Food preferences of the springtail *Cryptopygus antarcticus* from Signy Island

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Abstract

The Maritime Antarctic collembolan *Cryptopygus antarcticus* (Isotomidae) shows a high degree of dominance throughout the Antarctic Peninsula/Scotia Arc area (70-90% of the fauna in moss habitats (Tilbrook, 1970)).
The ecological significance of collembolan in the Antarctic and more especially *C. antarcticus* calls for a more detailed investigation of their role in this ecosystem. Early statements postulated that this Collembola species was a non selective microbial or detritus feeder (Tilbrook, 1970). However, using gut contents, faecal pellets analyses and microbial techniques, more recent studies (Broady, 1979) suggested that *C. antarcticus* was able to select its food items (filamentous fungi and algae being the most important at Signy Island (Block, 1984)).
In order to clarify the feeding preferences of this particular springtail, this study aims (1) to determine if *C. antarcticus* is an omnivorous organism, (2) to clarify the feeding selection ability of the species, and is so, (3) to assess its feeding preferences among a restricted number of diets using laboratory feeding observations and stable isotope ratios techniques.
It is essential to know both what is consumed and what is assimilated by the organisms in order to be able to assess the exact role of *C. antarcticus* within the Antarctic ecosystem.
With this intention, three experiments were carried out in order to investigate (1) the feeding preferences among labeled (^{13}C and ^{15}N) diets-6 choice experiment, (2) the preference between two different diets (labeled and non labeled)-2 choice experiment. The diets tested were composed of one alga species *Prasiola crispa*, two lichen species *Umbilicaria decussata*, *Usnea Antarctica*, and three mosses species *Brachytecium glaciale*, *Pohlia nutans*, and *Sanionia uncinata*. The proportion of springtails found on or close to each diet was used for the analysis and collembolan stable isotope ratios composition was measured. It appears that *C. antarcticus* exhibits a preference for the diet alga Prasiola, nevertheless it consumes as well lichens but mosses species are hardly eaten.

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Acknowledgments

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I- Presentation of the research institute: The NIOO-KNAW

The Netherlands Institute of Ecology (NIOO-KNAW) is the largest research institute of the Royal Netherlands Academy of Art and Sciences (KNAW). The core task of the institute is to carry out basic and strategic research into individual organisms, populations, communities and ecosystems.
The NIOO-KNAW employs more than 250 people at three research centers: research at the Centre for Estuarine and Marine Ecology (CEME) in Yerseke concentrates on ecosystems in brackish and salt water, the Centre for Limnology (CL) in Nieuwersluis focuses on freshwater ecology, while the Centre for Terrestrial Ecology (CTE) in Heteren emphasizes land-based ecology.

The Centre for Estuarine and Marine Ecology (CEME) is situated in the village of Yerseke on the Oosterschelde estuary in the province of Zeeland. CEME conducts research in estuaries and coastal waters in Europe, Africa, Asia, and the Polar regions. CEME consists of three departments: Ecosystem Studies, Marine Microbiology and Spatial Ecology.

The spatial Ecology department aims at describing and modeling the dynamics of estuarine and coastal habitats. In particular, the interactions between the estuarine communities on intertidal mudflats and salt marshes with their physical environment receive most attention. The department wants to provide models representing the dynamics of the estuarine landscape, based on field and laboratory process studies.

The group with which I worked with is the Unit for Polar Ecology which started in 2002, as a project group of the Department of Spatial Ecology. The Unit comprises personnel from various departments of NIOO, who spend part of their time on ecological research in Polar Environments, and contract staff who work full-time on polar ecology.
As the changes in temperature are especially apparent in the high latitudes, polar ecosystems are ideal for studies on the effects of a change in one of the key environmental factors.
The central research theme of the unit is the structure and functioning of, and the relations between terrestrial, limnetic and coastal marine ecosystems, using temperature change as a “natural” experimental variable. The Unit for Polar Ecology will use the responses of the ecosystems to climate change, to study the more fundamental processes occurring in ecosystems, such as functional diversity, structure and dynamics of food webs and carbon and nitrogen cycles and the interactions between marine, terrestrial and limnetic ecosystems.

The Unit for Polar Ecology has three main research lines:

- The first one focuses on functional diversity, like the study of the diversity of functional groups in the ecosystem and the diversity within functional groups. The study of the structure of the ecosystem prevails, but it has also a strong functional component.

- The second research line emphasizes on dynamics and evolution of food webs. The effects of abiotic factors, such as light and temperature, on the functioning of the food web are central, and any change in the food web due to changes in these factors, will directly affect the diversity within the constituent functional groups or the diversity of the functional groups itself. Therefore, climate change may lead to changes in the rate and extent of processes in terrestrial and limnetic ecosystems in the Antarctic, increasing the relative importance of certain trophic levels in food chains.

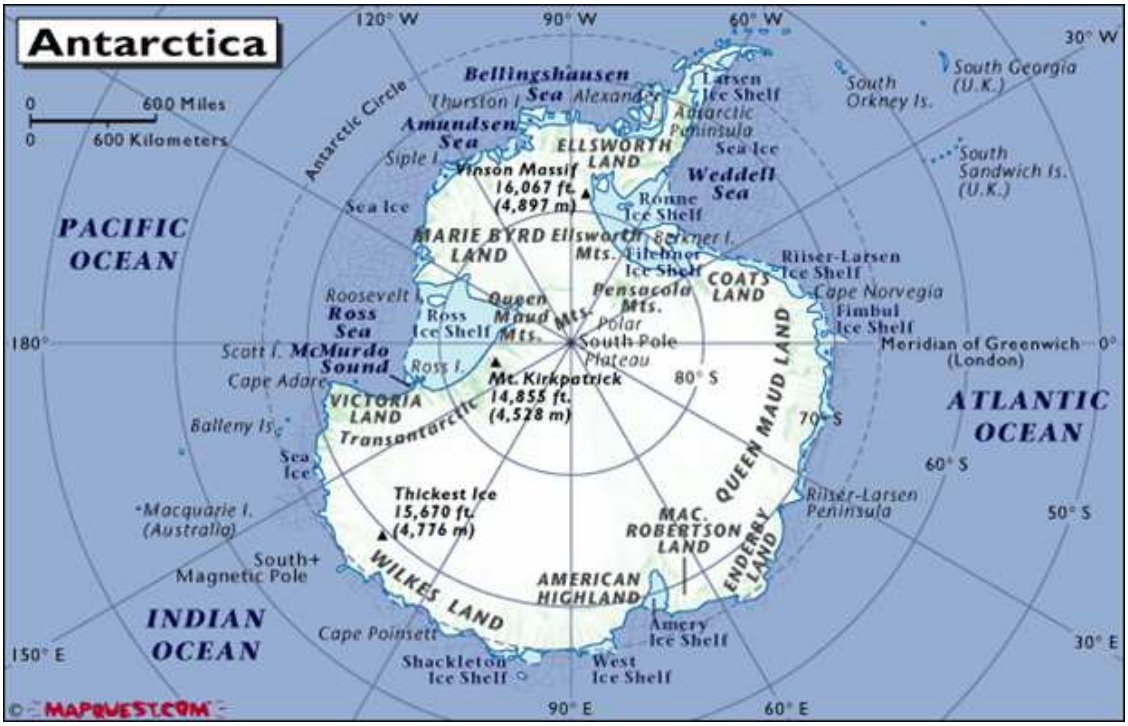
- At last, the third research line will try to determine the carbon and nitrogen cycles and their interactions between marine, terrestrial and limnetic ecosystems. The study of carbon and nitrogen fluxes in the ecosystem is inextricably connected with the structure and the function of the food web of the ecosystem. Moreover, the effect of temperature rise may be that the diversity within functional groups may change or that the trophic structure and thus the diversity of functional groups may change.

The Unit for Polar Ecology works on several projects, one of them is about the effects of environmental change on carbon and nitrogen fluxes in Antarctic Terrestrial Ecosytems (FATE). Terrestrial ecosystems in the higher latitudes of the Maritime Antarctic are structurally simple with only two trophic levels well developed: primary producers and decomposers. Nitrogen and other minerals are mostly imported from the marine ecosystem (sea spray, precipitation, marine birds and mammals). These ecosystems are largely oligotrophic, with primary production and decomposition limited by water availability and temperature. The presence of liquid water is linked to temperature. Therefore, temperature is the key factor controlling the cycling of carbon and nitrogen. The hypothesis according to which, as a result of the increasing temperatures in this region, terrestrial ecosystem structure and processes will become more complex and will ultimately develop increasing similarity with counterparts currently found in lower latitudes, has been made. The objectives of this project are to measure C, N isotopic ratios of organic matter to known origin and identify organic biomarkers indicative of terrestrial and marine ecosystems, in order to describe the origin, diagenesis and transport pathways of organic matter in the terrestrial ecosystem, as well as to quantify the imports of carbon and nitrogen from the marine ecosystem into the terrestrial ecosystem and identify the role of snow/ice in their storage and subsequent release. The use of stable isotope analyses and identified biomarkers will be done in order to quantify rates of decomposition and describe fluxes of organic matter through key trophic elements of the terrestrial food chain. The main objective is to identify the influence of elevated temperature on decomposition and the transport of carbon and minerals. (www.nioo.knaw.nl/PROJECTS/UPE/fate/ and www.riscc.aq/projects.html)

II-Introduction

1. The Antarctic environment

The Antarctic continent consists of an area of about $14x10^6$ km^2, less than 3% of this is ever free of permanent snow and ice. Large fluctuations in the total area of ice are due to seasonal changes (being at a minimum in January/February) in the sea-ice cover surrounding the continent. Over 50% of the continent is over 2000m and about 25% is more than 3000m above sea level. The highest point, in the Sentinel Range of the Ellsworth Mountains, rises to 5140m. The mean ice thickness is 1880m and the ice sheet contains over 90% of the world's ice (Walton, 1984).



F*igure 1:* Antarctica map
source: www.myblumberg.com/Antarctica/Map%20of%20Antarctica.png

Three types of bare ground can be distinguished on the Antarctic continent:

(1) areas permanently free of snow in which precipitation is virtually absent
(2) areas normally with some snow cover in winter which largely melts off in the summer to provide free water
(3) exposed areas of rock associated with mountains isolated amongst snow and ice

We can distinguish three areas within the Antarctic continent: the continental Antarctic, the maritime Antarctic and the sub-antarctic region. As this study focus mainly on a species living in the maritime Antarctic, only this region will be described. The Maritime Antarctic region includes the western coastal fringe of the Antarctic Peninsula and adjacent islands, South Shetland Islands, South Orkney Islands, South Sandwich Islands

and Bouvetoya (Tilbrook, 1970). The total area of the Maritime Antarctic region covers approximately an area of 40000 km^2, roughly 90% of this consists of permanent ice or snowfields (Tilbrook, 1970).

Snow-free ground in summer in the maritime Antarctic islands varies considerably both between islands and between groups. In the South Orkney Islands the extent of snow-free ground varies between 50% for Signy Island and less than 5% for Coronation Island (Walton, 1984).

Some details from Signy Island give an indication of the relative proportions of the various habitat divisions within this region: of a total area of 19,4 km^2, approximately 13 km^2 is snow free in summer and this probably represents 16,5 km^2 of actual ground surface (Tilbrook, 1970).

1. Climate

The climate of the Maritime Antarctic region is oceanic (Tilbrook, 1970). Cold moist maritime climate, mean monthly temperatures exceed 0°C for 3-4 months in summer, but rarely fall below -10°C in winter; precipitation of 350-500 mm per annum with much falling as rain in summer (R.I.Lewis Smith, 1984).

Short cold summers followed by long, very cold winters typify the continental temperature regime. The mean annual air temperature range lies between 45 and 55 degrees for coastal stations and 64 to 68 degrees for ice shelf and plateau stations. (Walton, 1984).

The major part of the Antarctic can be described as arid. Not only is there virtually no available unfrozen water but precipitation is very low. In summer radiative heating on a dark rock surface is sufficient to cause local melting, and it is on nunataks and in some of the coastal areas the plant and invertebrate life is to be found (Walton, 1984).

2. Water

Most water on the continent remains unavailable in the form of snow and ice. In some of the oasis areas radiative heating causes sufficient melt for pond formation (Walton, 1984). The presence of a permafrost layer of varying thickness in all continental and maritime Antarctic sites has important effects on drainage patterns, leaching and soil development (Walton, 1984).The critical biological factor in these areas is the presence of free water for at least part of the summer.(Walton, 1984)

3. Wind

Polar microclimates are greatly affected by wind, the paucity of shelter making plants and animals especially vulnerable to its effects. The wind has three principal effects: disturbance of local temperature and humidity profiles, abrasion damage by transport of

ice crystals and mineral particles, and transport of living propagules on both micro and macro scales (Walton, 1984).
Cold dense air slides from the high interior ice fields towards the lower areas along the coasts. At the edges of the ice plateau the winds accelerate, thereby lifting and blowing clouds of snow high into the air (www.quarkexpeditions.com/antarctica/anviro.shtml). These sudden and unexpected winds are called katabatic (gravity driven), or down slope, winds (www.quarkexpeditions.com/antarctica/anviro.shtml). These winds create dramatically low effective temperatures, due to the wind-chill factor (www.quarkexpeditions.com/antarctica/anviro.shtml).

4. Soil types

Three major classes of Antarctic soil types can be recognized –ahumic, ornithogenic, and very small patches of humic soil occurring beneath the limited moss and grass stands. Sub-Antarctic soils are generally humic, often with well developed profiles although primitive ahumic soils may occur at high altitudes (Walton, 1984).

The character of the exposed land surface ranges from bare rock to pure mineral soil and varying proportions of mineral matter and vegetation (Tilbrook, 1970).

Nutrient availability does not appear to be limiting to the system (Allen and Northover, 1967; Northover and Allen, 1967; Northover and Grimshaw, 1967 in Tilbrook, 1970) and it is climate and substrate stability which are the dominant factors controlling both the establishment of vegetation and the development of soils (Allen et al., 1967).

5. Vegetation

The environmental factors controlling the present-day distribution of the terrestrial vegetation fall into three categories: climatic (temperature, availability of moisture, wind effect…), edaphic (types of substratum available), and biotic (influence of other organisms, mainly animals) (Lamb, 1970).
The terrestrial vegetation of Antarctica, in order of decreasing abundance of representation, consists of bacteria, lichens, mosses, algae, hepatics, fungi and flowering plants:

- *Lichens*: About 400 species of lichens have been recorded from Antarctica. Lichens are well fitted to polar habitats by their ability to colonize completely bare rock surfaces and their remarkably effective physiological adaptation to low temperatures. A number of lichen species, even in a saturated condition, have been found by Lange (1962) to be capable of surviving temperatures as low as -75°C for prolonged periods. Lichens are well adapted to the Antarctic environment, being able to photosynthesize and respire at lower light, temperature and tissue moisture contents than other plants. Their metabolism is regulated by thallus water content so that at high surface temperatures or light intensities the thallus dries out and becomes metabolically inactive. The high concentrations of pigments and lichen acids in the thallus prevent it from freezing even at -10°C and permit limited photosynthesis at that temperature (R.I.Lewis Smith, 1984).

The minimum temperature at which CO_2 exchange has been recorded is -18.5°C in *Usnea* (R.I.Lewis Smith, 1984).

- *Mosses*: 72 species of mosses have been listed from the Antarctic Botanical zone by Steere (1961). Mosses are most frequently encountered in Antarctica in wetter areas of drainage channels or seepage slopes, on scree and morainic detritus and in crevices of rock-faces. Among the commoner Antarctic mosses are *Polytrichum alpinum*, *Sanionia uncinata*, *Bryum antarcticum*, *Pohlia nutans* and *Sarconeurum glaciale*. Bryophyte photosynthesis is often maximized at relatively low light intensities (under snow cover), and inhibited by high intensities (Convey, 2003). We can also note that in Antarctica, mosses endure freezing temperatures for long periods, this implies that the Antarctic mosses either are permanently cold conditioned (frost resistant) or require no cold hardening, as vascular plants do (Rastorfer, 1972). Nevertheless, the ability of Antarctic mosses to endure frequent and rapid temperature changes and their apparent high lipid to soluble carbohydrate ratio may suggest cold endurance mechanisms different from those proposed for vascular plants (Rastorfer, 1972).

- *Algae*: approximately 360 species of non-marine algae have been recorded from Antarctica (Greene, et al., 1967), but here, as with the lichens, the actual number is probably lower. The groups represented belong to the divisions Cyanophyta, Chrysophyta, Xanthophyta, Bacillariophyta (Diatoms), and Chlorophyta, and display a variety of morphological organization including unicellular, colonial, filamentous, and thalloid types. The habitats which they occupy are terrestrial (rocks, detritus, sometimes also artificial solid substrata), aquatic (freshwater lakes, ponds and pools), or, in the case of the so-called cryoplankton, snow or ice. Alga occurs also, of course, inside the thalli of lichens, to which they are mostly peculiar, apparently very seldom occurring in the free-living condition.
The supralittoral, green alga *Prasiola* is one of the most widespread and conspicuous alga species in coastal regions. *P. crispa* forms sheet-like thalli on the surface of wet ground; in winter the algae are permanently frozen and undergo repeated freeze/thaw cycles in the spring and autumn (Jackson, 1997). In addition to that, *P. crispa* is also subject to considerable fluctuations in salinity due to influx of freshwater from snow melt, exposure to salt spray and nutrient enrichment from birds (Jackson, 1997). In spite of these factors, *P. crispa* has successfully colonized large areas of Antarctica (Jackson, 1997). Davey (1989) has already suggested, on the basis of evidence that photosynthesis in *P.crispa* continued down to -15°C (Becker, 1982), that this alga might contain large quantities of cryoprotectants (Jackson, 1997).

