op een blad en plaats deze in de droogstoof bij 103 °C.
- Na 1 uur zijn de schaaltjes voldoende afgekoeld en kunnen gewogen worden. (as gew.)

Berekening:

$$\%\ as \in droge\ stof = \frac{as\ gew. - leeg\ gew.}{inweeg\ gew. * \frac{\%\ DS}{100}} * 100\ \%$$

Alle gewichten zijn in grammen.

De toegestane verschillen zijn:

gehalte	nauwkeurigheid
< 5 %	0,5 % abs.
5-10 %	10 % rel.
> 10 %	5 % rel.

Bepaling van N.D.F..

Het monster wordt gekookt in een buis met N.D.R. (neutral detergent reagent)-oplossing. De celinhoud lost hierdoor op. Na filtreren blijft de celwandfractie, N.D.F. (neutral detergent fiber) achter in de kroes. Deze fractie wordt uitgespoeld, gedroogd en gewogen.

Benodigdheden:
- buizen van 250 ml.
- kookblok
- glasfilterkroezen met manchetten (kroezen G 2)
- standaard met afzuigtrechters
- kookblok
- droogstoof en kroezentang
- balans
- buisjes met ijs
- aceton

Werkwijze:

- Homogeniseer het monster en weeg 0,9 gram af op het weegschuitje met lange hals en breng het monster over in een 250 ml. buis met behulp van een kwastje.
- Voeg m.b.v. een dispenser 100 ml. N.D.R.-oplossing toe aan elke buis.
- Zet het kookblok op 170 °C en plaats bijv. elke 5 minuten een buis in het kookblok. Hang er een buisje met ijs in (dit om verdamping tegen te gaan).
- Noteer van elke buis het tijdstip van koken.
- Vervang het buisje met ijs na 30 minuten koken.
- Na totaal 60 minuten gekookt te hebben wordt het monster gefiltreerd over een glasfilterkroes welke geplaatst is op een afzuigtrechter met manchet (op de eerste rij).
- Spoel de restanten van het monster uit de buis over met heet demiwater. De glasfilterkroes wordt nog drie maal gespoeld door deze voor driekwart te vullen met heet demiwater en vervolgens door te spoelen.
- Plaats de glasfilterkroes op een trechter van de achterste rij en spoel het monster twee maal door met een beetje aceton. Vul vervolgens het kroesje voor de helft met aceton en laat dit tenminste één minuut staan.
- Plaats de kroezen in de droogstoof gedurende 8 uur bij 103 °C.
- Weeg de kroezen één voor één zoals beschreven staat bij de drogestof bepaling.
- Plaats de kroezen (max. 45) op volgorde in de moffeloven, op de metalen slede. Sluit de deur en zet de oven aan.
- Laat de schaaltjes gedurende 3 uur verassen bij 550 °C. (let op het opwarmen van de moffel is niet bij deze tijd inbegrepen).
- Zet vervolgens de schaaltjes op volgorde op een blad en plaats deze in de droogstoof bij 103 °C.
- Na 1 uur zijn de schaaltjes voldoende afgekoeld en kunnen gewogen worden (as gew.).

Berekening:

% celwand in de organische stof

$$\% NDF = \frac{droog.\ gew. - as\ gew.}{inweeg\ gew. * \frac{\%\ ds.}{100} * \frac{\%\ cs.}{100}} * 100\ \%$$

Alle gewichten zijn in grammen.

Benodigde oplossingen:

N.D.R.-oplossing met EDTA.
- 465,25 gram Na_2EDTA (Titriplex III) (T2) in 2 maal 2 liter demi-water (onder verwarmen).
- 170,25 gram $Na_2B_4O_7.10H_2O$ (natrium boraatdecahydraat)(borax) (N11) in 2 maal 2 liter demi-water (onder verwarmen).
- 114,00 gram Na_2HPO_4 (natrium monowaterstoffosfaat) (N6) in 2 maal 2 liter demi-water (onder verwarmen).
  Of 142.90 gram $Na_2HPO_4.2H_2O$ (natrium monowaterstoffosfaat) (N6) in 2 maal 2 liter demi-water (onder verwarmen).
  Of 287,41 gram $Na_2HPO_4.12H_2O$ (natrium monowaterstoffosfaat) (N6) in 2 maal 2 liter demi-water (onder verwarmen).
- Drie porties van 250 gram $C_{12}H_{25}NaSO_4$ (dodecyl natriumsulfaat) (D2) afwegen in drie bekerglazen en oplossen in warm demiwater.
- Alle oplossingen m.b.v. een trechter overbrengen in het vat van 25 liter.
- Om schuimen te beperken 250 ml. 2-ethoxy ethanol (E2) in porties toevoegen.
- Vul aan tot ca. 24 liter en laat afkoelen.
- Vul aan tot 25 liter en meng goed.
- Meet de pH, deze moet tussen de 6,9 en 7,1 zijn.
- Zonodig korrigeren met 0,1 N H_2SO_4 of 0,1 N NaOH.

WERKWIJZE

Van de te onderzoeken monsters ruwvoer wordt 0.5 g gemalen lucht droog materiaal (deeltjesgrootte < 1 mm) nauwkeurig afgewogen en in centrifugebuizen overgebracht. Hetzelfde wordt in triplo gedaan met een standaardmonster van tamelijk hoge en met een standaardmonster van tamelijk lage verteerbaarheid.
Van beide standaardmonsters is de verteringscoëfficient voor de organische stof berekend als gemiddelde van tenminste 10 bepalingen in triplo. Met elke serie worden tevens 3 blanco bepalingen meegenomen.
De buizen worden in een stoof van 38-39 °C voorgewarmd. Van de fosfaat-bicarbonaat buffer wordt 2.5 liter tot 38-39 °C verwarmd in een waterbad, waarna de pH van de oplossing op 6.9 gebracht wordt door er een flinke stroom CO_2-gas door te leiden (ca. 20 min). Tijdens het CO_2-doorleiden wordt de buffer goed gemengd met een roermotor.
Om ... uur (2 uur na het voeren) wordt van twee fistelhamels ca. 1 liter (elke hamel 500 ml) pensvloeistof genomen, die direct in van te voren tot 38-39 °C verwarmde termosflessen wordt overgebracht. Direct daarna wordt de pensvloeistof door dubbel kaasdoek gefiltreerd in een stoof van 38-39 °C. De laatste resten vloeistof worden uit de kaasdoek geperst, zodat daarin slechts een droge massa achterblijft.
Aan de gefiltreerde pensvloeistof wórdt 625 ml aan de met CO_2 verzadigde fosfaat-bicarbonaat buffer toegevoegd. Het mengen van de vloeistof en het CO_2-inleiden worden voortgezet.
Aan elk monster ruwvoer wordt nu met de automatische pipet 50 ml van het mengsel pensvloeistof-buffer toegevoegd, waarna de buis snel wordt afgesloten en in een stoof van 38-39 °C geplaatst. Na een uur wordt de inhoud van de buizen goed gemengd, zorg ervoor dat zo weinig mogelijk vaste deeltjes aan de glaswand blijven plakken.
Gedurende de incubatietijd van 46 uur wordt de inhoud van de buizen 2 maal per dag gemengd en wel om 8 en om 17 uur. Na de incubatie met pensvloeistof-buffer wordt met een verdeelpipet aan elke buis 5 ml Na_2CO_3 oplossing 10 % toegevoegd, gemengd en gedurende 15 min. gecentrifugeerd bij 2500 t.p.m. De bovenstaande vloeistof wordt afgeschonken over eens stukje nylondoek, dat op een Buchnertrechter (doorsnede 6 cm) is bevestigd.
De deeltjes, die op het nylon achterblijven, worden met pepsine-zoutzuur (38-39 °C) via een trechter met korte steel in de buis teruggespoten.
De deeltjes, die aan de glaswand kleven, worden met een glasstaaf, voorzien van een rubber wissertje, los gemaakt en de wand met pepsine-zoutzuur (38-39 °C) schoongespoeld.
Het residu op de bodem van de buis wordt met een glasstaaf losgewerkt en goed gemengd met de pepsine-zoutzuur, waarna de buis met pepsine-zoutzuur (38-39 °C) wordt aangevuld tot 50 ml.
De inhoud van de buis wordt gemengd, waarna de buis wordt afgesloten en in een stoof van 38-39 °C geplaatst. Tweemaal per dag (om 8 en 17 uur) wordt de inhoud weer gemengd.

GLASFILTERKROEZEN.
Alleen deze kroezen dienen te worden gebruikt i.v.m. verassen bij hoge temp.

CENTRIFUGE
2500 T.P.M
Maximaal 8 buizen van 100 ml

THERMOSFLESSEN (500 ML)

ROERMOTOR
Deze moet voorzien zijn van een roerder met vrij grote schoepen, zodat bij een lage snelheid van de motor de vloeistof toch goed gemengd wordt. Een hoge motorsnelheid kan beschadiging van de micro-organismen tot gevolg hebben.