- *Fungi:* fungi are few in Antarctica. One Myxomycete, three Ascomycetes, and six Basidiomycetes have been listed by Singer (in Greene et al., 1967), from the west coast of the Peninsula and the South Shetland Islands. Most of them grow among mosses.

- *Flowering plants*: there are only two species of flowering plants native to Antarctica, *Colobanthus quitensis* (Kunth) Bartl. and *Deschampia Antarctica* Desv., both occurring only in the Peninsula sector.

Climatic, topographic and edaphic variations are responsible for a wide range of different habitats and consequently of cryptogamic community types, in the maritime Antarctic. The relationship between bryophyte growth-form and moisture supply in the habitat has been stressed by Longton (1967) (Gimingham, Smith, 1970). A striking feature throughout mosses is the preponderance of dense types of colony (cushions and turfs). It has been demonstrated that these play a part in restricting evaporation during periods of exposure (Gimingham, 1967; Smith, 1968). Hence species possessing a cushion form, most effective in this respect, are predominant (Gimingham, Smith, 1970).

6. Community and trophic structure

Antarctic food webs are relatively simple in community structure (Figure 2) and have a low species diversity compared to mid-latitude and equatorial ecosystems (Convey, 2003).

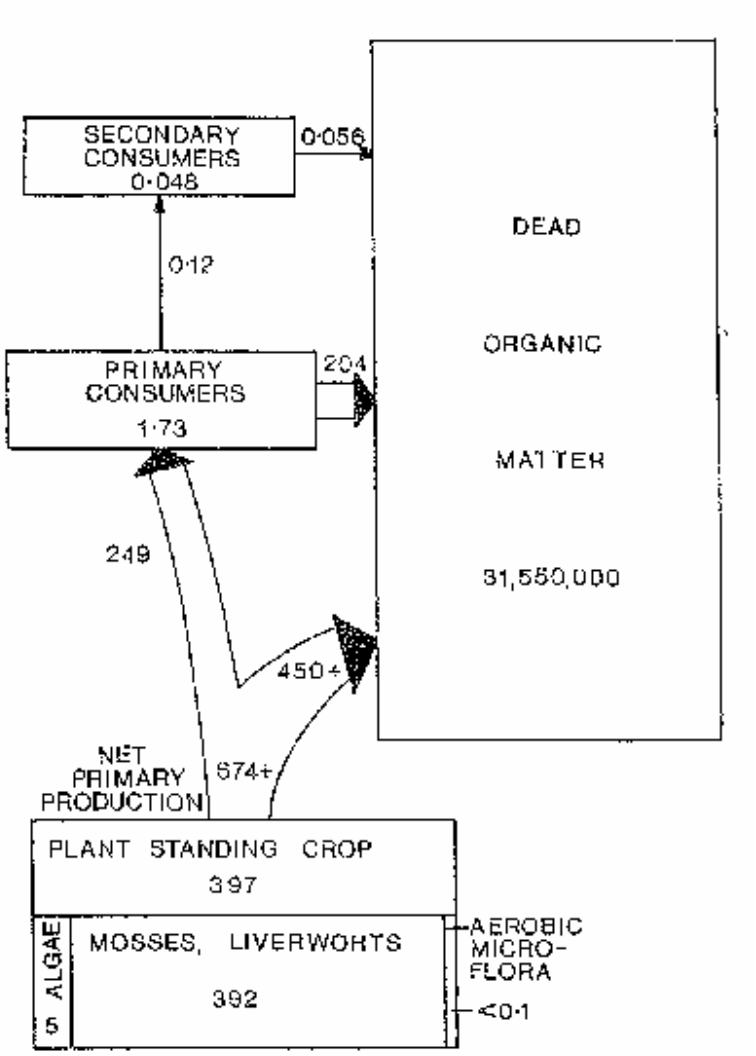


Figure 2: Generalized diagram of energy flux in bryophyte-dominated ecosystems of the maritime Antarctic (After Davis 1981). Units are g m^2 $year^{-1}$ dry wet.

In terrestrial communities of the maritime Antarctic, organic matter resides in three compartments: autotrophs (primary producers), heterotrophs and dead organic matter. The primary producers are chiefly mosses, liverworts and lichens together with both uni- and multi-cellular algae. The heterotrophs are comprised of saprotrophs (bacteria, yeasts, and filamentous fungi), primary consumers (protozoa, rotifers, tardigrades, nematodes, mites, and springtails), and secondary consumers (three carnivorous species: a nematode, *Coomansus gerlachei*, a tardigrade, *Macrobiotus furciger*, and a mite, *Gamasellus racovitzai*) (Figure 2). The dead organic matter pool receives contributions from both auto- and heterotrophic components, but consists mainly of dead plant material (Block, 1985).
Even the most complex Antarctic terrestrial communities are formed by, at most, two phanerogams (plants that produces seeds), a limited number of cryptogams (mosses, liverworts, lichens), micro arthropods (mites, springtails), microscopic invertebrates (nematodes, tardigrades) and microbiota (algae, fungi, cyanobacteria, bacteria) (Convey, 2003).
Antarctic terrestrial habitats have a community composition with few interacting species, but often comprising large numbers of individuals (Block, 1985).
Due to a disharmonic flora and a patchy distribution of organic material and microorganisms, microfaunal organisms are less widely distributed; hence, interactions involved are more limited in space and diminished than in species-rich environments (Bolter, 1997).

Invertebrates are by far the most important heterotrophs within undisturbed terrestrial food webs in the Sub-Antarctic. The invertebrates occur in several habitats: intertidal zones; among piles of kelp and other jetsam on the shore; within colonies of seabirds and seals; and, in a fairly wide spectrum of terrestrial plant communities (moss and lichen) (Holdgate, 1977; Somme, 1986). Both Gressitt (1970) and Abbott (1974) suggested that the physical and biotic environments of the islands governed the invertebrate faunistic composition. Hence, careful studies of the food webs and niches of the terrestrial invertebrates are needed to make meaningful conclusions about island biogeography in the Sub-Antarctic.
Antarctic terrestrial invertebrates inhabit some of the most extreme environments in the world, particularly in respect of temperature and moisture (Block, 1982). A range of strategies is adopted to survive sub-zero temperatures and often drought conditions during both summer and winter. Among the most successful species are those arthropods belonging to the Acari (mites, especially *Cryptostigmata* and *Prostigmata*) and the Collembolan (wingless insects termed springtails). Successful species, both resident and colonist, have to optimize their performance during short periods in the austral summer when relatively warm and moist conditions prevail (Block, 1982).
There are 17 species of Collembolan in the continental and maritime Antarctic, with 37 species in the richer sub-Antarctic zone. The total Antarctic collembolan fauna numbers 47 species, of which 70% are endemic (Block, 1982).

Despite the apparent simplicity of Antartic terrestrial environments and its impoverished fauna, little is known about the food preferences of soil arthropods in Antarctic ecosystems. In the summer 2003/2004 a joint Dutch/English research project called

FATE has started. The main research focus is on the effects of environmental change on the community structure and on carbon and nitrogen flows through the system. To mimic possible environmental change effects Open Top Chambers (OTC) will be placed in a tundra and fell field type vegetation at three different locations along a latitudinal gradient (Falkland Islands, Signy Island (South Orkney Islands) and Anchorage Island (Marguerite Bay)). The OTC's will increase the temperature in the vegetation by 2-3^{0}C.
Each year a survey will be made of the community structure and how this might change due to the artificial warming treatments. To find out what the role and place of the different species is, it is necessary to know what the feeding preferences of certain species are. With this information it is possible to construct a more precise food web. Changes in community composition due to environmental change could then be easily linked to certain ecosystem functions.
Therefore, my research lies within the scope of this project by tempting to clarify the main diet and food preferences of springtails species from Antarctica. My research will mainly focus on the species *Cryptopygus antarcticus*, which belongs to the Collembolan classis (Superclassis: Hexapoda).
These species play an important role in biodegradation because they contribute to the nutrients pool in the soil; they speed up the process of decay and deposit nutrient rich feces back into the earth. In addition, they may distribute fungal spores and mycelial fragments, and doubtless bacteria, both in their faeces and on their bodies, and thus indirectly promote decomposition processes (Healey).

The absence of above-ground herbivores and the reduced invertebrate component of the fauna in Antarctic have profound effects on both the structure and functioning of the terrestrial ecosystem. It shifts the emphasis of energy flux onto smaller organisms in the below-ground-soil sub-system. This, in turn, greatly enhances the role of the micro-flora, especially in polar ecosystems, and the micro-flora-invertebrate interface is one that warrants further study under these conditions (Block, 1982). The importance of Collembolan to soil processes has been discussed by several authors including Macfadyen (1965) and Hale (1967). Particular emphasis has been placed notably on the role of micro-arthropods in stimulating decomposition processes (Ghilarov, 1963).
C. antarcticus may play an important role as a consumer either of living micro-algae or of decomposer micro-organisms, and thereby aiding their decomposition (Block, 1982). At Signy Island, *C. Antarcticus* plays an important part in alga-dominated habitats as a consumer of living algal material (Broady, 1979). Dissemination of viable algal cells and micro-organisms in faecal pellets by such Collembolan may also be significant. (Block, 1982). In bryophytes sites, this role is often unimportant and *C. antarcticus* becomes a consumer of the decomposing micro-organisms (maceration of dead and decaying plant tissue probably aids its further microbial decomposition) (Broady, 1979). Faeces deposited on senescent plant material may serve as the initial innoculum of decomposing micro-organisms. It has already been postulated (Broady, 1977) that Collembolan are important to non-motile algae epiphytic on bryophytes (Broady, 1979).
Invertebrates appear less sensitive to annual variations in primary production, completing their life cycles when environmental conditions permit, and, hence, they may be more efficient at exploiting low temperature habitats. Thus, the principal grazing chain in the maritime Antarctic is ectothermic and invertebrate based (Block, 1982).

2. Generalities about Collembola

1. Morphology

Cryptopygus antarcticus belongs to the elongate species suborder; the first thoracic segment is reduced in size relative to the other thoracic segments, producing a "neck"-like structure (Figure 4). Also, the legs, antennae, and furcula are elongate, and the eyes well developed (Encyclopedia of Entomology) (Figure 3). *C. antarcticus* is a below-ground species; small, with short antennae and legs, with reduced eyes and a jumping apparatus (Encyclopedia of entomology, online version).

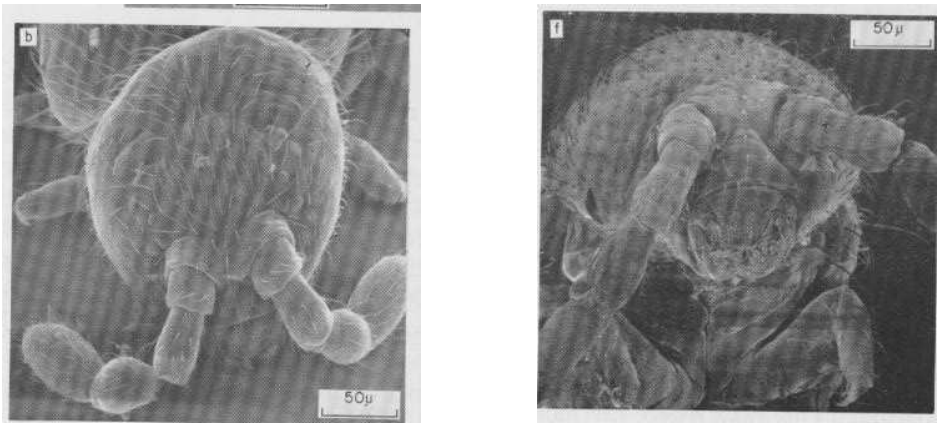


Figure 3: Scanning electron microscope pictures of *C. antarcticus*: dorsal view of head and anterior aspect of head (Source: The biology of *C.antarcticus*, Tilbrook, 1970)

Collembolans are small animals, measuring only between 1040 and 1134 μm in length, and usually less than 5 mm long. They are either elongate or globular in body form. Their color varies, and though often obscure, some are brightly colored. The antennae are short to medium in length, and consist of 4 to 6 segments. The compound eyes are small, with only a few facets per eye. The mouthparts are basically the biting (chewing) type, but sometimes extensively modified, and somewhat enclosed by the head. The legs are small, and lack extensive modifications. Wings are always absent. The abdomen consists of 5 to 6 segments. Springtails often are equipped with a jumping apparatus that serves as the basis for the common name. This apparatus consists of a fork-like furcula originating near the tip of the abdomen that is flexed ventrally and held by a catch, the tenaculum. When the tenaculum releases, the furcula springs with a snap that propels the insect forward. In addition, springtails possess a small ventral tube-like structure on the first abdominal segment, called a collophore or ventral tube. The collophore has various functions, including water absorption and excretion, and possibly adhesion to smooth surfaces. Springtails generally lack trachea, with gas exchange occurring through the integument (Encyclopedia of entomology).

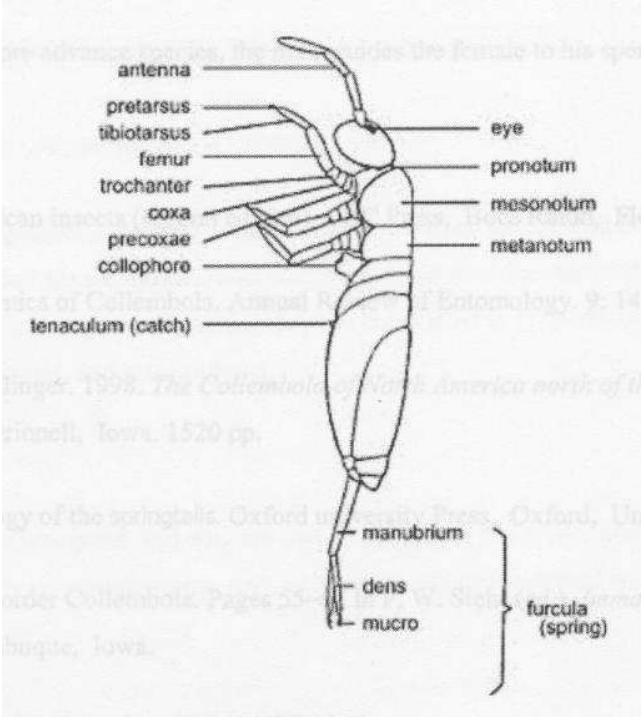


Figure 4: diagram of a springtail showing a lateral view, (source: Encyclopedia of entomology (online version))

The reproductive behavior of springtails is consistent with other entognathous insects. Sperm transfer is indirect, with sperm deposited in stalked droplets on the ground. In most cases, sperm uptake by the female is a passive process, but in some of the more advance species, the male guides the female to his sperm (Encyclopedia of entomology, online version). Metamorphosis may be lacking or incomplete. Collembolans often display 6 to 8 moults during their life, with sexual maturity attained after the fifth moult. Thus, unlike insects, they continue to molt as adults. The immature usually resemble the adults in external morphology (Encyclopedia of entomology, online version).

Springtails are hemidaphic (live in the superficial soil layers and leaf litter) and ectothermic species (they use heat acquired from the environment and possess a behavioral adaptation to regulate their body temperature). They occur in numerous habitats, including the water surface of fresh water, the tidal region of salt water, in soil, leaf debris, bird nests, beneath bark, under snow field, and occasionally on foliage (Encyclopedia of entomology, online version).

From a behavioral view, collembola often aggregate in large groups, and this can be observed on the surface of water, snow, or on organic material. The purpose or cause of aggregations is unknown (Encyclopedia of entomology, online version).

Water availability is recognized as one of the most important limits on the distribution of terrestrial organisms (Hayward, 2004). Collembolans are considered to be highly

susceptible to desiccation because they rely on gas exchange across the cuticle for respiration in the absence of a tracheal system (Hayward, 2004).

The majority of micro arthropods are generalist primary consumers or detritivores (Convey, 1997). Thus, they can be herbivorous, carnivorous or omnivorous. Numerous studies confirmed that collembolans are opportunists regarding their feeding: they select among various food sources but fungi are among the most often consumed (Varga, 2002). Omnivory (used here in the sense of feeding on more than one trophic level) is probably the prevailing feeding strategy in collembolan (Filser, 2001).
Many Collembolans should probably be regarded as "herbivores" in the system in that they are grazers on the "primary producers", the fungi and bacteria, whose growth is perhaps stimulated by their grazing activity. But the same species may also be coprophilous or feed directly on detritus (Healey).

The food sources of most species of Collembolan is known in a general way to consist largely of fungal hyphae and spores, and sometimes algae and faeces of larger invertebrates, although decaying higher plant material is eaten occasionally (Dunger, 1959; Macnamara, 1924; Poole, 1959; Scott and Stojanovich, 1963). The presence of quite large mineral particles (often up to 30 μm) in the gut suggests that they may be indiscriminate feeders. Superficially at least it seems that, with the exception of a few forms with specialized mouthparts which are probably suctorial feeders, there is little specialization in feeding in the group.
However, some facts need to be enlightened, as it is difficult to draw clear conclusions from experimental observations; for instance the gut contents of the ten commonest species in a habitat may be extremely similar, and the proportion of different foodstuffs in the gut on different occasions may vary collectively. Furthermore, it is not clear what nourishment the animals obtain from their food. Spores and hyphal fragments bigger than a few μm long can be shown to germinate freely after passage through the Collembolan gut (Healey, 1965), and there are indications that forms feeding on humus do not alter its chemical composition (Naglitsch, 1965). Therefore, it is not surprising that no estimates of assimilation efficiency have been made and only few estimates of feeding rates (Dunger, 1956; Healey, 1965).

2. Biology and ecology of the Collembolans *C. antarcticus*

Following the classification of Salmon (1964) the systematic position of *C. antarcticus* within the Collembolan is:

Suborder: Arthropleona
Super-family: Entomobryoidea
Family: Isotomidae
Sub-family: Anuraphorinae

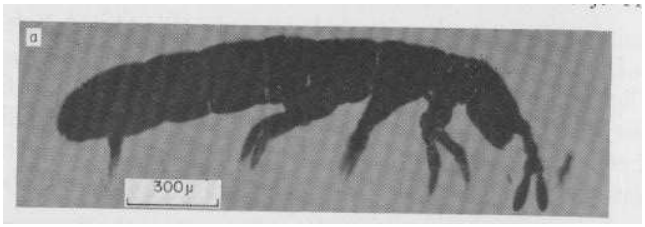


Figure 5: Lateral view of *C. antarcticus* as seen under the light microscope to show normal body form (Source: The biology of *C.antarcticus*, Tilbrook, 1970)

3. Distribution

The genus *Cryptopygus*, represented by 20 species is restricted to the Southern Hemisphere. *C. antarcticus* is a common Maritime Antarctic collembolan and has a circumpolar distribution in the Subantarctic (Tilbrook, 1970). The distribution of *C. antarcticus* is widespread in the Antarctic Peninsula and the islands of the Scotia ridge and has also been found on Bouvetoya, Archipel de Kerguelen and Heard Island. The springtail *C. antarcticus* (Collembolans, Isotomidea) is ubiquitous in the maritime Antarctic, occurring wherever there is vegetation (Block, 1984).

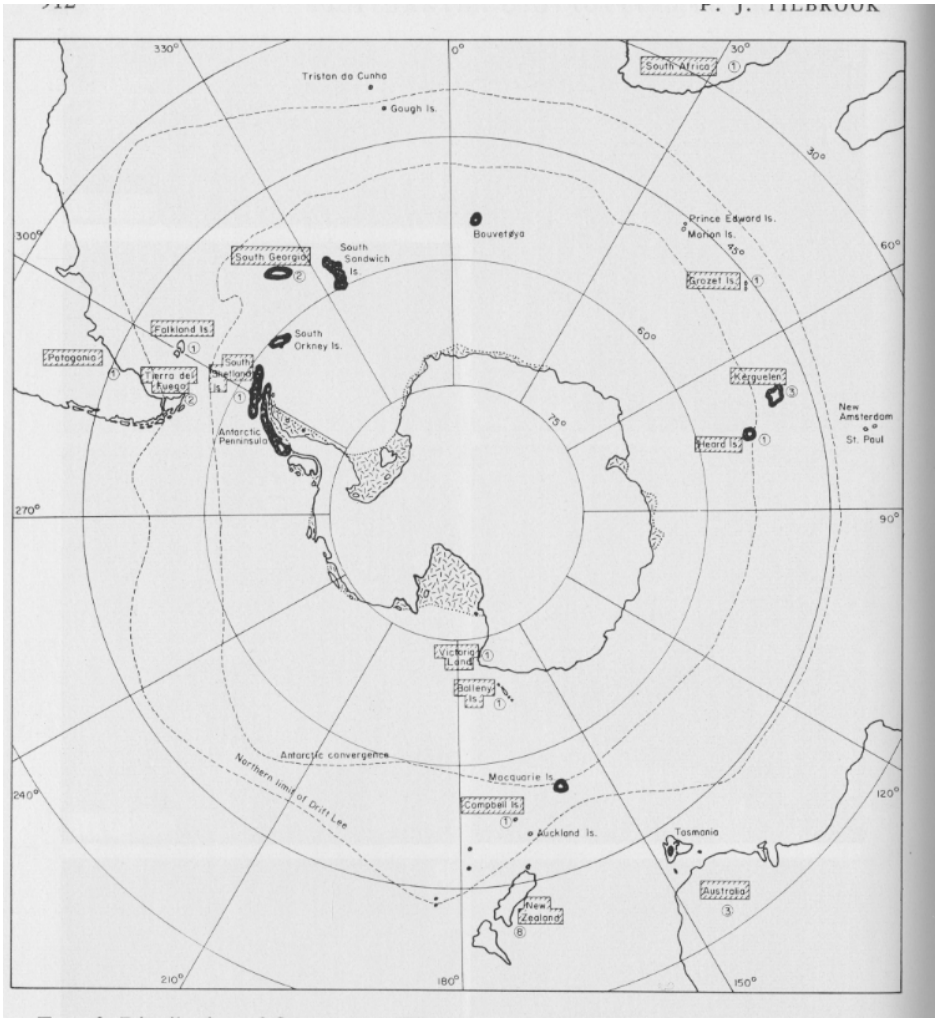


Figure 6: Distribution of *Cryptopygus*. The present distribution of *Cryptopygus antarcticus* is shown with areas surrounded by black and the place names surrounded by cross-hatching indicate where other species of the genus *Cryptopygus* occur (Source: The biology of Cryptopygus antarcticus, P.J. Tilbrook, 1970).

4. Habitat

The hemiedaphic habit (live in the superficial soil layers) of this species is indicated by the presence of a furcula, fairly long antennae, dense pigmentation and well-developed eyes (Tilbrook, 1970). In the Maritime Antarctic the species has been found in almost all terrestrial habitats free from permanent snow and ice (Tilbrook, 1977); they are found in recently deglaciated areas and fell fields, well-developed moss banks and algal-dominated habitats close to the shore and near penguin rookeries and seal wallows (Block, 1985).

In a study of Tilbrook at Signy Island, it appeared that 24% of the *C. antarcticus* population in the top 6 cm occurred in the 3-6 cm layer, but there were significant variation between different moss types. This author found a correlation of depth distribution with available pore space (Tilbrook, 1977).

The abundance of the species is a function of humidity, and the species show a high degree of dominance among the ecosystem, making up 70-90% of the fauna in moss habitats and only 5-20% in lichen habitats (greater degree of exposure and lower relative humidity).
At Signy Island, 4 springtail species were found in a detailed study by Tilbrook (1967, 1973), and data on the seasonal abundance of the dominant *C. antarcticus* obtained for 3 moss-lichen vegetation types. Maximal numbers were $116x10^3$ individuals m^{-2} in a stand of *Pohlia nutans* (Block, 1986). *C. antarcticus* was exctracted from 68 out of 70 qualitative samples and it had the highest relative abundance in 81% of these samples (Block, 1986).
Evidence is accumulating on the association between species density and vegetation cover and, from the core data, the distribution of *Cryptopygus* in the moss turf and carpet is very clumped. Significantly higher numbers of *C. antarcticus* occur in core samples of moss turf containing *Polytrichum* alone, and *Chorisodontium* and surface lichens together, than in dead mosses and bare peat (Block, 1982).

5. Ecology

There are 7 major constraints which influence the ecology of such terrestrial species: low temperature (often with freezing conditions), freeze-thaw events, drought and dryness, wet-dry cycles, snow cover, seasonal light climate, increased UV-B in certain areas (Block, 1982). In addition, seasonal changes in light levels, light quality and intensity, as well as in UV-B, may be critical for different life stages of such species, and these constraints may be most important during the spring and summer periods in the Antarctic (Block, 1982). These constraints result in a short active season with variable food supply for most terrestrial micro arthropods, but there appears to be little interspecific competition due to low species diversity of the fauna (Block, 1982).
According to some experiments, the temperature causing heat death is somewhere between 33°C and 43°C, which is similar to other collembolan species (Tilbrook, 1970). Optimum feeding activity occurs at around 10°C, and maximum moult rate between 10 and 15°C (Hayward, 2003), suggesting that conditions most favorable for development are within this range. However, tolerance of these temperatures will largely depend on moisture availability (Hayward, 2003).

6. Feeding habits

The mouth parts of *C.antarcticus* are of a common collembolan type with a grinding area on the mandibules. Examination of gut contents suggests that many different types of food are taken (Tilbrook, 1970). It is well known that Collembolan in general are non-selective in their feeding habits and indeed that many things are taken into the gut which are either of no nutritional value or pass out unchanged. In some cases this may, however, represent just a residue, after any bacteria and fungi present have been digested (Tilbrook, 1970). When offered a variety of fungi isolated from Signy Island soil and vegetation some were consumed and others ignored. Also, food accepted on one occasion was often rejected on another. The Collembolans feed primarily on fungal hyphae and dead plant material (Block, 1985). Four principal food sources can be distinguished: the

dominant foods for such micro-arthropods are algae, both micro- and macro-forms, which comprise a highly diverse and easily assimilated food resource (Block, 1985). Broady (1979) recorded upwards of 162 species of algae from a range (122) of terrestrial habitats at Signy Island. Analyses of gut contents of *C. antarcticus* suggest that this species feeds extensively in the field on unicellular green algae, which grow epiphytically on the live shoots of certain mosses species (Block, 1982). A second order resource is the micro-flora, with yeasts and fungi being the main items. The third food component is dead organic matter, and the fourth resource is entirely animal material as prey for the single arthropod predator. Mosses, liverworts and lichens are therefore not consumed by the arthropods to any significant extent (Block, 1985). Thus algae, together with the micro-flora and detritus, provide the main fraction of the diet of many of the primary consumers, especially the micro-arthropods (Block, 1985).
Under experimental conditions the Collembolan *Cryptopygus antarcticus* has a preference for micro-algae, but nevertheless also eats fungi and in the laboratory will feed on homogenized moss turf. In the field, examination of gut contents and faecal pellets reveals that filamentous fungi and micro-algae are the most important food sources. Eventually, we can note that feeding rates changed rapidly over 0°C-5°C (Block, 1985).

7. Nutritional ecology of collembolans

Lichens and mosses

Lichens and mosses are phylogenetically unrelated groups that differ in numerous chemical, structural, and physiological characteristics (Slansky, 1987). Mosses are green plants fully capable of photosynthesis, and lichens are fungi that must obtain their food from colonies of captured algae maintained in the lichen body (thallus) (Slansky, 1987). The lichen thallus is therefore a dual structure, and a herbivore may feed on the entire thallus or specialize on either algal or fungal portions of the thallus (Slansky, 1987). However, there are numerous ecological similarities between lichens and mosses: these plants tend to be relatively small, long-lived, and perennial, thus, it means that they are exposed to ecologically similar groups of herbivores (Slansky, 1987).

Suitabilty of lichens and mosses as food:

The relatively low susceptibility of lichens and mosses to arthropod attack is generally explained in two ways: (1) lichens and mosses are low in nutritional value and make poor foods compared with vascular plants

(2) lichens and mosses produce secondary compounds that inhibit invertebrate grazers (Slansky, 1987).

- Suitability of lichens: a number of collembolans have been reported to feed on lichens, although the details of the interactions are very poorly known (Slansky, 1987). Lichens are apparently an important food source for many species of springtails. Despite a high variability in nutrient concentrations reported for different species and growing regions, protein content is usually low, along with calcium and potassium concentrations, on the other hand fiber

content is generally high (Slansky, 1987). In addition, there are age-specific differences in lichen nutrient content; with young lichen tissue having the highest nutrient levels (Slansky, 1987). Defense compound production by lichens probably also influences their susceptibility to herbivores (Slansky, 1987). Lichens produce numerous secondary compounds, many of which are not found in other organisms. Most are weak phenolic acids of fungal origin that form water-insoluble extracellular deposits on lichen fungal cell walls. Although lichen compounds have very low water solubilities (Slansky, 1987), they are apparently soluble enough to be biologically active (Slansky, 1987). These compounds are able to afford lichens a general protection against grazers, thus lichen compounds have a potential herbivore deterrent role (Slansky, 1987).

- Suitability of mosses: Mosses appear to be consumed by arthropods even less frequently than lichens (Slansky, 1987). Nevertheless, Collembola have been observed feeding on mosses in Antarctica (Pryor, 1962; Janetschek, 1967) and have been reared on mosses in the laboratory (Slansky, 1987). Furthermore, McMillan and Healey (1971) found moss fragments in the gut contents of the collembolan *Tomocerus* (Slansky, 1987). Mosses are similar to other green plants in their nutritional composition; they contain the same sugars as higher plants, although some unknown sugars are found in *Sphagnum* mosses (Slansky, 1987). The principal reserve foods of bryophytes are considered to be starches, lipids, or both (Rastorfer, 1972). Lipid concentrations are highest in spores (Slansky, 1987). Since mosses lack the storage organs of higher plants, a relatively higher lipid content would permit the maximum energy reserves in a limited space (Rastorfer, 1972). The caloric value of mosses is in the same range as that of higher plants (Slansky, 1987). In addition to that, the carbohydrate composition of mosses does not differ substantially from that of higher plants (Rastorfer, 1972); arachidonic acid, has unexpectedly been reported in a few mosses and vascular cryptogams, but it seems to be absent in higher vascular plants (Rastorfer, 1972). Concentrations of essential elements, especially nitrogen, vary extensively from group to group and the concentration of a given element in a moss may depend on the age of the segment assayed (Rastorfer, 1972). There is, however, some evidence for generally lower potassium and magnesium levels in mosses than in higher plants (Slansky, 1987). In a study of Rastorfer about the physiology of Antarctic mosses from Argentine Islands, it appeared that the species *P. nutans* contains relatively higher amounts of phosphorous, iron, boron, and aluminum. In the same study, the author found relatively high concentrations of aluminum and iron in *P. strictum* and *P. nutans*. These elements seem likely to be derived from the rock substratum, which has a high aluminum content (feldspar) and a smaller iron content (Rastorfer, 1972). Under the Antarctic conditions at Signy Island, Allen et al. (1967) found no appreciable difference between the element contents (phosphorous, sodium, calcium, magnesium, carbon, and nitrogen) of a living moss surface and of its underlying brown parent material except for the potassium content which was lower in the

underlying brown layer. This observation was applicable only to those taxa that accumulate thick layers of organic matter, such as *Polytrichum*, *Dicranum*, *Depanocladus*, and *Brachythecium* (Rastorfer, 1972). We can also note that in Antarctica, mosses endure freezing temperatures for long periods, this implies that the Antarctic mosses either are permanently cold conditioned (frost resistant) or require no cold hardening, as vascular plants do (Rastorfer, 1972). The ability of Antarctic mosses to endure frequent and rapid temperature changes and their apparent high lipid to soluble carbohydrate ratio may suggest cold endurance mechanisms different from those proposed for vascular plants (Rastorfer, 1972). Thus, bryophytes appear likely to contain a greater number and often greater amounts of non-essential elements than vascular plants (Rastorfer, 1972). We can then wonder why mosses are consumed so infrequently by arthropods. It is probably because of the low digestibility of most mosses and the production of inhibitory compounds. Compared with tree leaves, mosses generally contain lower concentrations of easily digestible soluble carbohydrates and hemicelluloses, and higher concentrations of structural components less easily digested, such as cellulose and polyphenolic lignin like compounds, although mosses do not produce true lignin (Erickson and Miksche, 1974; Miksche and Yasuda, 1978 in (Slansky, 1987). These compounds are known to have an antibiotic action, and several studies have shown the antibiotic activity of compounds extracted from mosses and liverworts (Slansky, 1987). Presence of these antibiotics is likely to have a negative effect on grazers either directly or indirectly (by inhibiting gut microorganisms) (Slansky, 1987). Lastly, it is interesting to note that mosses produce a highly unsaturated fatty acid, arachidonic acid, and information available suggests that ingestion of this compound affords protection of cell membranes against very low temperatures. If this is true, moss consumption in cold climate may be adaptive despite the low digestibility of mosses generally (Slansky, 1987). For instance, Prins (1981) observed that, at least among vertebrate herbivores, mosses are consumed relatively frequently in arctic and cold-temperate regions and practically not at all in warm climates (Slansky, 1987).

8. Importance of collembolan in the ecosystem

Counts for arthropods densities are reported from Byers Peninsula (Levingston Island), which showed population densities between 0 and 173 300 individuals/m^{-2} (Bolter, 1997) related to soil cover; non-vegetated sites showed low densities. The value found in the study of Bolter are comparable to those of Usher and Booth (1984) who found counts between 4300 and 81 400 individuals/m^{-2} in moss-turf samples from Signy Island. It was also noted by Usher and Booth (1984) that maximal counts can be found in the uppermost horizons (0-3 cm) rather than in deep layers.
Travé (1977) reported a mean density of micro arthropods for all the samples of 2673.1 per 1000 cm^3 from series of standardized samplings collected in the main biotopes of the Kerguelen Archipelago. The mean density was highly variable according to the habitats. The least inhabited of them is the moss peats, mainly that of the degraded area, 572 micro

arthropods only per 1000 cm^3. The richest are the halophilic mosses, 12652 arthropods per 1000 cm^3, and the freshwater and terrestrial algae, 8659.4 arthropods per 1000cm^3 (Travé, 1977). The relative proportions of the various groups are highly different according to the habitat. As a result, the marine algae in the intertidal zone contain almost exclusively terrestrial mites (98,5%) and very few collembolans (0,4%); the reverse is true for the freshwater and terrestrial algae (92,9% collembolans and 4,0% mites)(Travé, 1977).