VERDEELPIPET VAN 50 ML
Met onderverdelingen van elke 5 ml.

MAATCYLINDER, 500 ML

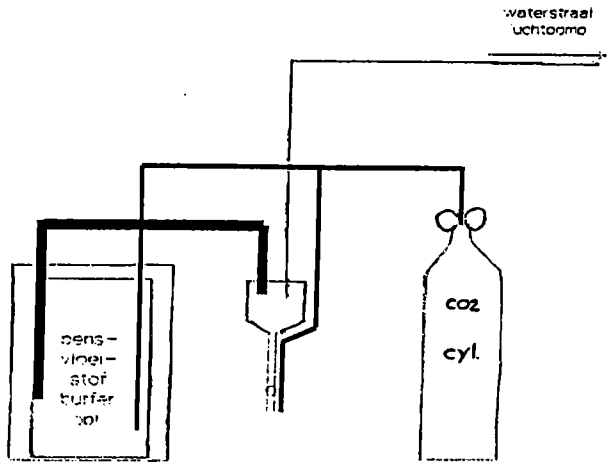
KAASDOEK, MET EEN FIJNHEID VAN CA. 200 OPENINGEN PER CM^2
NYLONDOEK
Zeer fijn geweven.(bv. voeringstof)

TRECHTER
Bovenzijde ca 6 cm; lengte steel ca. 1 cm.

TRECHTER
Doorsnede bovenzijde ca 15 cm.

ROERSTAAF
Met rubber wisser.

SITUATIE TEKENING:



****
VERTEERBAARHEID"IN VITRO" VAN GROENVOER (Tilly en Terry)
****

PRINCIPE VAN DE METHODE:

De techniek bootst de twee fasen na van het verteringsproces van de herkauwer. Groenvoer wordt eerst verteerd met anaerobe micro-organismen uit de pens van een fistelschaap en dan met pepsine-zoutzuur. Tijdens de eerste fase worden de celwand-koolhydraten (cellulose en hemi cellulose) door de enzymen van de pens-micro-organismen gedeeltelijk omgezet in oplosbare produkten. De meeste van de overige bestanddelen worden eveneens voor een deel afgebroken in deze fase.
De afbraak van het eiwit vindt slechts voor een gering deel plaats; daarom is een tweede fase, behandeling met zoutzuur en het proteolytische enzym pepsine, noodzakelijk om de eiwitten om te zetten in in water oplosbare produkten.
De niet opgeloste stof wordt afgefiltreerd, gedroogd en gewogen, waarna de verteringscoëfficient kan worden berekend.

REAGENTIA EN BENODIGDHEDEN:

FOSFAAT-BICARBONAAT BUFFER.
Deze wordt gemaakt van 3 moederoplossingen.
Oplossing 1: 46,5 g $Na_2HPO_4.12H2O$ (potnr.)
49,0 g $NaHCO_3$ (potnr.)
2.35 g NaCl (potnr.)
2.85 g KCl (potnr.)
Deze 4 stoffen worden opgelost in water en aangevuld tot 1 liter.
Oplossing 2 : $MgCl_2$-oplossing (6%): 12,8 g/100 ml (potnr.)
Oplossing 3 : $CaCl_2$-oplossing (4%) : 5,3 g/100 ml (potnr.)
Bereiding voor ca. 45 monsters :
500 ml oplossing 1 + 5 ml oplossing 2 + 5 ml oplossing 3
hierna met water aanvullen tot 3,5 liter.

PEPSINE-ZOUTZUUR OPLOSSING.
5 g pepsine (1:10.000) wordt opgelost in 2250 ml water. Aan deze oplossing wordt 250 ml HCl (1 N) toegevoegd (voor ca.45 bepalingen). Bereiding voor 1 liter HCl (1 N):
Voeg 85 ml toe aan ca. 800 ml water en vul aan tot 1 liter.
N.B. De oplossing is niet stabiel en moet steeds vers worden bereid voor iedere reeks bepalingen.

Na_2CO_3-OPLOSSING 10 %
Los 7 gram op in 100 ml water (potnr.)

PENSVLOEISTOF
Voor de levering van de pensvloeistof zorgen twee van een

APPARATUUR EN HULPMIDDELLEN

CENTRIFUGEBUIZEN (inw. doorsnede 3.2 cm; hoogte 11 cm.)

De buizen worden afgesloten met rubber stoppen, voorzien van een gas uitlaat ventiel, gemaakt naar het principe van het Bunson ventiel. Een kort stukje capillair (uitw. 0 4.5 mm; inw. 0 o.5 mm) wordt door een doorboorde stop gestoken, zodat de onderkant van het capillair gelijk is met de onderkant van de stop, terwijl de andere kant ca. 1.5 cm boven de stop uitsteekt.
Een stukje rubberslang van 4.5 cm (wanddikte: 2 mm) wordt over het uitstekende capillair geschoven, terwijl het boveneinde van de slang wordt afgesloten door een stukje glasstaaf.
In het midden van de rubber slang wordt een korfje van 6 mm gemaakt in de lengte richting van de slang. Dit gleufje is normaal gesloten en gaat alleen open om gas uit de buis te laten ontsnappen. Op elke centrifugebuis wordt een merkteken aangebracht op de plaats, waar het vloeistofoppervlak zich bevindt als de buis met 50 ml vloeistof is gevuld.

AUTOMATISCHE PIPET EN VOORRAADFLES (zie fig. 8)

Door opening A met de vinger af te sluiten wordt het mengsel van pensvloeistof en buffer in de pipet gezogen tot boven niveau B. Nadat de vinger bij A is weggenomen loopt de vloeistof terug in de voorraadfles, totdat in de pipet het niveau B is bereikt. De hoeveelheid vloeistof, die in de pipet blijft, kan worden geregeld door buis C naar boven of naar beneden te bewegen. Door kraan D te openen kan de vloeistof aan het te onderzoeken monster groenvoer worden toegevoegd.
Het vacuum wordt geregeld met een kraan en mag niet te sterk zijn, daar de vloeistof dan met te grote kracht in de pipet wordt gezogen, waardoor schuim ontstaat.

WATERBAD
Deze wordt ingesteld op 38-39 °C. De afmetingen van het waterbad moeten zodanig zijn, dat de hiervoor genoemde voorraadfles er in geplaatst kan worden.

CYLINDER MET CO_2-GAS
Deze moet voorzien zijn van een reduceerventiel.

DROOGSTOOF (103-105 °C)

BROEDSTOOF (38-39 °C)

MOFFELOVEN (550 °C)

BALANS
Met een gevoeligheid van 0.1 mg

Na 46 uur worden de buizen gedurende 15 min. gecentrifugeerd bij 2500 t.p.m., waarna weer wordt afgeschonken door nylondoek. De vaste deeltjes op het nylon worden met water in de buis terug gespoten. De inhoud van de buis wordt afgefiltreerd door een van te voren gegloeid (1,5 uur bij 550 °C) glasfilterkroesje. De vaste deeltjes, die aan de glaswand kleven, worden met een glasstaaf, voorzien van een wisser losgemaakt en met water in het kroesje overgespoeld.
De kroesjes worden gedurende 1 nacht bij 103-105 °C gedroogd (45 min in de exicator) en gewogen. Daarna wordt de inhoud gedurende 1,5 uur bij 550 °C verast waarna de kroesjes weer worden gewogen.

BEREIDING VAN DE OPLOSSINGEN

OPL.1	CHEMICALIEN	POTNR	NODIG VOOR 3L	NODIG VOOR 2.5 L
	$Na_2HPO_4 \cdot 12H_2O$ 2 H_2O	N16	139.5 G	116.25 G 57.80 N-16
	$NaHCO_3$	N14	147.0 G	122.5 G
	NaCl	N24	7.05 G	5.88 G
	KCl	K26	8.55 G	7.13 G

OPL.2	NODIG VOOR 100 ML
$MgCl_2$ $6H_2O$ (6%)	12.8 G

OPL.3	NODIG VOOR 100 ML
$CaCl_2$ $2H_2O$	5.3 G

		NODIG VOOR 1L	NODIG VOOR 2.5 L
Na_2CO_3 (10%)		10.6 G	26.5 G

		VOOR 2.5 L	VOOR 2 L	VOOR 1.5 L
HCl-opl	HCl (37%)	208 ML	167 ML	126 ML

BENODIGDHEDEN VOOR CA 120 BEPALINGEN

EERSTE DAG:
Bij het inzetten heb je een begin volume nodig van 12,5 liter

2100 ml oplossing 1
21 ml oplossing 2 --------> aanvullen tot 10,5 liter
21 ml oplossing 3

Aan deze oplossing wordt 25 g tripticase toegevoegd, vervolgens 2 liter penssap zodat je op een totaal volume komt van 2.5 liter.

DERDE DAG:
1100 ml Na_2CO_3-opl----> 5 ml/buis

5 g pepsine in 2250 ml water en hieraan 250 ml HCl 1N toevoegen. Deze opl. wordt 3 maal bereid.