Throughout the Antarctic Peninsula/Scotia Arc area, apart from South Georgia, the species *C. antarcticus* shows a high degree of dominance, making up 70-90% of the fauna in moss habitats, though the figure is lower at about 50% in the South Shetlands Islands (Tilbrook, 1985). In lichen habitats relative abundance is frequently as low as 5-20%, and this can be correlated with a greater degree of exposure and a lower relative humidity. The dominance of the species in other habitat divisions is very variable from region to region, but in general, percentage abundance is very high and may be from 90% to 100% in such specialized niches as penguin colonies, bare rock and soil. The important factor again appears to be the humidity (Tilbrook, 1985).
In the Maritime Antarctic moss carpet of Signy Island, there may be of the order of 10^4 individuals both of Collembola and Acari (Tilbrook, 1967). At 8 stations along a 150 m transect from the shore inland on Robert Island, South Shetland Islands (Etchegaray et al., 1977), Collembolans were the dominant faunal group (99%) and were found in the lower, warm and wet stations (Block, 1982). Field population in summer ranged from 19 000 (moss turf) through 39 000 (fellfield) to 180 000 (algal site) individuals m^{-2} at Signy Island (Burn, 1984) with annual mean values of 49 420 (moss turf) individuals m^{-2} (Block, 1982).

In many areas of the Antarctic *Cryptopygus antarcticus* is not only numerically the dominant arthropod but, because of its biomass and activity, is also one of the most important components in the energy cycle of the terrestrial ecosystem (Tilbrook, 1967). *Cryptopygus antarcticus* alone, may well account for almost half of the annual metabolism of metazoan invertebrates at the site of Signy Island (Tilbrook, 1977). It is clear that this species is relatively of much greater importance to energy flow within a simple Antarctic ecosystem than other collembolan species from temperate moorland habitats, but its contribution is still likely to be small by comparison to that of the microorganisms.

III-Research question: Importance of a possible selective feeding behavior

In spite of their potential importance in terrestrial food webs, relatively little is known about specific preferences in arthropods feeding strategies. Information on the feeding habits of *C.antarcticus* is limited and Tilbrook (1970) concluded that it is impossible to assess the true role of this species within the ecosystem until its food intake in the field has been evaluated (Broady, 1979).

As far as we know, *C. antarcticus* feeds on unicellular green algae, dead moss material and fungal hyphae (in order of preference) (Block, 1985). It probably takes in microphytes and detritus as well (Tilbrook, 1970).

Specificity

The degree of feeding specificity among arthropod consumers of lichens and mosses is probably very low, although generalizations are difficult to make (Slansky, 1987). Informations available at that time suggests that lichen and moss herbivores tend to be generalist feeders that frequently include a variety of other food items in their diets (Slansky, 1987). The reasons why there are so few herbivores specialized on lichens and mosses have not been thoroughly investigated but probably the low nutritional quality of most lichens and mosses and the presence of allelo-chemicals that deter herbivores could be part of the answer (Slansky, 1987). Given the general lack of specialized feeding on these plants, those few herbivores known to prefer lichens or mosses over other foods are extremely interesting (Slansky, 1987). Very little is known about the causes of preferences for lichens and mosses, and even less is known about how discriminatory feeding among lichens and mosses influences an animal's nutritional ecology (Slansky, 1987).

Selectivity

Among collembolan species, several feeding behaviors have been observed; hence it seems to be difficult to draw any general conclusion. In a feeding study of two collembolan species (Varga, 2002), it appeared that selective feeding occurs on moss inhabiting fungi by both investigated collembolan species. In the same study, microscopic observations of intestinal content revealed that the moss cushion might provide a complex food source for the investigated collembolan species. High rates of moss fragments and fungal propagules were found in collected collembolans, which indicated that grazing on moss is a general type of nutrition for them (Varga, 2002). Using gut contents, faecal pellets analyses and microbiological techniques, Broady (1979) determined that *C. antarcticus* selected its food items; filamentous fungi and algae being the most important at Signy Island.

In a study of Block and Tilbrook (1975) on energy budgets for both young and mature individuals of *C. antarcticus*, it appeared that both age classes assimilated algae better than moss peat, although individuals fed more rapidly on the latter, which had a larger indigestible component, thereby compensating for a lowered assimilation efficiency and achieving similar rates of growth on both substrata (Block, 1997). Collembolans may select a food type containing a particular source of a nutrient which they are not capable of producing themselves (Worland, 2000). Other Collembola have been shown to select food sources particularly rich in essential carbohydrates (fungi containing high levels of mannitol, which is an important cryoprotectant)(Worland, 2000).

When judging the effect of *Cryptopygus* species within the system, it is important to know both what is consumed and what is really assimilated, but it seems at present that only food-labeled experiments with tracers will provide meaningful data. Especially needed are investigations into the nutritional ecology of those few herbivores that successfully use lichens and mosses as food. What factors influence their food choices?

How do differences in the nutritional quality of lichens and mosses influence these choices? (Slansky, 1987). Are there differences in the digestion of the tested diets? To what extent do lichen/moss feeding patterns of herbivores result from differences in food composition? Is lichen/moss feeding of greater nutritional benefit to herbivores in harsh physical environments?
A combination of natural-abundance isotope surveys and isotope addition experiments appears to be a powerful approach for investigating both average patterns and interspecific variability in resource exploitation. In such experiments, use of multiple natural isotopes (Sullivan & Moncreiff, 1990, Riera et al., 1996, Peterson, 1999) or/and isotope-addition experiments (Middleburg et al., 2000) are often necessary to resolve diets.

IV-Material and methods

1. Material

The lichens were transported to The Netherlands in the desiccated state?? and stored at -20°C in a deep freezer. Previous investigations (Lange, 1969) have shown that the metabolic capability of frost and drought resistant lichens is not changed by such treatment.

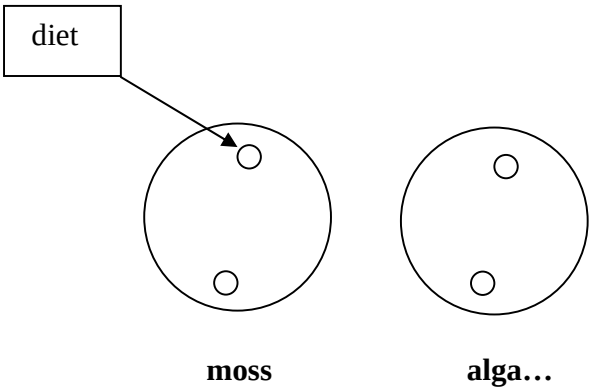
Three main experiments were carried out in order to determine for the species (1) the food preferences among diets-6 choice experiment, (2) the preference between two different diet- 2 choice experiment, and (3) isotopic signatures.

2. Experiment 1 , single diet experiment (non-labeled experiment)

This first experiment has been set up as a one food source experiment; only one type of diet (either algae, mosses or lichens) has been put in a circular jar (30 jars in total: 6 diets x 5replictaes for each diet) with a base of moist plaster of Paris) at two different places, and then 20 springtails have been added. This experiment has been conducted in a cold room at 2^0C with low overhead lighting. 5 replicates for each diet have been made.
Concerning the diets, six different types of diet were tested: one species of alga (*Prasiola crispa*), two species of lichen (*Umbilicaria decussata* and *Usnea Antarctica*) and three species of moss (*Brachythecium glaciale*, *Pohlia nutans,* and *Sanionia uncinata).*

Experiment: one food source experiment

- Experimental set up:
    - Circular feeding arena (polystyrene pots with a base of moist plaster of Paris)



- Pieces of filter paper covered with a layer of a single diet
- Animals are starved for 1 week before the beginning of the experiment
- 20 drops of sterile water are added in the middle of the arena
- 20 Collembolans are added in the middle of the arena
- For each diet tested, 5 replicates are made

Some observations were made during the experiment like the presence of moults, the number on each diet, the number of individuals on the specific diet, presence of the food source or absence, as well as the number of faecal pellets on each diet.

The duration of the experiment took 6 weeks. The distribution was recorded by counting the number of individuals within each diet zone.
At the end of the experiment, the animals were collected from the jars and put in a freezer for three days. Then the animals samples were freeze-dried for three days. Then, animals (4 for carbon isotope ratio analysis and 15 for nitrogen) were placed into ultra-clean tin capsules and analyzed by continuous flow isotope ratio mass spectrometry using a GC-c-IRMS for the stable isotope analyses.

3. Experiment 2, two-diet choice experiment (non-labeled experiment)

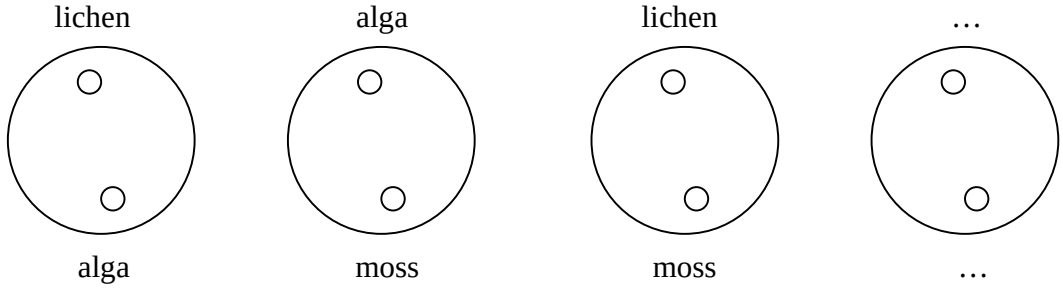
The 2-choice experiment was used to determine the preference between two different diets. Animals were starved for a week at 2°C prior to being placed in the jars. 20 springtails were kept in a plastic vial with plaster of Paris at the bottom. A piece of one type of diet was placed into the vial in 5 replicates for each combination of diets (75 jars in totals: 15 combinations x 5 replicates). Jars were placed in a controlled-environment room at 2°C with low overhead lighting. The number of individuals staying on the diet was counted for weeks. The proportion of springtails found on each diet was used for the analysis.

Aim: What are the feeding preferences of Antarctic springtails?
Experiment: two-choices experiments

- Species: Collembola (*C. antarcticus*)
- Diet types: lichen (*Umbilicaria decussata*, *Usnea antarctica*), mosses (*Brachytecium glaciale*, *Pohlia nutans*, *Sanionia uncinata*), foliose green algae (*Prasiola crispa*)

- Experimental conditions: room at 2^0C; low overhead lighting
- Experimental set up:

    - Circular feeding arena (polystyrene pots with a base of moist plaster of Paris)



    - Pieces of filter paper covered with a layer of diet
    - 20 drops of sterile water are added in the middle of the arena
    - 20 Collembolans are added in the middle of the arena
    - For each combination of diets 5 replicates are made

- **Observations:** similar to the previous experiment

At the end of the experiment, the animals were collected from the jars and put in a freezer for three days. Then the animals samples were freeze-dried for three days. Then, animals (4 for carbon isotope ratio analysis and 15 for nitrogen) were placed into ultra-clean tin capsules and analyzed by continuous flow isotope ratio mass spectrometry using a GC-c-IRMS for the stable isotope analyses.

For the following experiments, labeled food sources are use; therefore, we firstly labeled the different food sources used in this study following this procedure:

Carbon labeling of the food sources (algae, lichen, mosses)

Carbon dioxide absorbed by plants:

CO_2 production: sodium bicarbonate labeled (20 mg) + chloridric acid (HCl) for 3-4h. .

Carbon dioxide will be supplied via sodium bicarbonate addition ($NaHCO_3$ see below).

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow H^+ + H^+ CO_3^{-2}$$

- Put the food sources to be labeled in a dessicator with light on it (in Petri dishes)
- Food sources have to be moisturized before the experiment

- In a glass Petri dish: $NaHCO_3$ labeled (20 mg) in a glass Petri dish (solution of sodium bicarbonate with distilled water) and add carefully with a syringe the solution of HCl diluted (1 M) with distilled water (about 2 cm^3 or 0,002 l of HCl, end volume: 22ml) until the pH of the solution reaches the value of 2 (checking with a pH indicator)
- Close the dessicator for 3-4 hours
- Labeling in the climate room

Nitrogen labeling of the food sources (algae, lichen, mosses)

Nitrogen is taking up by the leaves of the algae and mosses:

- Preparation of the 20% ^{15}N labeled solution: cf. Prasiola medium (Schlasser, 1997); preparation of 100ml of solution containing 0,89 g of ^{15}N labeled (98 atom % excess ^{15}N) as KNO_3:

For 1l medium solution:

- Stock solutions: - $CaCl_2.2H_2O$: 2,5 g for 1l of medium: 10 ml of this stock solution for 1l medium
  - $MgSO_4.7H_2O$: 7,5 g for 1l of medium: 10 ml of this stock solution for 1l medium
  - NaCl: 2,5 g for 1l of medium: 10 ml of this stock solution for 1l medium
  - $K_2HPO_4.3H_2O$: 7,5 g/l for 1l of medium: 10 ml of this stock solution for 1l medium
  - KH_2PO_4: 17,5 g/l for 1l of medium: 10 ml of this stock solution for 1l medium

- KNO_3 (^{15}N labeled): 0,89g for 1l of medium (0,178g $K^{15}NO_3$ + 0,712g KNO_3)

- Micronutrients: - 0,75 g Na_2EDTA in 1l
  Then add in sequence:
  - $FeCl_3.6H_2O$: 97 mg
  - $MnCl_2.4H_2O$: 41 mg
  - $ZnCl_2.6H_2O$: 5 mg
  - $NaMoO_4.2H_2O$: 4 mg
  - $CoCl_2.6H_2O$: 2 mg

  Use 6 ml of this solution for 1l of medium

- In 1l bottle, add 10ml of $CaCl_2.2H_2O$, 10ml of $MgSO_4.7H_2O$, and 10ml of NaCl
- Autoclave this bottle for about 2 hours
- In another bottle, add 0,89g $K^{15}NO_3$, 10ml $K_2HPO_4.3H_2O$, and 10ml KH_2PO_4.
- Filter the solution of $K^{15}NO_3$, HPO_43H_2O, KH_2PO_4 because this solution precipitate, in a anoxic atmosphere (with syringe in the bottle)

- Spraying of the different food sources by a ^{15}N labeled solution of potassium nitrate
- Food samples put in a Petri dish (moisturizing of the food sources before and regularly; everyday)
- Spraying once a day during 2 weeks
- Samples used in the feeding experiments, some used as controls and analyzed for their isotopic composition

NaNO3 has been replaced by KNO3 from the initial medium for the labeling. Initialy, 0,75g/l of NaNO3 is added to the medium, but in this case 0,89g of KNO3 has been added to the 1l medium.

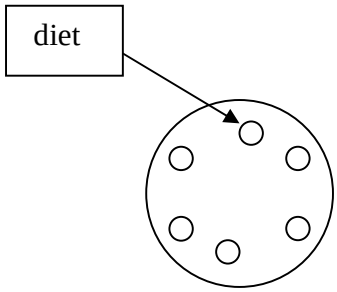
4. Experiment 3, cafeteria experiment (labeled experiment)

In the 6-choice experiment, one piece of each of the 6 diets were placed in a circle (cafeteria) and 20 springtails were released into every vial. There were 5 replicates (vials) per species. The number of individuals on diet was counted daily for 5 successive days. The food has been labeled beforehand in order to get a clearer pattern of the food choice. This experiment lasted 4 weeks and observations of the animals in the jars were made regularly.

Experiment: cafeteria experiment (6-choice experiment)

- Species: Collembola (*C. antarcticus*)
- Diet types: lichen (*Umbilicaria decussata*, *Usnea antarctica*), mosses (*Brachytecium glaciale*, *Pohlia nutans*, *Sanionia uncinata*), foliose green algae (*Prasiola crispa*)
- Experimental conditions: room at 2^0C; low overhead lighting
- Experimental set up:

  - Circular feeding arena (polystyrene pots with a base of moist plaster of Paris)



- Pieces of filter paper covered with a layer of a single labeled diet (^{13}C and ^{15}N labeled)
- Animals are starved for 1 week before the beginning of the experiment
- 20 drops of sterile water are added in the middle of the arena
- 20 Collembolans are added in the middle of the arena
- For each diet tested, 5 replicates are made

- Observations: similar to the previous experiments

The duration of the experiment was 4 weeks. The distribution was recorded by counting the number of individuals within each diet zone.

For the stable isotope analyses, the animals were collected from the jars and put in a freezer for three days. Then the animals samples were freeze-dried for three days. Then, animals (4 for carbon isotope ratio and 15 for nitrogen) were placed into ultra-clean tin capsules and analyzed by continuous flow isotope ratio mass spectrometry using a GC-c-IRMS for the stable isotope analyses.