BEREKENING

$$V = 100 - \frac{10.000 * (E - C - B)}{P * D(1 - 0.01 * A)}$$

WAARIN:

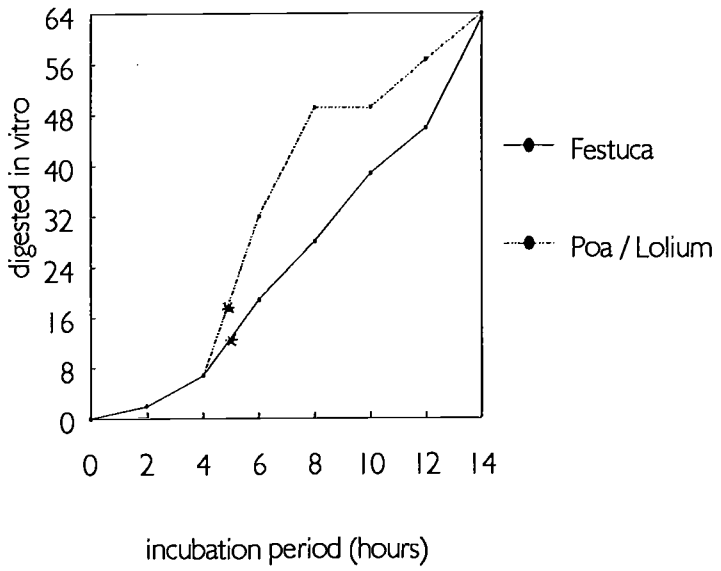
A = % AS IN DE DROGE STOF VAN HET MONSTER.
B = VERSCHIL VAN HET GEMIDDELDE GEWICHT VAN DE DROGE RESIDU'S EN DE GEGLOEIDE RESIDU'S VAN DE BLANCO BEPALINGEN IN GRAMMEN
C = GEWICHT VAN HET KROESJE+RESIDU NA HET GLOEIEN IN GRAMMEN.
D = % DROGE STOF IN HET MONSTER
E = GEWICHT VAN HET KROEJE+RESIDU NA HET DROGEN IN GRAMMEN
P = AFGEWOGEN HOEVEELHEID STOF IN GRAMMEN
V = VERTERINGSCOEFFICIENT

De verteringscoëfficiënten voor de organische stof van de standaardmonsters, die berekend zijn als gemiddelde van tenminste 10 bepalingen in triplo, worden grafisch uitgezet tegen de gemiddelde verteringscoëfficiënten van de standaard monsters, die gelijk met de serie te onderzoeken monsters zijn bepaald.
De rechte lijn, die door de gevonden punten wordt getrokken, geeft het verband aan tussen de werkelijke en de gevonden verteringscoëfficiënten van de onderzochte monsters. Met behulp van deze lijn worden de gevonden verteringscoëfficiënten gecorrigeerd.
De verterings coëfficiënten worden gerapporteerd tot op 0.1.
Het maximum toelaatbare verschil tussen de duplo's bedraagt 2 eenheden.

Appendix 11

Berekening in vitro waarden:

Tijdens de in vitro bepaling volgens Tilly & Terry wordt plantmateriaal 2 *24 uur geincubeerd. Na aanleiding van de gegevens verzameld door W. van Marken Lichtenbelt (1981) kozen we voor 6 uur incubatie. Zie ook figuur 1. De *-jes in de figuur zijn gevonden vivo verteringswaarden. Verwacht werd dat de op deze wijze verkregen data, de vertering van het plantenmateriaal door de rotganzen, het beste zou benaderen. Verder werd ook verwacht dat de verschillen tussen de verteerbaarheid van de onderzochte plantensoorten beter zichtbaar zouden zijn bij 6 uur ipv 48 uur incubatie.



*Figuur 1 In vitro verteringwaarden van Festuca en Poa /Lolium bij verschillende incubatie tijden. *-tjes geven gevonden vivo verteringswaarden weer. (Marken Lichtenbelt, 1981)*

Omdat bij de in vitro bepaling volgens Tilly & Terry gebruik gemaakt wordt van penssap van levende runderen, corrigeert men de aan het eind gevonden data voor verschillen in penssap. Verschillen in penssap agv verschillende individuen; hooi of gras gevoed etc Dit gebeurt mbv ijkmateriaal waarvan de vitro-verteringswaarden bekend zijn en die mee genomen worden tijdens de bepaling: Wilgentakken [63809], vers gras [1475], beheershooi [1593] en Engels raai gras [1611]. Om de correctie voor verschillen in penssap ook bij 6 uur incubatie toe te kunnen passen is de volgende berekening uitgevoerd:

LUW file:
6 uur incubatie tijd nr 15 t/m 88
24 uur incubatie tijd nr 89 t/m 102
30 uur incubatie tijd nr 103 t/m 116
48 uur incubatie tijd nr 117 t/m 130
48+ uur incubatie tijd nr 131 t/m 144

Vitro 6 uur - Vitro 48 uur
Beide bekend, tegen elkaar uitgezet.
Regressie lijn: Y=0.85X - 7.18 [R^2= 0.88, N=6, Y=vitro 6, X=vitro 48]

Aangenomen dat verhouding tussen **Vitro 6 uur - Vitro 48 uur**
gelijk is aan verhouding tussen **Vivo 6 uur - Vivo 48 uur**
daarom nieuwe ijklijn; na berekening **Vivo 6** (0.85*E152-7.18) en verder
van x= vitro 6
y= in vivo 6

Y=0.83x +0.82 [R^2=0.87, N=6]

Met behulp van deze vergelijking kunnen de gevonden waarden van Festuca, Puccinellia ed bij 6 uur incubatie gecorrigeerd worden voor verschillen in penssap ten opzichte van andere in vitro bepalingen.

	VIVO 48 UUR	VITRO 48 UUR	VITRO 6 UUR	VIVO 6 UUR
Wilgentakken [63809]	31.9	35.6	25.64	19.94
Vers gras [1475]	75.3	80.3	56.76	56.82
Beheershooi [1593]	48.1	66.1	39.07	33.71
Engels raai gras [1611]	80.9	86.6	71.45	61.59
Hazebrokken	64.3	81.8	66.56	47.47
Luzernebrokken	54.9	59.6	47.08	39.49

Appendix 12

T1 EilPuc	X̄ cover %	SE	N	Date
Limvul	11,2	2,21	20	05-06-95
Glamar	2,25	0,94	20	05-06-95
Asttri	0,35	0,10	20	05-06-95
Artmar	4,25	2,94	20	05-06-95
Fesrub	1,17	0,77	20	05-06-95
Suemar	1,45	0,48	20	05-06-95
Junger	0	0	20	05-06-95
Pucmar	13,97	3,24	20	05-06-95
Placor	0	0	20	05-06-95
Plamar	0,62	0,14	20	05-06-95
Spaang	0,15	0,06	20	05-06-95
Atrpor	0,45	0,19	20	05-06-95
Atrpro	0	0	20	05-06-95
dood mat.	1,02	0,26	20	05-06-95
onbedekt	52,95	5,55	20	05-06-95
Agrsto	0	0	20	05-06-95
Armmar	0,02	0,02	20	05-06-95
Elyfar	0	0	20	05-06-95
Elyath	0	0	20	05-06-95
Spemar	6,1	2,51	20	05-06-95
Trimar	0	0	20	05-06-95
Salmar	0,77	0,16	20	05-06-95
Sagmar	0	0	20	05-06-95
Entero	2,7	1,28	20	05-06-95
Trirep	0	0	20	05-06-95
leont	0	0	20	05-06-95

WH10	X̄ cover %	SE	N	Date
Limvul	13,55	5,60	10	06-05-95
Glamar	0,65	0,28	10	06-05-95
Asttri	0,75	0,17	10	06-05-95
Artmar	7,65	3,90	10	06-05-95
Fesrub	21,2	7,19	10	06-05-95
Suemar	0,5	0	10	06-05-95
Junger	0,7	0,29	10	06-05-95
Pucmar	0,5	0,19	10	06-05-95
Placor	0	0	10	06-05-95
Plamar	6,8	2,38	10	06-05-95
Spaang	0,05	0,05	10	06-05-95
Atrpor	5,4	3,27	10	06-05-95
Atrpro	0	0	10	06-05-95
dood mat.	6,55	3,11	10	06-05-95
onbedekt	33,3	9,01	10	06-05-95
Agrsto	0,1	0,1	10	06-05-95
Armmar	0,05	0,05	10	06-05-95
Elyfar	0,05	0,05	10	06-05-95
Elyath	0	0	10	06-05-95
Spemar	2,5	1,06	10	06-05-95
Trimar	0	0	10	06-05-95
Salmar	2,2	1,43	10	06-05-95
Sagmar	0	0	10	06-05-95
Entero	0,75	0,40	10	06-05-95
Trirep	0	0	10	06-05-95
leont	0	0	10	06-05-95

FO9	X̄ cover %	SE	N	Date
Limvul	5,75	1,89	20	8/15-06-9
Glamar	2,82	2,22	20	8/15-06-9
Asttri	0,22	0,10	20	8/15-06-9
Artmar	8,47	4,21	20	8/15-06-9
Fesrub	46,92	7,28	20	8/15-06-9
Suemar	0,17	0,05	20	8/15-06-9
Junger	7,95	4,81	20	8/15-06-9
Pucmar	0,32	0,15	20	8/15-06-9
Placor	0,75	0,29	20	8/15-06-9
Plamar	6,4	0,99	20	8/15-06-9
Spaang	0	0	20	8/15-06-9
Atrpor	0,1	0,04	20	8/15-06-9
Atrpro	0,15	0,06	20	8/15-06-9
dood mat.	4,65	0,79	20	8/15-06-9
onbedekt	3,35	0,63	20	8/15-06-9
Agrsto	18,42	5,07	20	8/15-06-9
Armmar	1,25	0,23	20	8/15-06-9
Elyfar	0,57	0,49	20	8/15-06-9
Elyath	0,8	0,48	20	8/15-06-9
Spemar	0,5	0,15	20	8/15-06-9
Trimar	0	0	20	8/15-06-9
Salmar	0,05	0,03	20	8/15-06-9
Sagmar	0,1	0,05	20	8/15-06-9
Entero	0	0	20	8/15-06-9
Trirep	0	0	20	8/15-06-9
leont	0	0	20	8/15-06-9

T2 laag	X̄ cover %	SE	N	Date	T2 Eil	X̄ cover %	SE	N	Date	T2 F78	X̄ cover %	SE	N	Date
Limvul	6,77	2,38	20	4/13-6-95	Limvul	10,9	3,33	10	02-05-95	Limvul	8,34	2,90	19	7/12-06-9
Glamar	0,02	0,02	20	4/13-6-95	Glamar	1,3	0,40	10	02-05-95	Glamar	0,89	0,25	19	7/12-06-9
Asttri	0	0	20	4/13-6-95	Asttri	0,5	0,18	10	02-05-95	Asttri	0,39	0,11	19	7/12-06-9
Artmar	0	0	20	4/13-6-95	Artmar	30,55	8,97	10	02-05-95	Artmar	0,97	0,36	19	7/12-06-9
Fesrub	0	0	20	4/13-6-95	Fesrub	7	4,40	10	02-05-95	Fesrub	59,47	6,80	19	7/12-06-9
Suemar	0,27	0,05	20	4/13-6-95	Suemar	0,5	0	10	02-05-95	Suemar	0,36	0,11	19	7/12-06-9
Junger	0	0	20	4/13-6-95	Junger	1,2	0,98	10	02-05-95	Junger	23,31	4,25	19	7/12-06-9
Pucmar	9,17	3,75	20	4/13-6-95	Pucmar	0,8	0,2	10	02-05-95	Pucmar	0,18	0,10	19	7/12-06-9
Placor	0	0	20	4/13-6-95	Placor	0	0	10	02-05-95	Placor	0	0	19	7/12-06-9
Plamar	0	0	20	4/13-6-95	Plamar	2	0,93	10	02-05-95	Plamar	8,94	2,94	19	7/12-06-9
Spaang	1	0,53	20	4/13-6-95	Spaang	0,15	0,07	10	02-05-95	Spaang	0	0	19	7/12-06-9
Atrpor	0,02	0,02	20	4/13-6-95	Atrpor	0,15	0,07	10	02-05-95	Atrpor	0,07	0,04	19	7/12-06-9
Atrpro	0	0	20	4/13-6-95	Atrpro	0	0	10	02-05-95	Atrpro	0,05	0,03	19	7/12-06-9
dood mat.	1,15	0,27	20	4/13-6-95	dood mat.	2,4	1,41	10	02-05-95	dood mat.	15,57	3,92	19	7/12-06-9
onbedekt	81,45	4,76	20	4/13-6-95	onbedekt	38,9	12,9	10	02-05-95	onbedekt	3,39	0,85	19	7/12-06-9
Agrsto	0	0	20	4/13-6-95	Agrsto	0	0	10	02-05-95	Agrsto	4,44	1,87	19	7/12-06-9
Armmar	0	0	20	4/13-6-95	Armmar	0	0	10	02-05-95	Armmar	0,44	0,10	19	7/12-06-9
Elyfar	0	0	20	4/13-6-95	Elyfar	0	0	10	02-05-95	Elyfar	0,02	0,02	19	7/12-06-9
Elyath	0	0	20	4/13-6-95	Elyath	0	0	10	02-05-95	Elyath	0,10	0,04	19	7/12-06-9
Spemar	0,55	0,12	20	4/13-6-95	Spemar	0,45	0,05	10	02-05-95	Spemar	0	0	19	7/12-06-9
Trimar	0,1	0,04	20	4/13-6-95	Trimar	2,5	1,28	10	02-05-95	Trimar	0,02	0,02	19	7/12-06-9
Salmar	0,7	0,12	20	4/13-6-95	Salmar	0,5	0	10	02-05-95	Salmar	0,42	0,04	19	7/12-06-9
Sagmar	0	0	20	4/13-6-95	Sagmar	0	0	10	02-05-95	Sagmar	0	0	19	7/12-06-9
Entero	1,45	0,51	20	4/13-6-95	Entero	0,25	0,08	10	02-05-95	Entero	0	0	19	7/12-06-9
Trirep	0	0	20	4/13-6-95	Trirep	0	0	10	02-05-95	Trirep	0,36	0,31	19	7/12-06-9
leont	0	0	20	4/13-6-95	leont	0	0	10	02-05-95	leont	0	0	19	7/12-06-9
Carnig										Carnig	0.05	0.04		
Carare										Carare	0.03	0.03		
Carext										Carext	0.05	0.04		

T3 p45	$\bar{X}$ cover %	SE	N	Date	T3 Eilhoog	$\bar{X}$ cover %	SE	N	Date	T3 Fest	$\bar{X}$ cover %	SE	N	Date
Limvul	6,55	2,86	20	09-06-95	Limvul	22,22	7,15	10	21-06-95	Limvul	19,05	2,99	20	15/21-6-9
Glamar	0	0	20	09-06-95	Glamar	0,22	0,12	10	21-06-95	Glamar	1,60	0,34	20	15/21-6-9
Asttri	0	0	20	09-06-95	Asttri	0	0	10	21-06-95	Asttri	0,07	0,04	20	15/21-6-9
Artmar	0	0	20	09-06-95	Artmar	12,33	6,11	10	21-06-95	Artmar	12,76	4,43	20	15/21-6-9
Fesrub	0	0	20	09-06-95	Fesrub	0	0	10	21-06-95	Fesrub	30,55	7,07	20	15/21-6-9
Suemar	3,05	1,30	20	09-06-95	Suemar	1,55	0,22	10	21-06-95	Suemar	0,36	0,06	20	15/21-6-9
Junger	0	0	20	09-06-95	Junger	0	0	10	21-06-95	Junger	20,92	4,20	20	15/21-6-9
Pucmar	0,75	0,14	20	09-06-95	Pucmar	0,33	0,08	10	21-06-95	Pucmar	0,23	0,06	20	15/21-6-9
Placor	0	0	20	09-06-95	Placor	0	0	10	21-06-95	Placor	0	0	20	15/21-6-9
Plamar	0	0	20	09-06-95	Plamar	0	0	10	21-06-95	Plamar	1,47	0,49	20	15/21-6-9
Spaang	1,42	0,74	20	09-06-95	Spaang	0	0	10	21-06-95	Spaang	0	0	20	15/21-6-9
Atrpor	1,05	0,46	20	09-06-95	Atrpor	47,55	8,35	10	21-06-95	Atrpor	1	0,52	20	15/21-6-9
Atrpro	0	0	20	09-06-95	Atrpro	0	0	10	21-06-95	Atrpro	0,05	0,03	20	15/21-6-9
dood mat.	1,85	0,49	20	09-06-95	dood mat.	6,22	2,52	10	21-06-95	dood mat.	19,71	4,23	20	15/21-6-9
onbedekt	84,7	3,40	20	09-06-95	onbedekt	20,66	6,87	10	21-06-95	onbedekt	1,60	0,33	20	15/21-6-9
Agrsto	0	0	20	09-06-95	Agrsto	0	0	10	21-06-95	Agrsto	2	1,78	20	15/21-6-9
Armmar	0	0	20	09-06-95	Armmar	0	0	10	21-06-95	Armmar	0,26	0,10	20	15/21-6-9
Elyfar	0	0	20	09-06-95	Elyfar	0	0	10	21-06-95	Elyfar	0	0	20	15/21-6-9
Elyath	0	0	20	09-06-95	Elyath	0	0	10	21-06-95	Elyath	0,13	0,05	20	15/21-6-9
Spemar	0,07	0,03	20	09-06-95	Spemar	0,05	0,05	10	21-06-95	Spemar	0,07	0,04	20	15/21-6-9
Trimar	0,02	0,02	20	09-06-95	Trimar	1,05	0,42	10	21-06-95	Trimar	0,05	0,03	20	15/21-6-9
Salmar	1,5	0,19	20	09-06-95	Salmar	0,61	0,18	10	21-06-95	Salmar	0,26	0,05	20	15/21-6-9
Sagmar	0	0	20	09-06-95	Sagmar	0	0	10	21-06-95	Sagmar	0	0	20	15/21-6-9
Entero	0,45	0,12	20	09-06-95	Entero	0	0	10	21-06-95	Entero	0	0	20	15/21-6-9
Trirep	0	0	20	09-06-95	Trirep	0	0	10	21-06-95	Trirep	0,02	0,02	20	15/21-6-9
leont	0	0	20	09-06-95	leont	0	0	10	21-06-95	leont	0	0	20	15/21-6-9