5. Experiment 4, single diet experiment (labeled experiment)

The experimental design of this experiment is similar to the one of experiment 1, but beforehand, the different food sources have been labeled (^{13}C and ^{15}N). This experiment lasted 4 weeks and observations of the behavior of the individuals were made regularly.
The distribution was recorded by counting the number of individuals within each diet zone.

For the stable isotope analyses, the animals were collected from the jars and put in a freezer for three days. Then the animals samples were freeze-dried for three days. Then, animals (4 for carbon isotope ratio and 15 for nitrogen) were placed into ultra-clean tin capsules and analyzed by continuous flow isotope ratio mass spectrometry using a GC-c-IRMS for the stable isotope analyses.

6. Experiment 5, two-diet choice experiment (labeled experiment)

The experimental design of this experiment is similar to the one of experiment 2, except that the food sources have been labeled beforehand (^{13}C and ^{15}N).

The 2-choice experiment was used to determine the preference between two different diets. Animals were starved for 4 days at 2°C prior to being placed in the jars. 20 springtails were kept in a plastic vial with plaster of Paris at the bottom. A piece of one type of diet was placed into the vial in 5 replicates for each combination of diets (75 jars in totals: 15 combinations x 5 replicates). Jars were placed in a controlled-environment room at 2°C with low overhead lighting. The number of individuals staying on the diet was counted daily for 5 days. The proportion of springtails found on each diet was used for the analysis.

For the stable isotope analyses, the animals were collected from the jars and put in a freezer for three days. Then the animals samples were freeze-dried for three days. Then, animals (4 for carbon isotope ratio and 15 for nitrogen) were placed into ultra-clean tin capsules and analyzed by continuous flow isotope ratio mass spectrometry using a GC-c-IRMS for the stable isotope analyses.

7. Methods

➢ Stable isotope analysis techniques

Basics

Biological material contains carbon and nitrogen with various proportions of their naturally occurring stable isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). Nitrogen and carbon both occur naturally as mixtures of two isotopes, with the lighter isotope being much more abundant. Isotopic composition is given by the ratio of the heavier (minor) isotope to the lighter (abundant) isotope (O.Schmidt, 1997). Globally, the ambient ratio of $^{15}N/^{14}N$ is 0,37% and that of $^{13}C/^{12}C$ is 1,1%. Natural abundances of stable isotopes are normally expressed using the "delta per mil" (δ‰) notation. This is the difference, in parts per thousand, of the sample isotope ratio from the isotope ratio of a standard. Delta (δ) values are normally measured as sample-standard comparisons and not as absolute values. For $\delta^{15}N$, the standard is atmospheric di-nitrogen, and most natural values are within +/- 20‰ of the standard. For $\delta^{13}C$, the standard is a fossil carbonate (PD Belemnite) with significantly higher ^{13}C content than most plant samples. (O.Schmidt, 1997). Stable isotopes of the same element participate in chemical reactions at different rates, which results in a preferential net incorporation of heavier isotopes in the consumer's body, a process termed "fractionation" (McNabb, 2001).

Measurement

Differences in stable isotope composition at natural abundance levels are measured using an isotope rationing mass spectrometer. It is inconvenient to express stable isotope ratios as absolute values, especially when we are concerned with small differences in these ratios. Therefore, isotope ratios are expressed relative to a standard. The unit of isotopic ratio measurement is the delta value (δ) and is expressed as the deviation per mil (‰) from an arbitrary standard. Thus, $\delta = R_{sample}-R_{standard}/ R_{standard} \times 1000$ where R is the absolute isotopic ratio of either the sample or standard (Ehleringer, 1986).
$\delta^{15}N$ of ecological materials is usually measured by a continuous-flow isotope ratio mass spectrometer (CF-IRMS). A sample (typically containing around 100μg N) is combusted on-line in the analyzer of the CF-IRMS, reducing all forms of N in the sample to N_2, on which R_{sample} is determined (Robinson, 2001). GC-c-IRMS comprises a GC equipped with a capillary column that is used to separate the compounds of interest at high resolution. The outlet of the column is attached to a miniature oxidation reactor where the organic molecules are combusted to CO_2, N_2 and H_2O gas. A reduction reactor is included for ^{15}N analysis to convert oxidized nitrogen species to N_2 gas. Water is removed on-line and the purified CO_2 and N_2 are led into an isotope ratio mass

spectrometer (IRMS). Because of its design, an IRMS measures the isotopic ratios between the heavy and light isotopes ($^{13}C/^{12}C$ for carbon and $^{15}N/^{14}N$ for nitrogen) and results are always calibrated against an international standard or derived reference material (Boschker, 2002).

Applications

This is a technique which has only recently been adopted to investigate trophic relations of soil animals, hence natural abundance stable isotope ratio techniques can be used as a tool for the investigation of the feeding ecology of soil animals and trophic relations in soil food webs (Schmidt, 2004). This technique is relatively fast and easy, with the additional advantage of taking into account not ingested food, but food that is really assimilated (Ponsard, Arditi, 2000). This technique is based on the fact that animal tissues are built with atoms of the food they assimilate, and therefore reflect the food's isotopic composition (Ponsard, Arditi, 2000). It has also been shown that, for carbon (DeNiro and Epstein, 1978) and above all for nitrogen (DeNiro, and Epstein, 1981), "you are what you eat…plus a few per mil": animal tissues tend to be slightly enriched in the heavier isotope compared with their diet (Ponsard, Arditi, 2000). DeNiro and Epstein (1981) and Schoeninger and DeNiro (1984) showed the $^{15}N/^{14}N$ ratio tend to increase much more markedly with the trophic level than the $^{13}C/^{12}C$ ratio (Ponsard, Arditi, 2000). This is because rates of fractionation of carbon are lower than those of nitrogen. Animal tissue is usually enriched only slightly in ^{13}C compared to its food source (0.4 +/- 1.4 ‰); in contrast, levels of ^{15}N in organisms tend to be 3.4 +/- 1.1‰ higher than those in their food (Peterson & Fry, 1987; Ehleringer & Rundel, 1988; Ostrom et al., 1997 in McNabb, 2001).In fact, the natural abundance of $^{15}N/^{14}N$ ($\delta^{15}N$) in animals is generally thought to be up to 5‰ more ^{15}N-enriched than the N in their diets (R. Neilson et al., 1998). Where whole body ^{15}N-enrichment occurs relative to diet, it causes a stepwise increase of whole animal $\delta^{15}N$ at each trophic level. Thus, values of ^{13}C are largely conserved in food chains, and provide information about the identity of the energy base (McNabb, 2001). It follows that, the isotopic composition of the whole body in small animals gives an accurate estimate of the $\delta^{13}C$ value of their diet due to the short time period for dietary carbon to become incorporated in tissues (M.J.I. Briones et al., 1999). The $\delta^{13}C$ value of an animal's diet must approximate to that of the carbon assimilated, with fractionation effects occurring through assimilation (M.J.I. Briones et al., 1999) and through losses in respiration (Deiro and Epstein, 1978) and excretion (M.J.I. Briones et al., 1999). This lead to the observed discrepancy between the isotopic values of the animal and the feed, resulting in a slight enrichment in $\delta^{13}C$ of the animal by about 1‰ in relation to the diet (DeNiro and Epstein, 1978) (the carbon isotopic composition of organisms becomes more positive moving up the food chain) and represents one of the major problems in using stable isotopes techniques (M.J.I. Briones et al., 1999). While $\delta^{15}N$ is less useful than $\delta^{13}C$ for directly tracing food sources, it is frequently useful for ranking animals into their relative trophic levels (R. Neilson et al., 1998). Variations in nitrogen stable isotope ratios may also be used to analyze the nutritional status of animals. Starvation results in an enrichment of ^{15}N in animal tissue (Oelbermann, 2002). High levels of ^{15}N in starving or nutritionally stressed animals are thought to be a consequence of recycling of body nitrogen (Oelbermann, 2002).

Ecological studies have been informed by using stable isotopes at naturally occurring levels (called “natural abundance”) and at levels well outside the natural range of values (called “enriched” levels); enriched isotope studies therefore use “labeled” substances (Dawson, 2002).
For studies using enriched materials, the labeled substance added (e.g. $^{15}NO3$) has an isotopic composition that significantly differs (usually exceeds) from any natural occurring level. The expression of the isotopic composition of this type of material is referred to in “atom %” (Ab) which is defined as,

$A_b = X_{heavy}/X_{heavy}+X_{light} = 100 \times (R_{sample}/R_{sample}+1), \%$ (1)

Where X_{heavy} and X_{light} are the numbers of heavy and light atoms present in the sample and R_{sample} is the isotope ratio. Equation (1) is most commonly used when values of A_b exceed 0.5 atom % (or 500%). Atom % is thus the percentage contribution of the heavy isotope to the total number of atoms of that element in the sample (Dawson, 2002). The more ^{15}N-enriched a sample, the more positive (or less negative) its $\delta^{15}N$.

V-Results

- Isotopes analysis

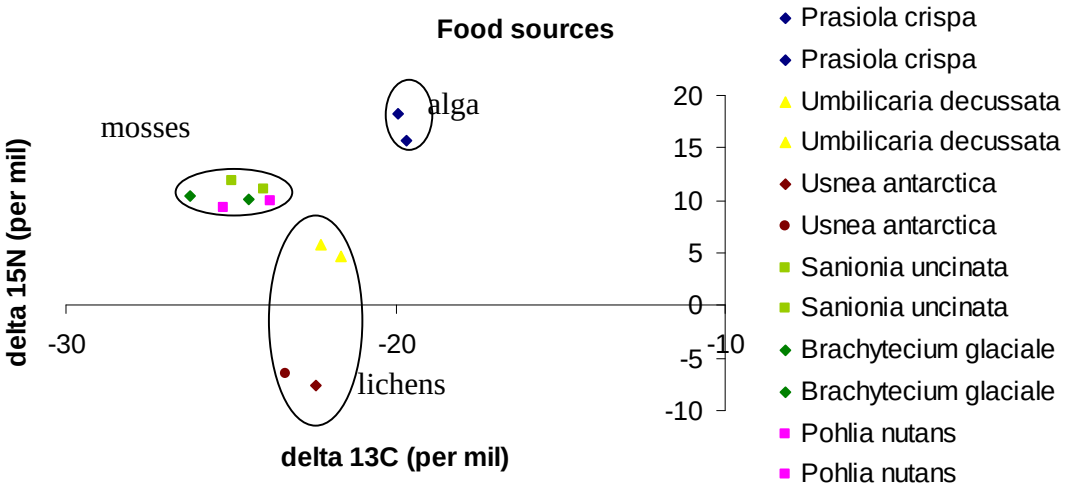


Figure 5.1.: isotopic signatures of the different food sources used in the study

As we can see on the graph, the different food sources are well differentiated according to their ^{13}C and ^{15}N isotopic content. The values fluctuate between -19,9 and 19,6‰ for carbon and between 15,6 and 18,2‰ for nitrogen for the diet *Prasiola crispa*. The two lichen species are situated between -23,3 and -21,6‰ for carbon and between -6,5 and 4,7‰ for nitrogen. At last, the three mosses species are situated between -26,2 and -23,7‰ for carbon and between 9,3 and 11,9‰ for nitrogen.

1. Experiment 1, single diet experiment (non-labeled experiment)

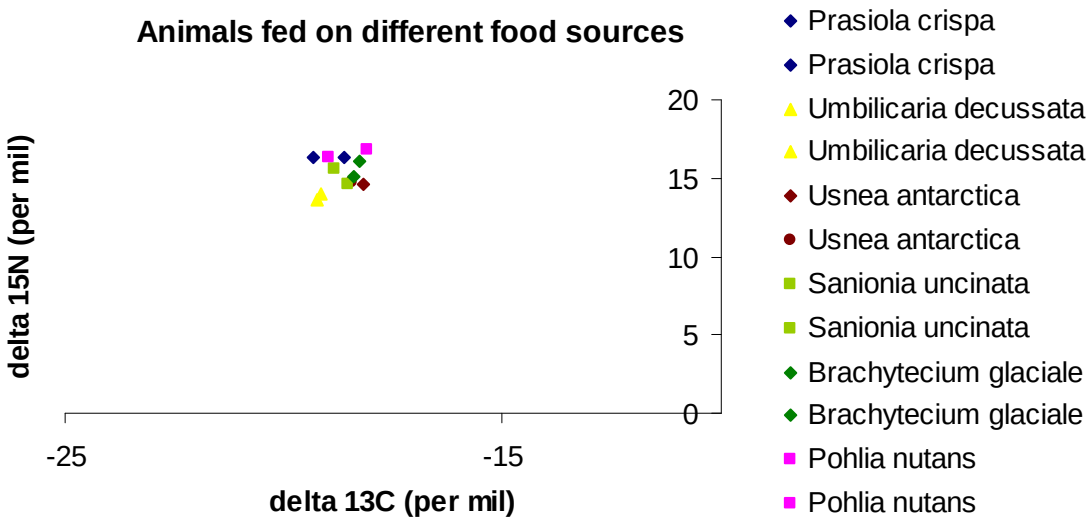


Figure 5.1.1: Isotopic signatures of the animals fed on different food sources

As we can see from this figure 5.1.1, the isotopic content of the animals fed on different diets does not show a large spread in the nitrogen values neither in the carbon ones, all the animals have an isotopic signature value between -18 and -19,3‰ for carbon and between 13 and 16,7‰ for nitrogen. It seems that the animals did not or barely incorporate the isotopic signature of their food sources. If we plot both the different diets types versus the animals isotopic content, we clearly see that the animals' isotopic signatures are all very close together, close to the *Prasiola* diet values (Figure 5.1.2).

As we cannot use an analysis of samples variances (ANOVA) with only two values for each experiment, we can use the Kruskall Wallis test to test whether or not there are significant differences between the ^{13}C and ^{15}N delta values of the animals fed on different diet types. For the delta ^{13}C values as well as for the ^{15}N values, there are no significant differences among the diets (Chi square=7, df=5, p=0,221 for carbon values and Chi square=10, df=5, p=0,075 for nitrogen values).

Fractionation of ^{15}N between food source (A) and consumer (B) can be calculated, it is described in terms of the difference in delta (δ) values using the Δ notation, where $\Delta=\delta_B-\delta_A$. A positive Δ value indicates a relatively greater concentration of the isotope with the higher mass for B, and therefore has a lower isotopic value than A.

Consumer	Diet	$\Delta\delta^{13}C$	$\Delta\delta^{15}N$
Cryptopygus antarcticus	*Prasiola crispa*	0,3	0,6
	Prasiola crispa	1,3	-1,9
	Umbilicaria decussata	3,0	8,1
	Umbilicaria decussata	2,4	8,8
	Usnea antarctica	4,2	22,1
	Usnea antarctica	4,8	21,2
	Sanionia uncinata	6,1	3,6
	Sanionia uncinata	5,4	3,5
	Brachytecium glaciale	7,8	4,7
	Brachytecium glaciale	6,1	5,8
	Pohlia nutans	5,7	6,8
	Pohlia nutans	6,2	6,9

Table 5.1.: Trophic shift for *Cryptopygus antarcticus* fed on different diets

From this table, we note a trend according to which the trophic shift is similar for a type of food, for instance the carbon trophic shift is very small for the alga Prasiola, and much larger when moss species are used as food sources. We observe the same trend for the nitrogen trophic shift, except that the largest shift is observed for the lichen Usnea. Unfortunately, this table does not provide much more information from relationships between the $\delta^{15}N$ values of the whole bodies of animals and the $\delta^{15}N$ values of their diets: because firstly, the animal nitrogen is in most cases enriched in ^{15}N relative to the diet nitrogen. Besides, the $\delta^{15}N$ values of different individuals of a species raised on the same diet can differ. At last, The Δanimal-diet values for a species fed different diets are similar (DeNiro and Epstein, 1981).
The different food sources were supplied as single diets ad libitum. They supported biomass increase and reproduction of the animals. Possible sources of variation in $\delta^{15}N$ fractionation therefore might be differences in the type and quality of food or in the metabolism and physiological status of consumer species.

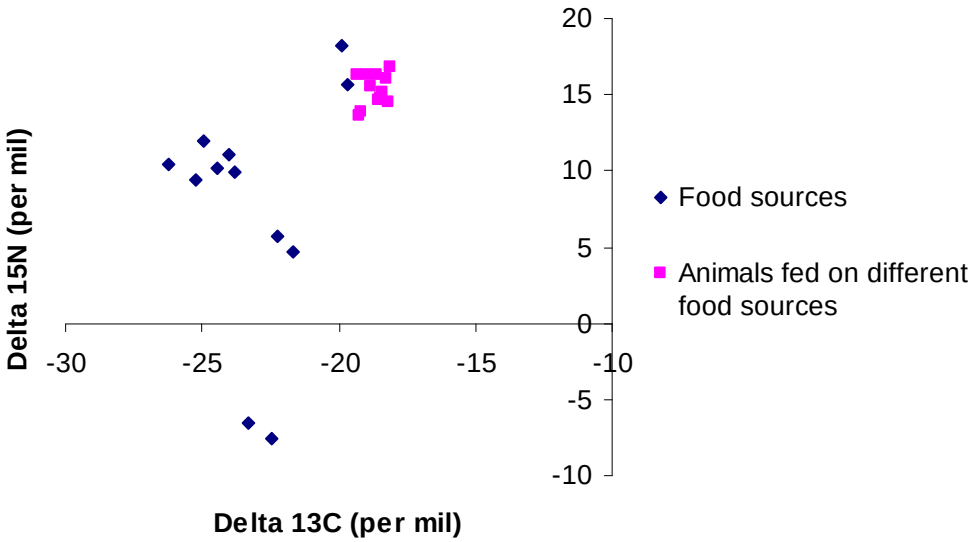


Figure 5.1.2: isotopic signatures of the different food sources and of the animals fed on

From this graph, we thus see the narrow range of isotopic signatures of the different animals fed on diverse food types. Nevertheless, if we look closer, we can distinguish some emerging patterns.