Appendix 13

Energy gain per bite

Table 1 Average bite size for the period of May in mm/bite (or in mm^2/bite indicated with *)

species	Lim*	Ent	Atr*	Pla	Ast*	Spa	Spe	Tri	Ely	Jun	Fes	Puc
bite size	89.47	-	40.3	17.38	38.6	9	10.4	14	14	14	14.48	8.93
source	1	1	1	3	1	1	1	2	1	1	3	3

1. This study 2. Egas, 1995 3. Prop & Deerenberg, 1991

Festuca rubra as example of the calculation by with values of table 5 are obtained:

Dry weight per lenght	= 0.40 * 10-'	mg/mm
Bite size	= 14.48	mm
Bite weight	= 0.58	mg/bite

The percentage **NDF, WSC, Protein** and **Ash** of this plant species found were found with the chemical analyses. No lipid analysis was carried out and a constant value of **5%** for all plant species was assumed.

One bite so 0.58 mg Festuca rubra consist of

0.58/100*43.6 =0.253 mg NDF
0.58/100*22.7 =0.132 mg WSC
0.58/100*20.5 =0.119 mg Protein
0.58/100 *5 =0.029 mg Lipid
0.58/100 * 7 =0.041 mg Ash

Since geese retain their food for only a short period in the alimentary track, not all material can be digested. The Apparent Digestability (AD) of NDF is assumed to be 29%, WSC 8.5%, Nitrogen 62.5% and Lipid 28.6% (Buchsbaum, 1986). See also table1. The values chosen (⇨) are derived from one experiment and take a middle position between other values found in literature.

after digestion:

0.253/100* 29 = 0.07 mg NDF
0.132/100* 68.5 = 0.09 mg WSC
0.119/100* 62.5 = 0.07 mg Protein
0.029/100* 28.6 = 0.01 mg Lipid
0.041/100* 0 ⇨ can't be digested

Energy obtained from the plant material digested:
NDF 13.2 KJ/g (Buchsbaum, 1986). WSC 17.6 KJ/g, Protein 17.8 KJ/g and Lipid 39.3 KJ/g (Schmidt-Nielsen, 1975)

0.07 * 13.2= 0.924 Kjoule obtained from NDF
0.09 * 17.6= 1.584 Kjoule obtained from WSC
0.07 * 17.8= 1.246 Kjoule obtained from Protein
0.01 * 39.5= 0.395 Kjoule obtained from Lipid

Total energy obtained after digestion of 1 bite Festuca =
0.924 + 1.584 + 1.246 + 0.395=4.15 Kjoule

Appendix 14

Contributions of cell wall, soluble carbohydrates, protein and lipid to the total energy obtained of 1 gram of their plant food.
An example of the calulation by with values of table 6 page 21 are obtained:

Species		Perc	gram	Digested (gram)	KJoule	Perc
Fesrub	NDF	43,6	0,44	0,13	1,67	23,03
	WSC	22,7	0,23	0,16	2,74	37,76
	Protein	20,5	0,21	0,13	2,28	31,46
	Lipid	5	0,05	0,01	0,56	7,75
	Ash	7	0,07			
	Total	98,8	0,99		7,25	100

Perc = percentage NDF, WSC, Protein and Ash of this plant species found with the chemical analyses. No lipid analysis was carried out and a constant value of 5% for all plant species was assumed.

gram= 1 gram Festuca rubra consist of

1/100*43.6 =0.44 gram NDF
1/100*22.7 =0.23 gram WSC
1/100*20.5 =0.21 gram Protein
1/100 *5 =0.05 gram Lipid
1/100 * 7 =0.07 gram Ash

Digested (gram) = Since geese retain their food for only a short period in the alimentary track, not all material can be digested. The Apparent Digestability (AD) of NDF is assumed to be 29%, WSC 68.5%, Nitrogen 62.5% and Lipid 28.6% (Buchsbaum, 1986). See also table 1. The values chosen (⇨) are derived from one experiment and take a middle position between other values found in literature.
Based on 1 gram Festuca rubra the following mass will be available for a Brent after digestion:

0.44/100* 29 = 0.13 gram NDF
0.23/100* 68.5 = 0.16 gram WSC
0.21/100* 62.5 = 0.13 gram Protein
0.05/100* 28.6 = 0.01 gram Lipid
0.07/100* 0 ⇨ can't be digested

Kjoule = Energy obtained from the plant material digested:
NDF 13.2 KJ/g (Buchsbaum, 1986). WSC 17.6 KJ/g, Protein 17.8 KJ/g and Lipid 39.3 KJ/g (Schmidt-Nielsen, 1975),

0.13 * 13.2= 1.67 Kjoule obtained from NDF
0.16 * 17.6= 2.74 Kjoule obtained from WSC
0.13 * 17.8= 2.28 Kjoule obtained from Protein
0.01 * 39.5= 0.56 Kjoule obtained from Lipid

Total energy obtained after digestion of 1 gram Festuca =
1.67 + 2.74 + 2.28 + 0.56=7.25 Kjoule

Perc = Of the total energy obtained after digestion of 1 gram of Festuca rubra
1.67*100/7.25= 23.03 % derived from NDF
2.74*100/7.25= 37.76 % derived from WSC
2.28*100/7.25= 31.46 % derived from Protein
0.56*100/7.25= 7.75 % derived from Lipid

Table 1: Apperent Digestability of cellcomponents by Brent geese

Component	Percentage	Source
Protein	70.5 %	1984, Deerenberg
	61.55 %	1985, Deerenberg
	75.23 %	1986 WD, Deerenberg
	87.60 %	1986 Texel, Deerenberg
	79.5 % ± 2.6	Buchsbaum, 1986
➪	62.5 % ± 5.0	Buchsbaum, 1986
	80 %	Bruns, 1988
NDF	22.91 %	1984, Deerenberg
	28.44 %	1985, Deerenberg
	29.95 %	1986 WD, Deerenberg
	41.63 %	1986 Texel, Deerenberg
	18.0 % ±10.3	Buchsbaum, 1986
➪	29.0 % ± 5.6	Buchsbaum, 1986
Lipid	51.4 % ± 6.0	Buchsbaum, 1986
➪	28.6 % ±10.4	Buchsbaum, 1986
	27.3 %	Prop & Vullink, 1991
Sol carbohydrates (WSC)	79.7 % ± 1.2	Buchsbaum, 1986
➪	68.5 % ± 3.5	Buchsbaum, 1986
	40 %	Bruns, 1988
	50 %	Bruns, 1988

Note: Deerenberg used values from Weijand (1976), Van Marken Lichtenbelt (1981) and Buchsbaum (1986).

Appendix 15

Comparing ash values:

plant species	percentage	methods	period	source
Juncus gerardi	**6.3**	550 ° C for 3h	**April-May**	**This study**
Juncus gerardi	10.3 ± 1.5	500 ° C for 3h	April-May	Buchsbaum et al., 1986
Festuca rubra	**7**	550 ° C for 3h	**April-May**	**This study**
Elymus sp	**7.1**	550 ° C for 3h	**April-May**	**This study**
Puccinellia maritima	**8.9**	550 ° C for 3h	**April-May**	**This study**
Puccinellia maritima	7.9 ± 0.5	MAFF, 1986	Sept-Okt	Summers et al., 1993
Spartina anglica	**10**	550 ° C for 3h	**April-May**	**This study**
Spartina alterniflora	10.8	500 ° C for 3h	April-May	Buchsbaum et al., 1986
Limonium vulgare	**11.5**	550 ° C for 3h	**April-May**	**This study**
Limonium vulgare	14.2 ± 1.4	MAFF, 1986	Sept-Okt	Summers et al., 1993
Triglochin maritima	**17.4**	550 ° C for 3h	**April-May**	**This study**
Triglochin maritima	22.8 ± 0.7	MAFF, 1986	Sept-Okt	Summers et al., 1993
Aster tripolium	**19.1**	550 ° C for 3h	**April-May**	**This study**
Aster tripolium	22.2 ± 5.2	MAFF, 1986	Sept-Okt	Summers et al., 1993
Plantago maritima	**24.7**	550 ° C for 3h	**April-May**	**This study**
Plantago maritima	33.9 ± 2.4	MAFF, 1986	Sept-Okt	Summers et al., 1993
Spergularia maritima	**26.6**	550 ° C for 3h	**April-May**	**This study**
Atriplex portulacoides	**29.1**	550 ° C for 3h	**April-May**	**This study**
Atriplex portulacoides	36.5 ± 1.4	MAFF, 1986	Sept-Okt	Summers et al., 1993
Enteromorpha	**33.4**	550 ° C for 3h	**April-May**	**This study**
Enteromorpha	24.8 ± 4.2	MAFF, 1986	Sept-Okt	Summers et al., 1993