- Feces

At the end of the experiment, the number of feces and moults were counted in each pot, the following graphs show the results of these counts:

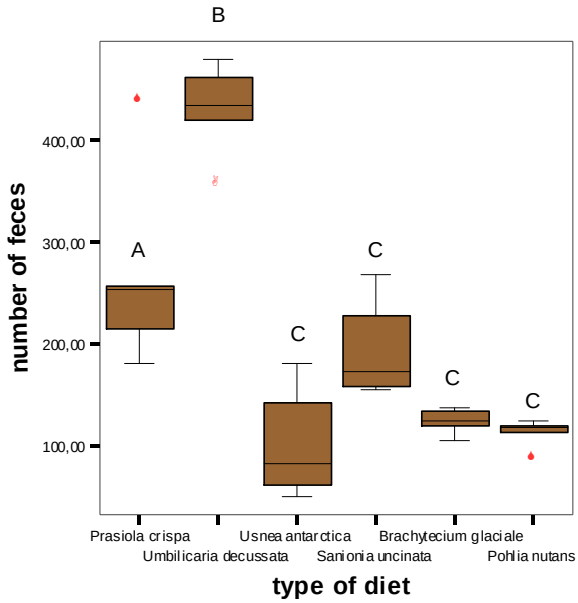


Figure 5.1.3.: Boxplot graph with the medians of faecal pellets produced by *C. antarcticus* on different types of diets

According to the Kolmogorov-Smirnov test of normality, it appeared that the data did not follow a normal distribution, and moreover the Leven's test of homogeneity of variances revealed that the variances between the groups were different. Thus, the Kruskal-Wallis non-parametric test (see appendix) was applied with the original data. The test indicates that there exists significant differences in the number of feces between the 6 different diet types (Chi square=23.250, d.f.=5, p=0.000). However, it is difficult to say which type differ significantly from the other 5; common sense should be used here, emphasize the highest and the lowest (*Umbilicaria decussata*/*Pohlia nutans* respectively), however, if we carry out a post hoc Scheffé test, we can see some significant difference; the number of feces appears to be significantly different between the alga diet (*Prasiola crispa*) and the rest of the diets, and the same difference appears for the number of feces counted with the lichen diet (*Umbilicaria decussata*). Thus, the number of feces on these two diets is significantly higher that on the others four diets with a higher number of feces on *Umbilicaria decussata* compared to *Prasiola crispa*. The fact that animals deposit a high amount of feces by consuming Prasiola and Umbilicaria diets would imply that either animals eat these diets and assimilate it as natural nutritious food source or eat it but do not assimilate it and deposit food feces leaving it untouched.

➢ Moults

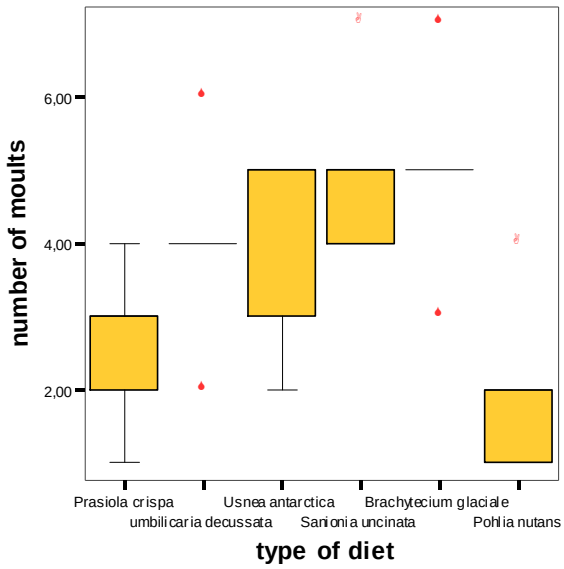


Figure 5.1.4.: Boxplot graph with the medians of moults produced by *C. antarcticus* on different types of diets

Here again, the Kolmogorov-Smirnov test of normality assessed that the data did not follow a normal distribution and the Leven's test of homogeneity of variances revealed that the variances between the groups were assumed to be equal. Thus, the Kruskal-Wallis non-parametric test (see appendix) was applied as well with the original data. The test indicates that there is a significant difference in the number of moults between the 6 different diet types (Chi square=14.197, d.f.=5, p=0.014). However, it is difficult to say which type differ significantly from the other 5; we can emphasize the highest and the lowest (*Brachytecium glaciale*/*Pohlia nutans*), but it will be difficult to say whether *Umbilicaria decussata* differ significantly from *Usnea antarctica* for instance. Here, the Scheffé's post hoc test does not give any significant difference between the different diets.

2. Experiment 2, two-diet choice experiment (non-labeled experiment)

Below the results are represented graphically:

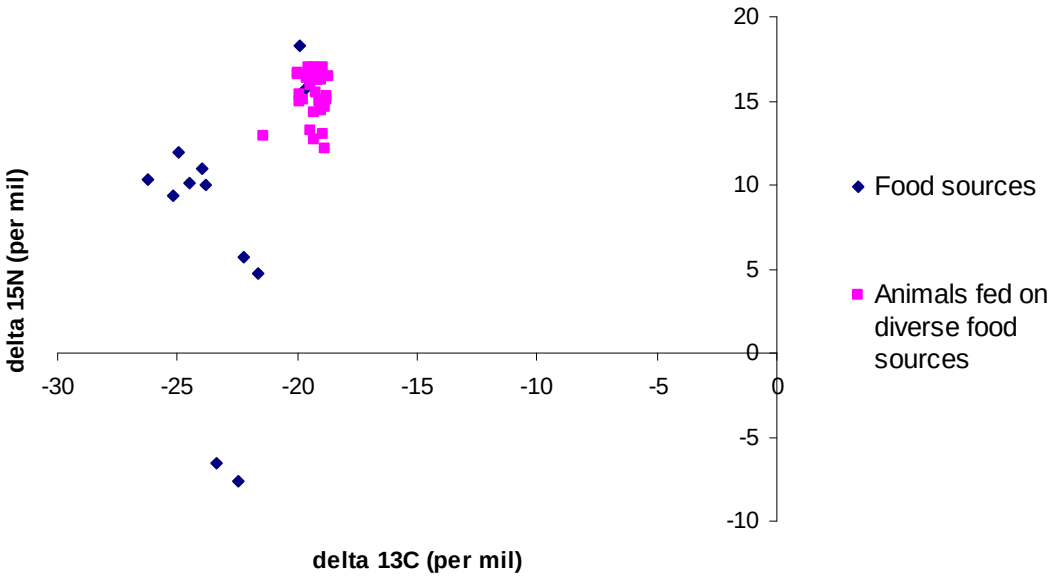
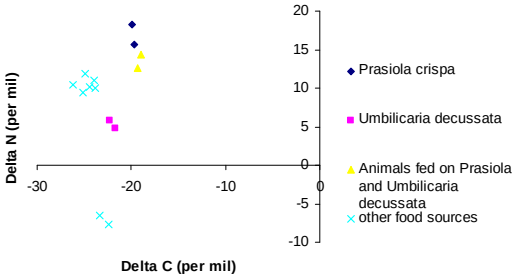
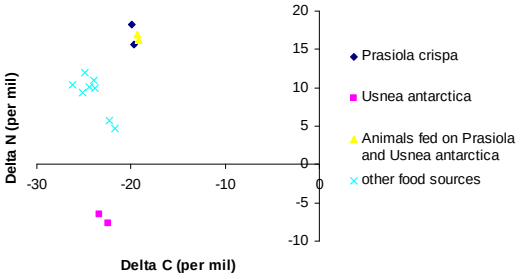


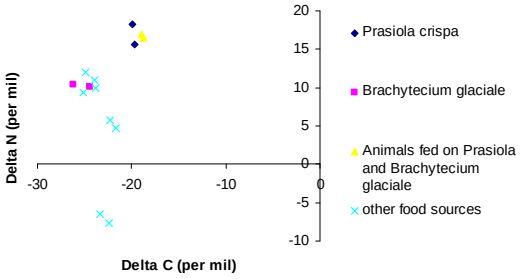
Figure 5.2.1.: Isotopic signatures of the different food sources and of the animals fed on a two-choices diets.

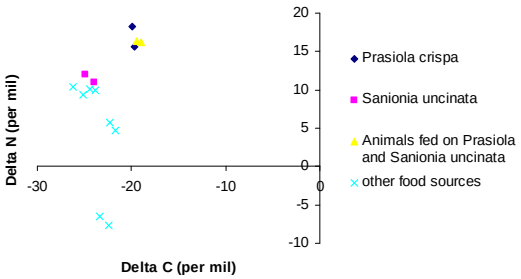
In this graph, the isotopic signatures of the animals are also clustered, nevertheless the animals values are more spread along the axis than in the first experiment. Delta ^{13}C values of the animals varies between -21,4 and -18,6‰ and delta ^{15}N values between 12,6 and 16,9‰. We can notice a wider variation range of values for ^{15}N than for ^{13}C.

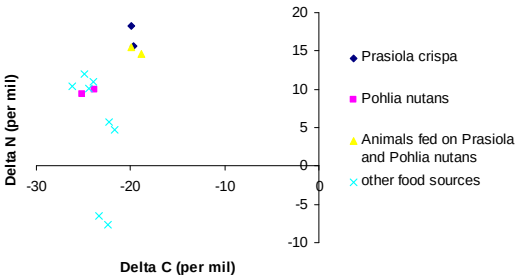
Below are presented some detailed results with all the diet combinations:

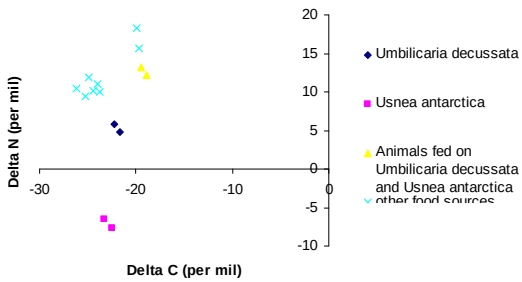

Delta N (per mil)
Delta C (per mil)
Prasiola crispa
Umbilicaria decussata
Animals fed on Prasiola and Umbilicaria decussata
other food sources

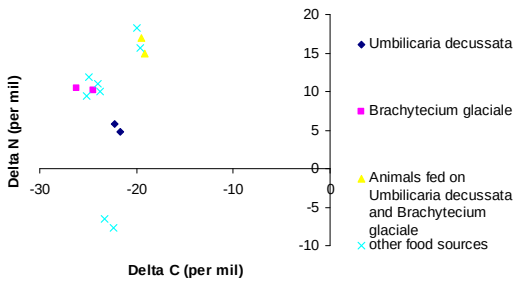

Delta N (per mil)
Delta C (per mil)
Prasiola crispa
Usnea antarctica
Animals fed on Prasiola and Usnea antarctica
other food sources

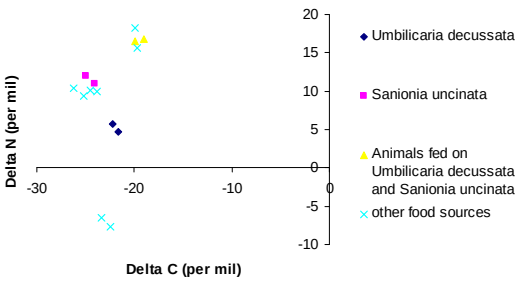

Delta N (per mil)
Delta C (per mil)
Prasiola crispa
Brachytecium glaciale
Animals fed on Prasiola and Brachytecium glaciale
other food sources


Delta N (per mil)
Delta C (per mil)
Prasiola crispa
Sanionia uncinata
Animals fed on Prasiola and Sanionia uncinata
other food sources


Delta N (per mil)
Delta C (per mil)
Prasiola crispa
Pohlia nutans
Animals fed on Prasiola and Pohlia nutans
other food sources


Delta N (per mil)
Delta C (per mil)
Umbilicaria decussata
Usnea antarctica
Animals fed on Umbilicaria decussata and Usnea antarctica


Delta N (per mil)
Delta C (per mil)
Umbilicaria decussata
Brachytecium glaciale
Animals fed on Umbilicaria decussata and Brachytecium glaciale
other food sources


Delta N (per mil)
Delta C (per mil)
Umbilicaria decussata
Sanionia uncinata
Animals fed on Umbilicaria decussata and Sanionia uncinata
other food sources

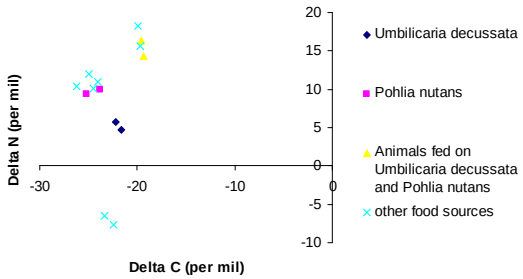

Delta N (per mil)
Delta C (per mil)
Umbilicaria decussata
Pohlia nutans
Animals fed on Umbilicaria decussata and Pohlia nutans
other food sources

Figure 5.2.2.: $\delta^{13}C$ and $\delta^{15}N$ values of several diet combinations and the delta values of the specimens fed on these combined diets

In the diet combinations where the alga *Prasiola crispa* is present (*Prasiola crispa/Umbilicaria decussata, Prasiola crispa/Usnea antarctica, Prasiola crispa/Brachytecium glaciale, Prasiola crispa/Sanionia uncinata, Prasiola crispa/Pohlia nutans*), the ^{13}C and ^{15}N values of the animals fed on these combined diets are close to the ones of the alga diet except for the combination *Prasiola crispa/Umbilicaria decussata* where the values of the animals shift to lower values.
If we look in more details, we can compare two combinations of diets where one of the same diet is present:

- *Prasiola crispa/Umbilicaria decussata* compared to *Prasiola crispa/Usnea antarctica*: the delta ^{15}N values of the animals fed on the combination *Prasiola crispa/Umbilicaria decussata* (mean of 13,5‰) are lower tan the ones of the animals fed on the diet *Prasiola crispa/Usnea antarctica* (mean of 16,6‰); the delta ^{13}C values do not change from a diet to another.
- *Umbilicaria decussata/Usnea antarctica* compared to *Umbilicaria decussata/Brachytecium glaciale*: the delta ^{15N} values of the animals fed on the diet *Umbilicaria decussata/Usnea antarctica* are lower (mean of 12,7‰) than the ones of the animals fed on *Umbilicaria decussata/Brachytecium glaciale* (mean of 15,9‰).
- *Umbilicaria decussata/Usnea antarctica* and *Umbilicaria decussata/Sanionia uncinata:* the delta ^{15}N values of the animals fed o on the diet *Umbilicaria decussata/Usnea antarctica* are lower (mean of 12,7‰) than the ones of the animals fed on *Umbilicaria decussata/Sanionia uncinata* (mean of 16,7 ‰).
- *Umbilicaria decussata/Usnea antarctica* and *Umbilicaria decussata/Pohlia nutans*: the delta ^{15}N values of the animals fed on the diet *Umbilicaria decussata/Usnea antarctica* are lower (mean of 12,7‰) than the ones of the animals fed on *Umbilicaria decussata/Pohlia nutans* (mean of 15,3‰).

Making all these comparisons would be too tedious but we can note that animals delta values shift to a larger extent when a lichen species is present within the combined diet, which does make sense because the isotopic signatures of the lichens are the lowest ones (for the ^{15}N axis) compared to the other food sources.

As we have only two samples analyzed for one experiment, it is not possible to use an ANOVA, but we can compare the ^{13}C and ^{15}N δ values differences among the different diet types with a non parametric test (Kruskall Wallis test). For the delta ^{13}C values of the animals fed on different combinations of diet there is no significant difference among the diets (Chi square=13,174, df=14, p=0,513). The same results come out with the delta ^{15}N values (Chi square=22,748, df=14, p=0,064).