Appendix 16

Comparing soluble carbohydrates values:

plant species	percentage	methods	period	source
Festuca rubra	**22.8 % ± 2.2**	Anthrone	**April-May**	**This study**
Festuca rubra	18 %	Anthrone	fall	E.S.Bakker, 1997
	28 %	?		Amat, 1991
Juncus geradi	**19.5 % ± 2.2**	Anthrone	**April-May**	**This study**
Juncus geradi	9.3 % ± 0.2	Chromatography	April-May	Buchsbaum et al., 1986
Puccinellia maritima	**18.8 % ± 2.4**	Anthrone	**April-May**	**This study**
Puccinellia maritima	20.3 % ± 0.7	WSC, MAFF 1986	Sept-Okt	Summers et al., 1993
Elymus sp	**18.6% ± 2.6**	Anthrone	**April-May**	**This study**
Enteromorpha	**14.3% ± 1.9**	Anthrone	**April-May**	**This study**
Enteromorpha	3.2 % ± 0.9	WSC, MAFF 1986	Sept-Okt	Summers et al., 1993
Triglochin maritima	**8.9 % ± 0.5**	Anthrone	**April-May**	**This study**
Triglochin maritima	5.8 % ± 1.6	WSC, MAFF 1986	Sept-Okt	Summers et al., 1993
Spergularia maritima	**6.1 % ± 1.7**	Anthrone	**April-May**	**This study**
Plantago maritima	**6.0 % ± 0.5**	Anthrone	**April-May**	**This study**
Plantago maritima	3.5 % ± 0.3	WSC, MAFF 1986	Sept-Okt	Summers et al., 1993
Aster tripolium	**5.1 % ± 0.7**	Anthrone	**April-May**	**This study**
Aster tripolium	28.2 % ± 1.0	WSC, MAFF 1986	Sept-Okt	Summers et al., 1993
Spartina anglica	**5.0 % ± 1.2**	Anthrone	**April-May**	**This study**
Spartina anglica	22 %	?		Lytle & Hull, 1980
Spartina alterniflora	22 %	Chromatography		Briens & Lahrer, 82
	7 %	Anthrone	fall	E.S.Bakker, 1997
	11 % ± 0.2	Chromatography	April-May	Buchsbaum et al., 1986
Limonium vulgare	**4.9 % ± 0.3**	Anthrone	**April-May**	**This study**
Limonium vulgare	6.5 % ± 2.8	WSC, MAFF 1986	Sept-Okt	Summers et al., 1993
Atriplex portulacoides	**4.7 % ± 1.8**	Anthrone	**April-May**	**This study**
Atriplex portulacoides	5.4 % ± 0.9	WSC, MAFF 1986	Sept-Okt	Summers et al., 1993

Appendix 17

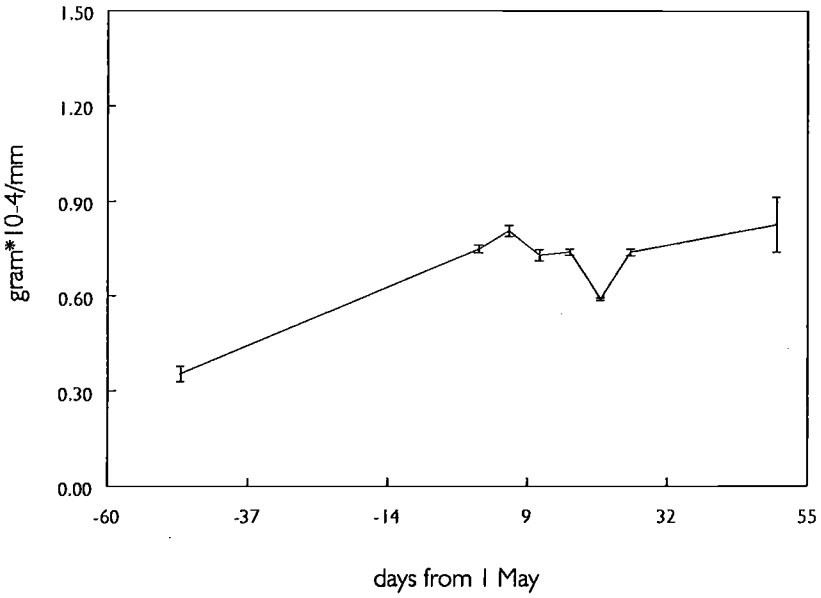
Comparing protein values:

plant species	percentage	methods	period	source
Triglochin maritima	**31.9 % ± 0.5**	Kjeldahl	**April-May**	**This study**
Triglochin maritima	40.1 %*	Kjeldahl	May	C. Deerenberg , 1985
Triglochin maritima	23.8 % ± 1.0	Kjeldahl	Sept-Okt	Summers et al., 1993
Limonium vulgare	**24.8 % ± 1.9?**	Kjeldahl	**April-May**	**This study**
Limonium vulgare	18.4 % ± 1.8	Kjeldahl	Sept-Okt	Summers et al., 1993
Puccinellia maritima	**24.5 % ± 1.6**	Kjeldahl	**April-May**	**This study**
Puccinellia maritima	23.5 % ± 4.1*	Kjeldahl	May	C. Deerenberg, 1985
Puccinellia maritima	15.0 % ± 0.9	Kjeldahl	Sept-Okt	Summers et al., 1993
Aster tripolium	**23.3 % ± 1.8**	Kjeldahl	**April-May**	**This study**
Aster tripolium	25.9 %*	Kjeldahl	May	C.Deerenberg, 1985
Aster tripolium	17.8 % ± 3.1	Kjeldahl	Sept-Okt	Summers et al., 1993
Elymus sp	**21.8 % ± 3.2**	Kjeldahl	**April-May**	**This study**
Spergularia maritima	**20.7 % ± 1.5**	Kjeldahl	**April-May**	**This study**
Festuca rubra	**20.5 % ± 2.3**	Kjeldahl	**April-May**	**This study**
Festuca rubra	25.8 % ± 0 .7	Kjeldahl	March-April	Faber, 1985
Festuca rubra		Kjeldahl		Koppel, 1996
Plantago maritima	**20 % ± 1.2**	Kjeldahl	**April-May**	**This study**
Plantago maritima	25.7 %*	Kjeldahl	May	C. Deerenberg, 1985
Plantago maritima	7.1 % ± 0.6	Kjeldahl	Sept-Okt	Summers et al., 1993
Atriplex portulacoides	**18.7 ?**	Kjeldahl	**April-May**	**This study**
Atriplex portulacoides	8.4 % ± 0.5	Kjeldahl	Sept-Okt	Summers et al., 1993
Juncus gerardi	**19.7 % ± 0.7**	Kjeldahl	**April-May**	**This study**
Juncus gerardi	13.9 % ± 0.3 *	Marks, 1985	April-May	Buchsbaum et al., 1986
Spartina anglica	**18.5 %**	Kjeldahl	**April-May**	**This study**
Spartina alterniflora	9.4 % ± 0.7 *	Marks, 1985	April-May	Buchsbaum et al., 1986
Enteromorpha	**10.6 % ± 2.1**	Kjeldahl	**April-May**	**This study**
Enteromorpha	29.3 % ± 3.2	Kjeldahl	Sept-Okt	Summers et al., 1993