➢ Feces

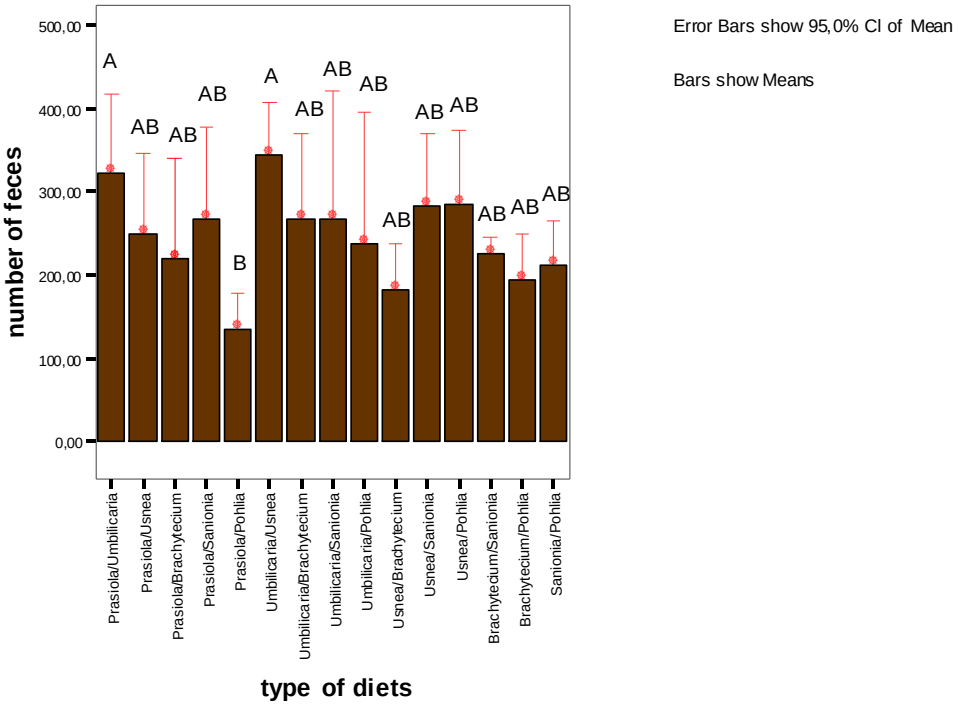


Figure 5.2.3.: Mean number of faecal pellets produced by *C. antarcticus* on different types of diets

The data followed a normal distribution according to the Kolmogorov-Smirnov test and the variances were assumed to be equal (Leven's test, t=1.521, df=14,60 p=0.131). Therefore, the ANOVA test of variances (see appendix) between groups was applied. This analysis enable us to say that there is significance between the number of feces on each diet type (F=2.513, df=14,60, p=0.007). Furthermore, it is possible to assess the differences among the different diets with the Tukey post-hoc test; the mean number of feces on *Prasiola/Pohlia* is significantly different from *Prasiola/Umbilicaria* and from *Umbilicaria/Usnea*, with a number of feces significantly higher with *Prasiola/Umbilicaria* and *Umbilicaria/Usnea* diet combinations compared to the number of feces on *Prasiola/Pohlia* diet.

➢ Moults

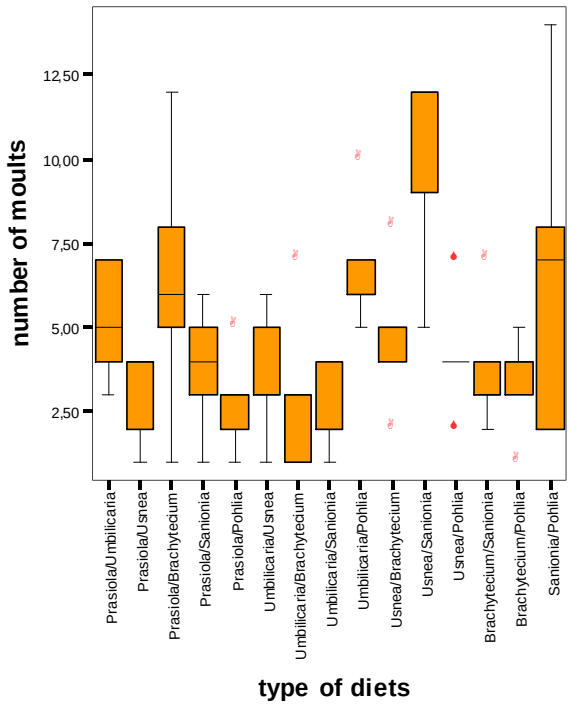


Figure 5.2.4.: Boxplot graph with the medians of moults produced by *C. antarcticus* on different types of diets

Here again, the Kolmogorov-Smirnov test of normality revealed that the data did not follow a normal distribution and the Leven's test of homogeneity of variances revealed that the variances between the groups were different. Thus, the Kruskal-Wallis non-parametric test (see appendix) was applied with the original data. The test indicates that there is a significance difference in the number of feces between the 6 different diet types (Chi square=27.584, d.f.=14, p=0.016). However, it is difficult to say which type differ significantly from the other 14; we can emphasize the highest and the lowest (*Usnea/Sanionia* and *Umbilicaria/Sanionia* respectively). Moreover, the Scheffé's post hoc test does not give any significant difference between the diets.

Labeling experiments

One way of increasing the ability to identify diets sources is to employ simultaneously additional tracers such as carbon and nitrogen isotopes. If two resources are offered, the proportion of carbon and nitrogen incorporated from each of the resources may be determined by using food materials with different stable carbon and nitrogen isotope signatures and then analyzing the resulting stable isotope signatures of the Collembolans (Bakonyi, Dobolyi & Thuy, 1995; Briones, Ineson & Sleep, 1999). It is then possible to apply mixing models for determining proportional contributions of different sources.

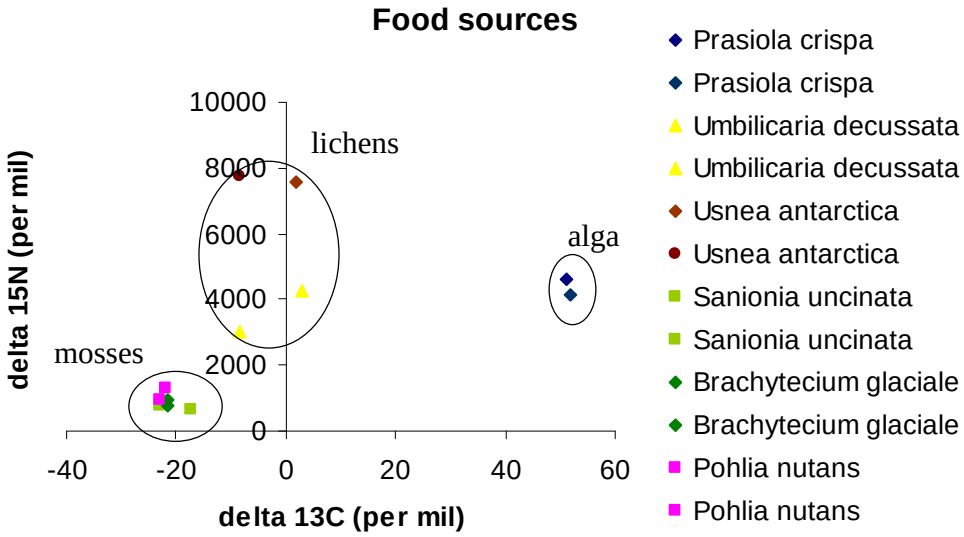


Figure 5.2.5.: isotopic signatures of the different food sources used in the study

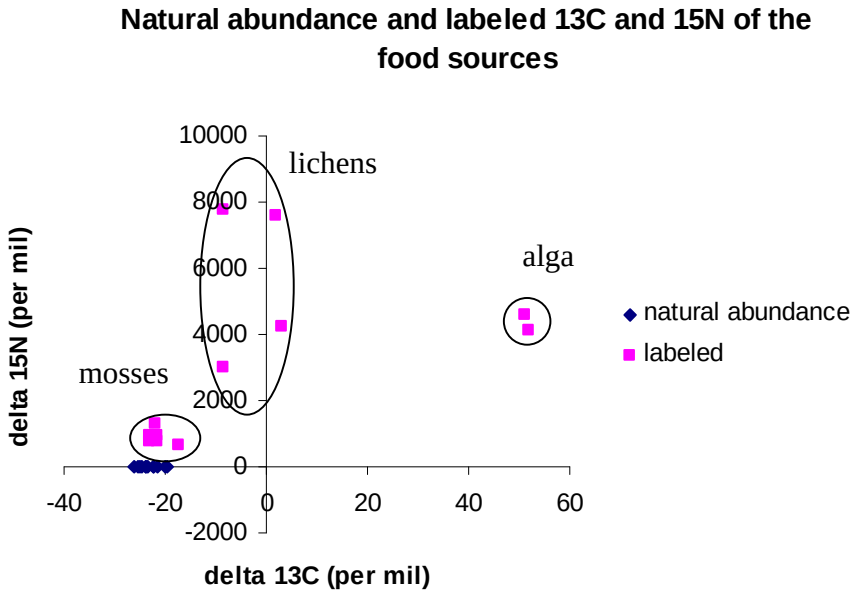


Figure 5.2.6.: isotopic signatures of the natural abundance and labeled food sources

From these two graphs, we can see that the labeling of the food sources did work (see appendix for ^{15}N content of the food sources before and after the labeling experiment) and that the different diets incorporated the enriched carbon and nitrogen.
According to the percentage of ^{15}N, the different food sources incorporated from 0,2% to 2,7% of ^{15}N depending on the type of diet (with the alga *Prasiola* and the lichen species incorporating 2 or 3 times more ^{15}N than the mosses species). For *Prasiola crispa*, the delta ^{13}C and ^{15}N values vary from -19 and -16‰ to 51 and around 4600‰ respectively.

Whereas for the lichen *U.decussata*, the delta values of ^{13}C and ^{15}N go rises from -22‰ to around 2‰ for the carbon and from 5‰ up to 4200‰ for nitrogen. The same range of increase is observed for the lichen *U.antarctica* which varies from -23 to 1‰ for delta ^{13}C and from -7 to 7700‰ for delta ^{15}N. On the other hand, mosses seem to incorporate much less of the label; ^{13}C delta values of *S.unciniata* slightly increase from -24 to -22 and ^{15}N delta values from 11 to 700. We observe the same small increment for the moss *B.glaciale* whom the delta ^{13}C values raise from -25 to -21 and delta ^{15}N values from 10 to 900, as well as for *P.nutans* whom values increase from -25 to -23 and from 9 up to 1300 for carbon and nitrogen delta values respectively.

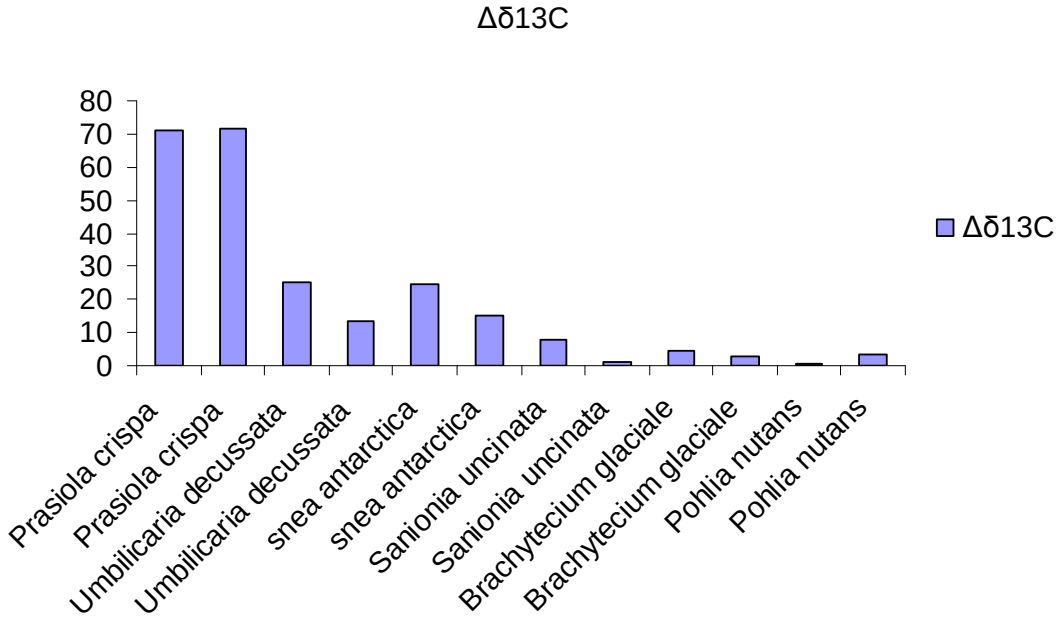


Figure 5.2.7.: increase in $\delta^{13}C$ in the different diet types

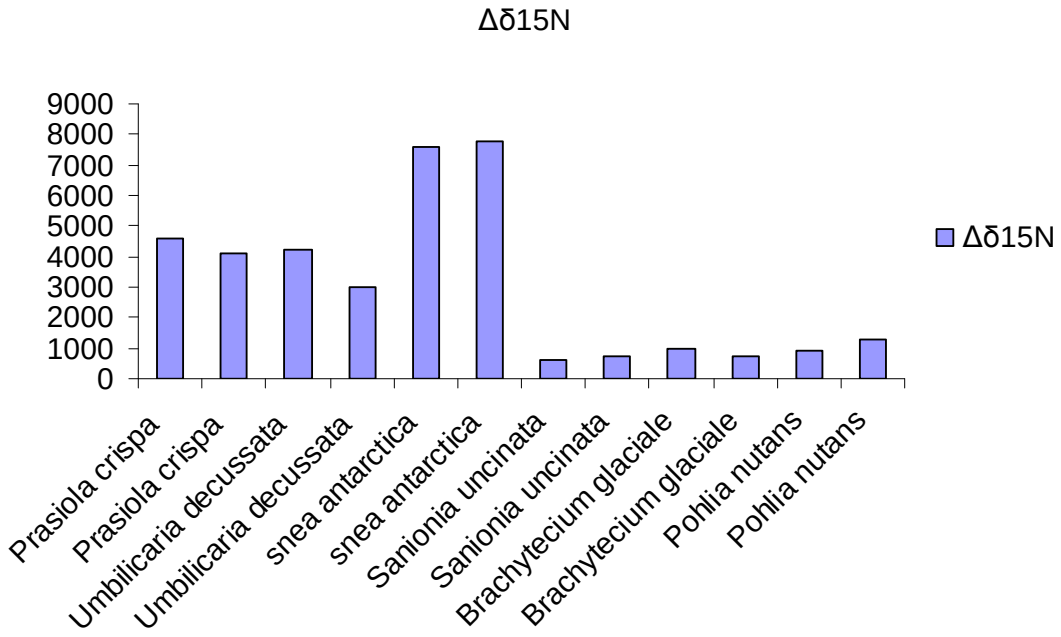


Figure 5.2.8.: increase in $\delta^{15}N$ in the different diet types

3. Experiment 3, Cafeteria experiment (labeled experiment)

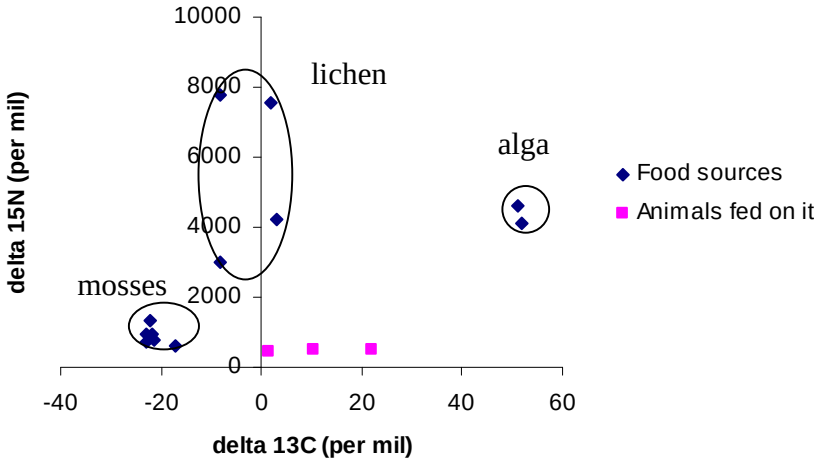


Figure 5.3.1.: delta 13C and 15N of the labeled food sources and of the animals fed on it

As we can see here, the shift for nitrogen is negligible, whereas, we see a noteworthy shift for the carbon values towards the *Prasiola* an lichens values on the right hand side of the graph. The values of the three animals analyzed are 424,3‰, 485,8‰ and 506,6‰ for delta N and 1,3‰, 10,2‰ and 21,8‰ for carbon values respectively. If we compare these values with the values of animals raised on a single labeled diet, we observe that animals fed on *Prasiola*, have delta N values around 750‰, the ones fed on the lichen

Umbilicaria decussata between 200 and 400‰ and the one fed on *Usnea antarctica* between 200 and 500‰, whereas the ones fed on mosses have delta N values much lower (around 25 or 35); thus, we see that animals fed with the 6 different diets have values closer to the animals' values fed either on *Prasiola* or lichens. For the $\delta^{13}C$ values, it is less obvious to make any conclusion because the values of the animals fed on the cafeteria are not very close to any of the values of animals fed on single diets; nevertheless, we can note that the values of these animals are positive and the only values positive for the animals fed on single diets are with the *Prasiola* diet (-16‰ and 8‰) and the moss *Sanionia uncinata* (10‰ and 13‰).