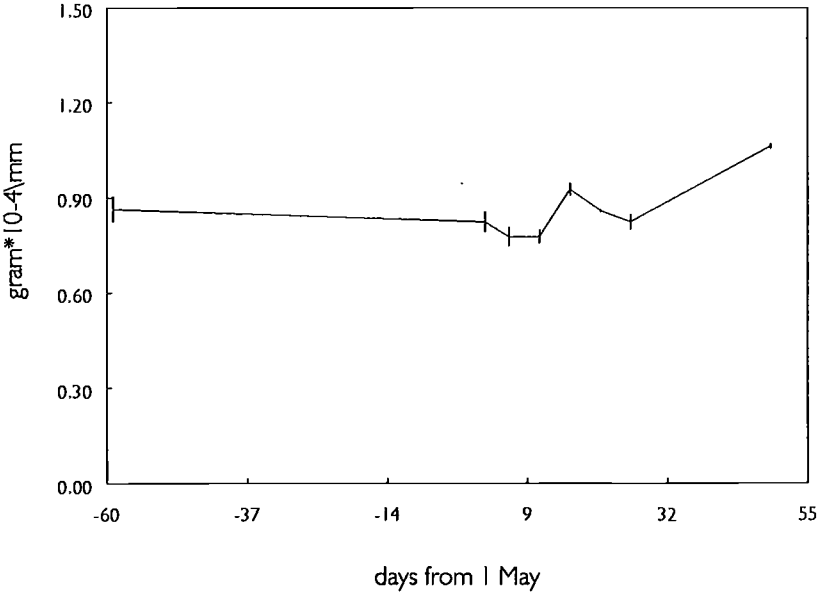
*based on ash free dry weight

Appendix 18

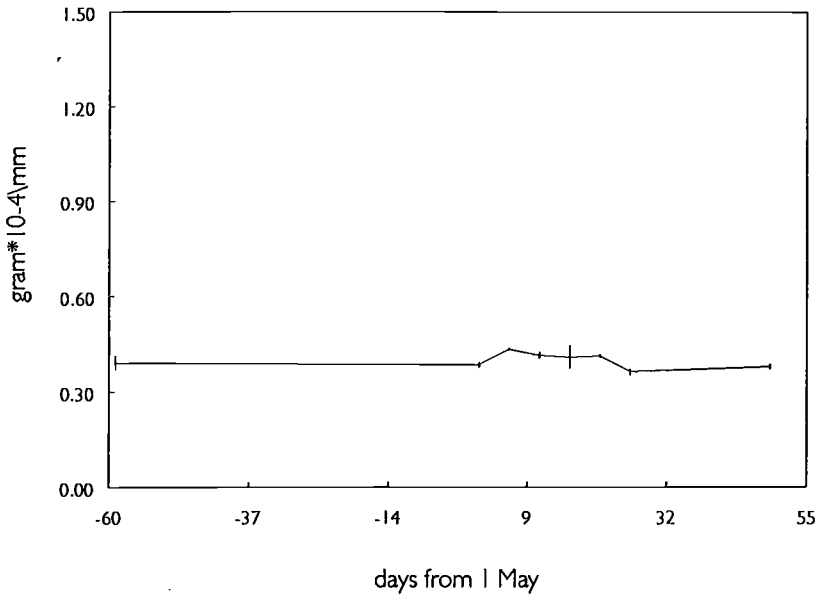
JUNCUS GERARDI



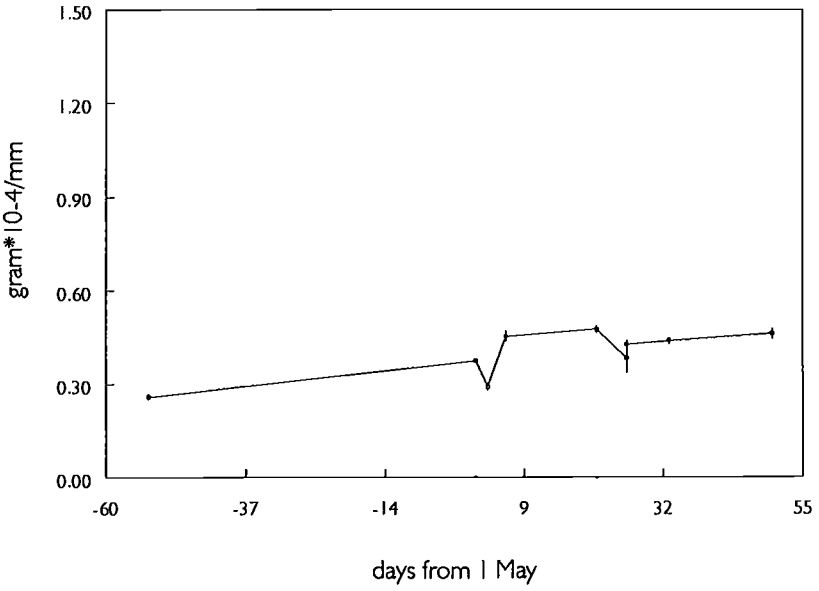
ELYMUS SP



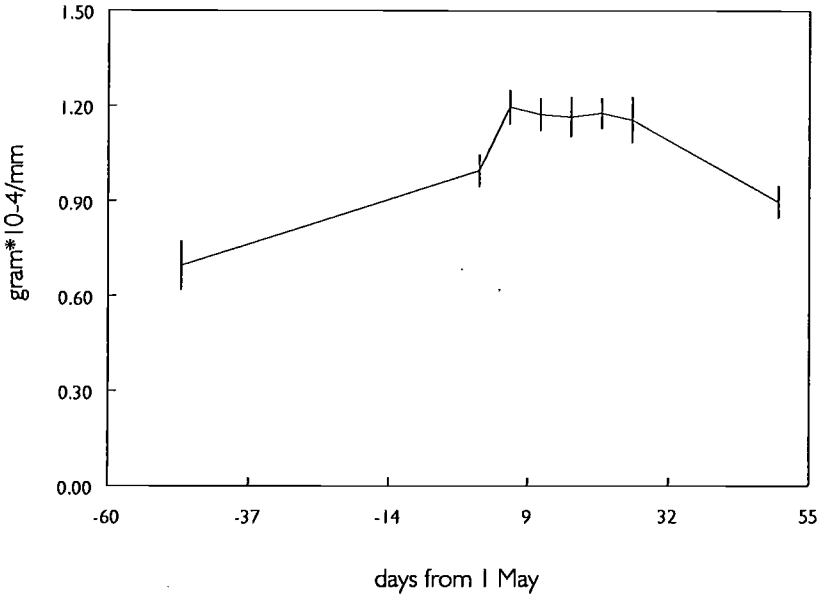
FESTUCA RUBRA



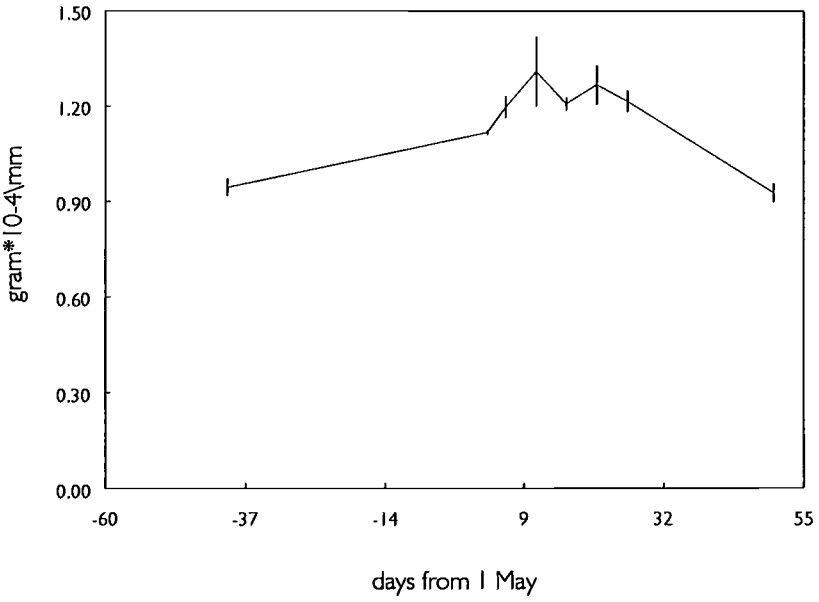
PUCCINELLIA MARITIMA



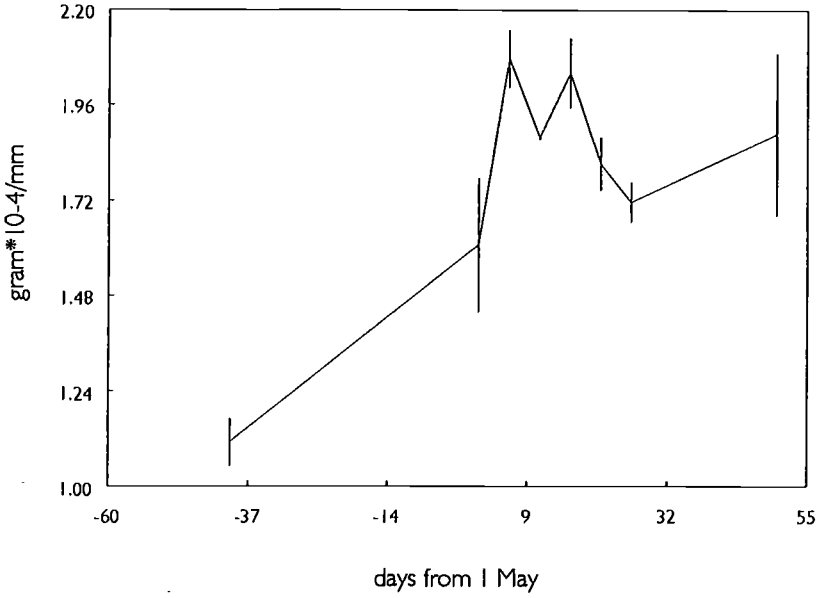
TRIGLOCHIN MARITIMA



SPERGULARIA MARITIMA

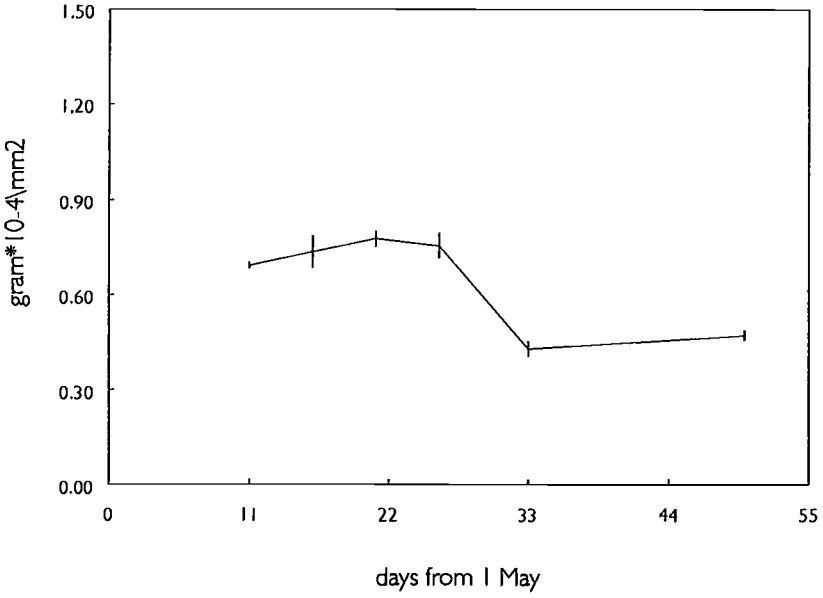


PLANTAGO MARITIMA

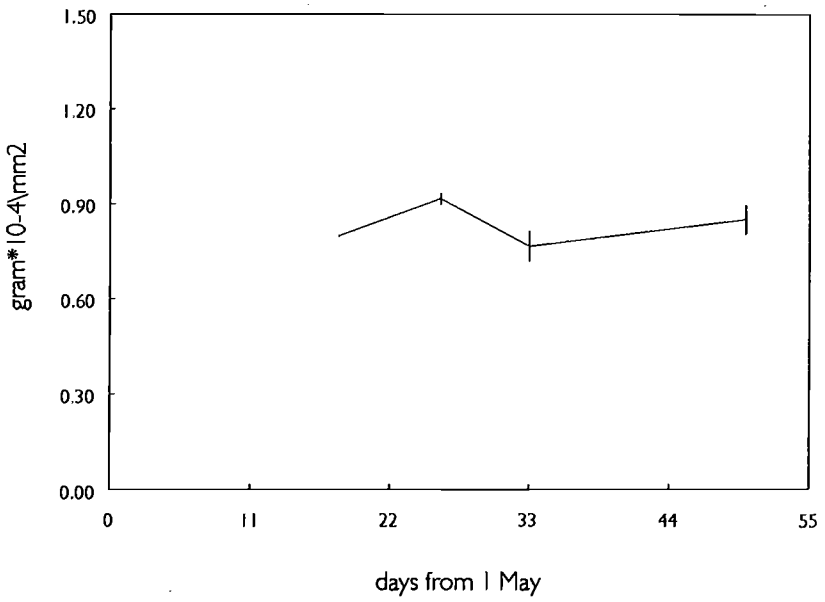


Note change in X and Y axis !

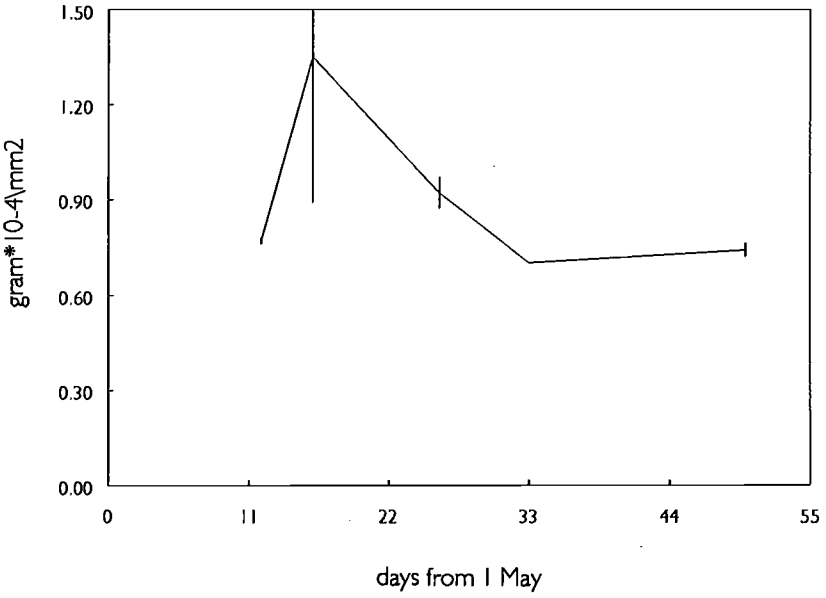
ASTER TRIPOLIUM



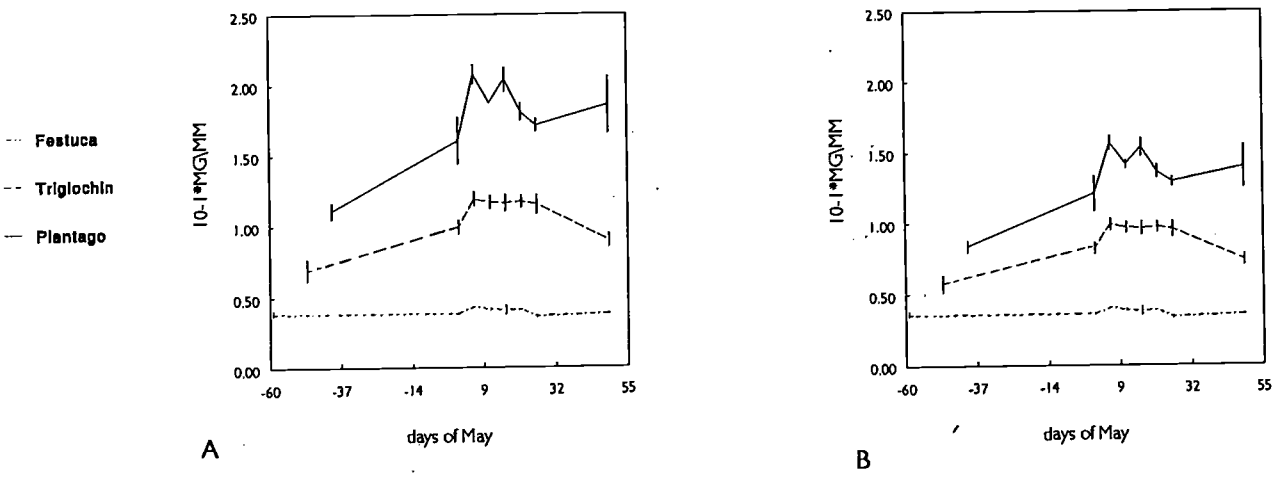
ATRIPLEX PROSTRATA



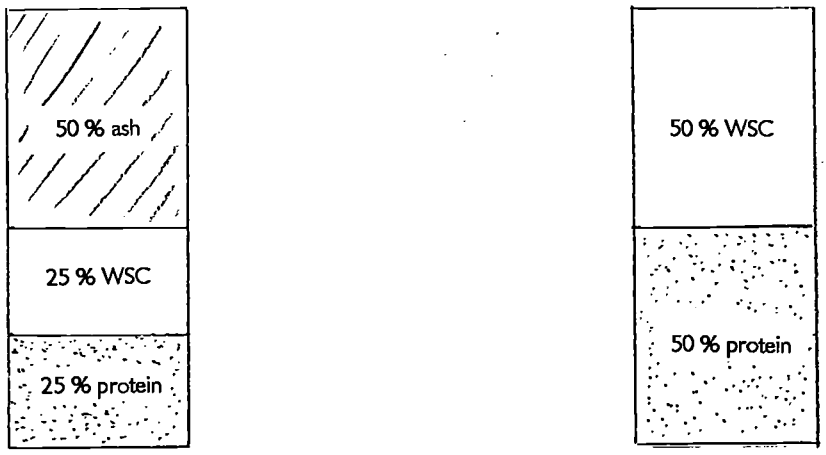
LIMONIUM VULGARE



Appendix 19



Dry weight (A) versus ash free dry weight (B) for *Plantago maritima*, *Triglochin maritima* and *Festuca rubra*



Effect on the percentges protein and WSC of plant species X after a correction for ash