4. Experiment 4, single diet experiment (labeled experiment)

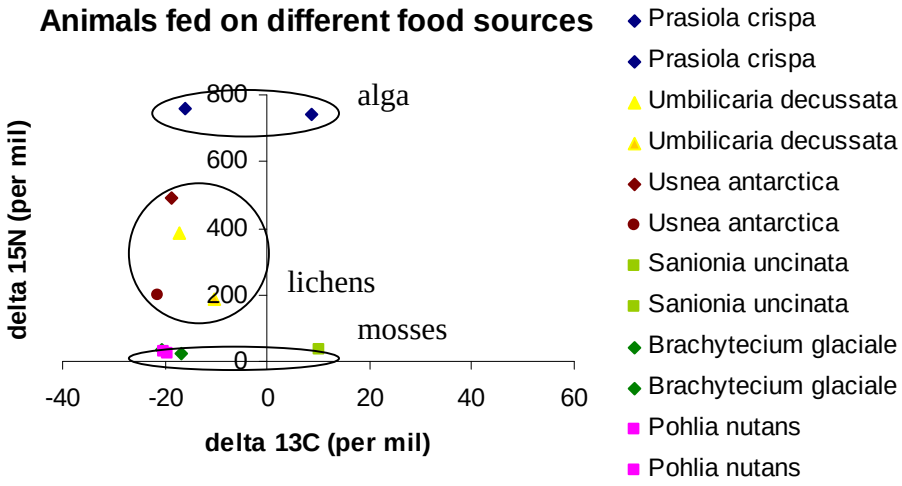


Figure 5.4.1.: values of delta 13C and 15N of the animals fed on different labeled food sources

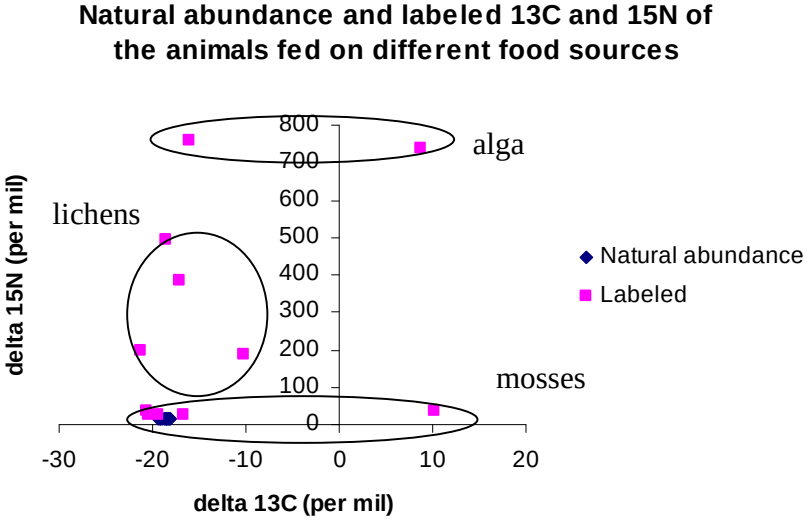


Figure 5.4.2.: values of ^{13}C and ^{15}N of animals fed on different food sources

Here again, with these two graphs we can notice that the isotopic signatures of the animals changed compared to their natural signature. And here again, as with the food sources, the increase in delta values for carbon and nitrogen vary according to the food ingested. In the case of the diet *Prasiola crispa*, delta ^{13}C values rise from -19 to -16‰ or even 8‰ and delta ^{15}N values from 16 to 760‰. For the two lichen species, the increase is similar for both species with delta ^{13}C values varying from -19 to -17‰ up to 10‰ for *U.decussata* and from -18 to -21‰ for *U.antarctica* and the ^{15}N delta values varying from 13 to 200‰ up to 400‰ for *U.decussata* and from 14 to 200 up to 500‰ for *U.antarctica*. For the animals fed on mosses, the increment is relatively small or inexistent compared to the values observed with animals fed on an alga diet or a lichen diet. For the moss *S.uncinata*, the ^{13}C delta values rise from -18 to 10‰ up to 13‰, for the moss *B. glaciale* the increase is very small or does not exist (from -18 to -16‰ or -20‰), the same is observed for the moss *P.nutans* with which there is no increase at all (values from -18 to -19‰).
Therefore, the range of increase is much larger for the delta ^{15}N values but we still find the same trend, with a higher increase of delta ^{15}N values for animals fed on alga diet or lichen diets compared to the increase of animals fed on mosses. For the animals fed on mosses species, delta ^{15}N values increase slightly from 15 to 37‰ for the moss *S. uncinata*, from 15 to 25‰ up to 35‰ for *B.glaciale* and from 16 to 28‰ for the species *P.nutans*. We can notice that the increment range in the delta values for both ^{13}C and ^{15}N of the animals fed on mosses is similar for the three different mosses diet.
The increase in carbon is very small compared to the big increment in nitrogen values.

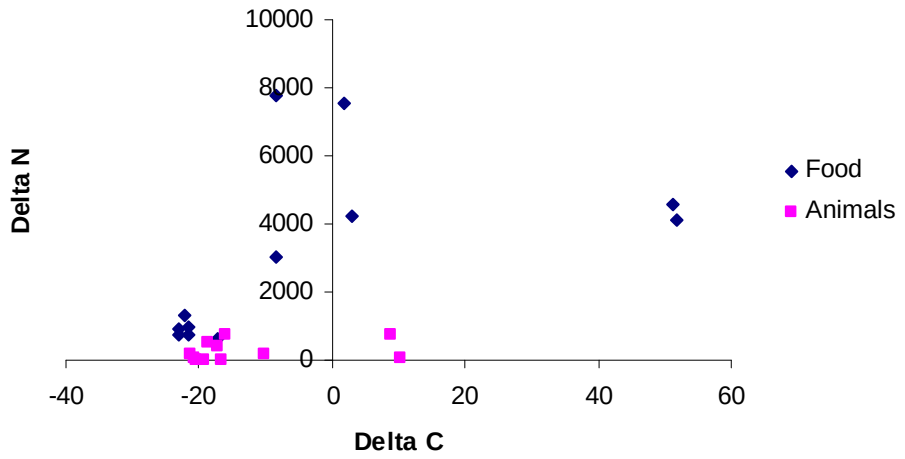


Figure 5.4.3.: isotopic signatures of the different food sources and of the animals fed on

If we compare this graph with the one from the experiment 1 (single diet experiment), we already see that the isotopic signatures of the animals are wider spread along the axis. So, it would be definitely easier to make conclusions about the food intake and the food choice of the animals.

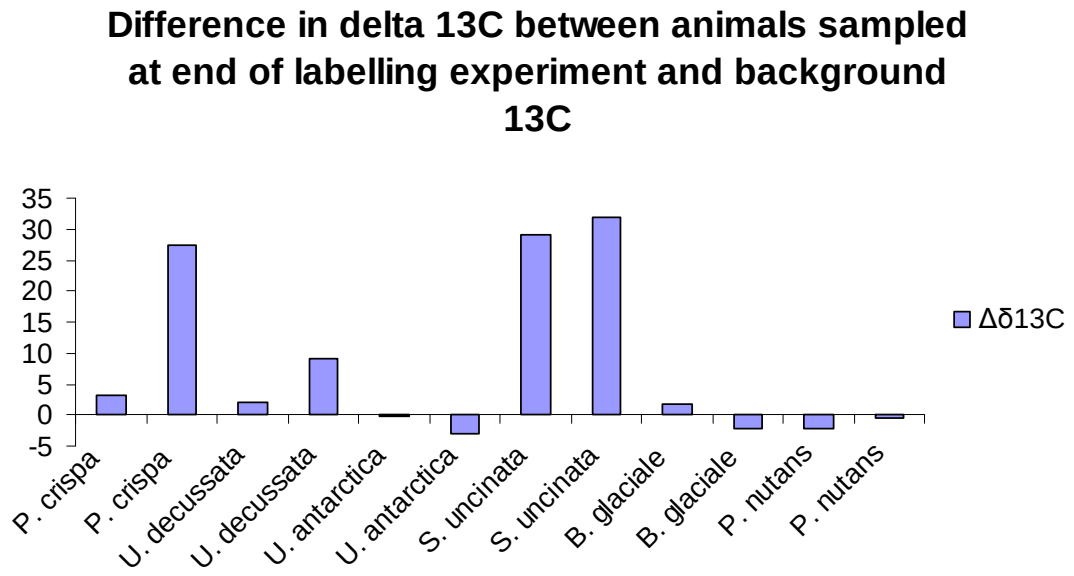


Figure 5.4.4.: $\Delta\delta^{13}C$ in individuals fed on different food sources, $\Delta\delta^{13}C$ is the difference in $\delta^{13}C$ between animals sampled at end of labeling experiment and background $\delta^{13}C$ measured for the animals fed on the same non labeled diets

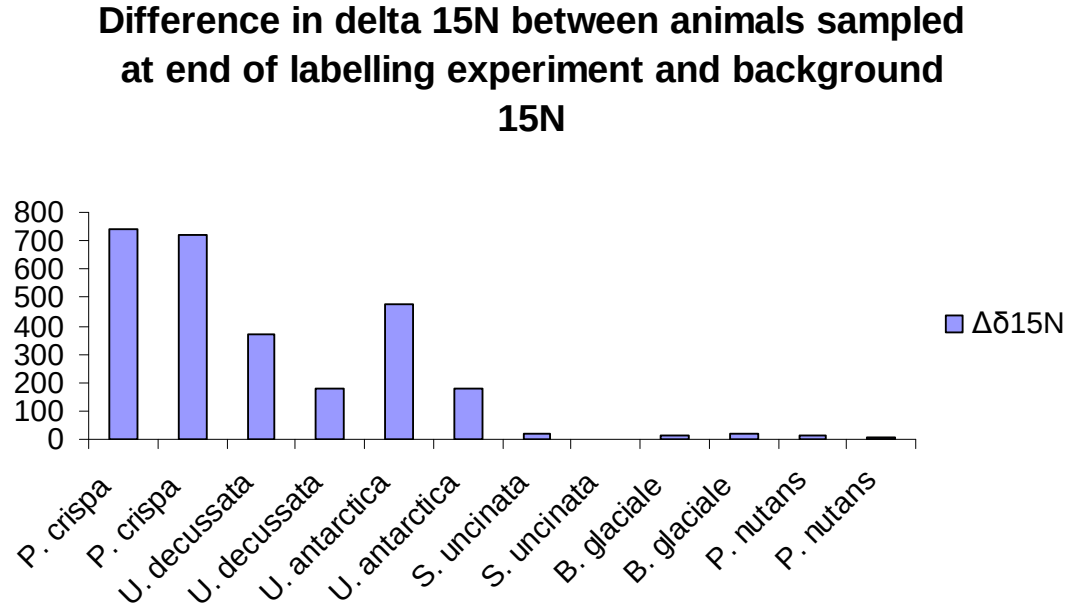


Figure 5.4.5.: $\Delta\delta^{15}N$ in individuals fed on different food sources, $\Delta\delta^{15}N$ is the difference in $\delta^{15}N$ between animals sampled at end of labeling experiment and background $\delta^{15}N$ for the animals fed on the same non labeled diets

This difference is expressed as $\Delta\delta^{13}C$, the difference in $\delta^{13}C$ of animals before and after labeling. For the carbon values, we observe a net uptake for the animals fed on the alga

Prasiola crispa, as well as for the animals fed on the lichen *Umbilicaria decussata* and on the moss *Sanionia uncinata*. For the nitrogen values, we note a specific uptake for the animals fed on the diet *Prasiola crispa*, the lichens *Umbilicaria decussata* and *Usnea antarctica*. If we combine the two values, we see a net uptake of the label by the animals fed on *Prasiola crispa* and *Umbilicaria decussata*. From these graphs, we also observe that the increase range of nitrogen uptake (from 9,2‰ to 742,8‰) is much more important than the one for carbon (from 0‰ to 32,0‰) as we observed for the food.

If we statistically compare the delta ^{13}C and ^{15}N values of the animals fed on different labeled diets, we see with a non parametric Kruskall Wallis test that none of the values are significant either for the carbon (Chi square=8,846, df=5, p=0,115) or nitrogen (Chi square=9,227, df=5, p=0,1) values.

We can look into each type of diet into more details in order to detect any preference feature:

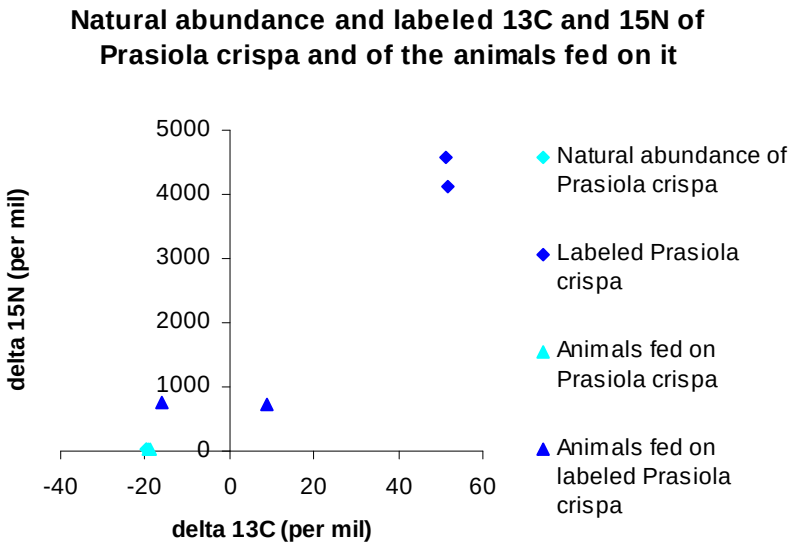


Figure 5.4.6.: values of delta ^{13}C and ^{15}N of the enriched and natural Prasiola diet and of the animals fed on the natural and enriched Prasiola diet

In this graph are plotted the natural abundance and labeled ^{13}C and ^{15}N isotope content of the alga diet *Prasiola crispa* and the delta ^{13}C and ^{15}N values of the animals fed on both diets (natural and labeled ones). As we can see the delta values of the animals fed on the labeled food seem to follow the values of the food labeled; the delta values of the animals shifted from -19,33‰, -18,61‰ for ^{13}C to -16‰ up to 8,81‰ and from 16,35‰ and 16,28‰ to 759,2‰ and 739,6‰ for the delta ^{15}N values. We can notice that the increase in the delta values are much greater for nitrogen compared to carbon. Thus, the labeling worked out and the animals seem to have incorporated the labeled signatures of the labeled food source, in this case *Prasiola crispa*.

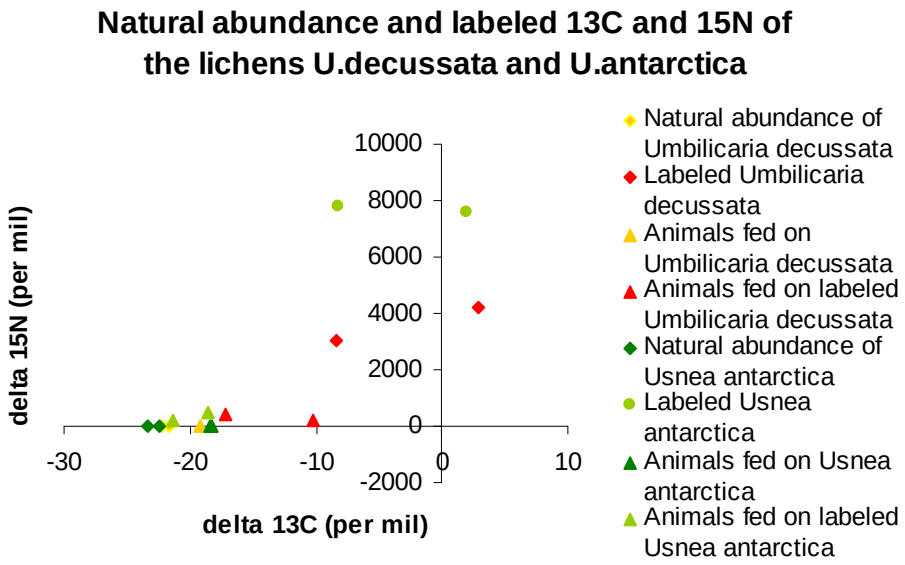


Figure 5.4.7.: values of delta ^{13}C and ^{15}N of the enriched and natural lichens diets and of the animals fed on the natural and enriched lichens diet

With this second graph, we can proceed the same way by comparing the delta values of the animals fed on the natural lichens and the labeled ones. For the lichen *U. decussata*, the delta ^{13}C values of the animals shifted from -19,1‰ and -19,2‰ (animals fed on the natural lichen) to -17,1‰ and -10,2‰ (animlas fed on the labeled lichen) and from 13,9‰ and 13,5‰ to 384,3‰ and 189,3‰ for ^{15}N delta values. According to these values, it seems that the animals increased their delta ^{13}C and ^{15}N values once fed on the labeled food source, with a much larger increase for nitrogen as with *Prasiola*. For the lichen *U. antarctica*, the delta ^{13}C values of the animals shifted from -18,1‰ and 18,4‰ to -18,5‰ and -21,3‰ and from 14,5‰ and 14,3‰ to 492,4‰ and 195,8‰. Therefore, the delta ^{13}C values did not increase but even decrease even more, but for nitrogen the values increased much more. So, if we compare the values between the two lichens species, we observe a shift in ^{13}C values for the moss *U.decussata*, while there is no change in the ^{13}C delta values of the animals fed on *U. antarctica*. For the ^{15}N values, we observe an increase in the nitrogen delta values of animals fed on both lichens species, and the range of increment is comparable for both lichens.

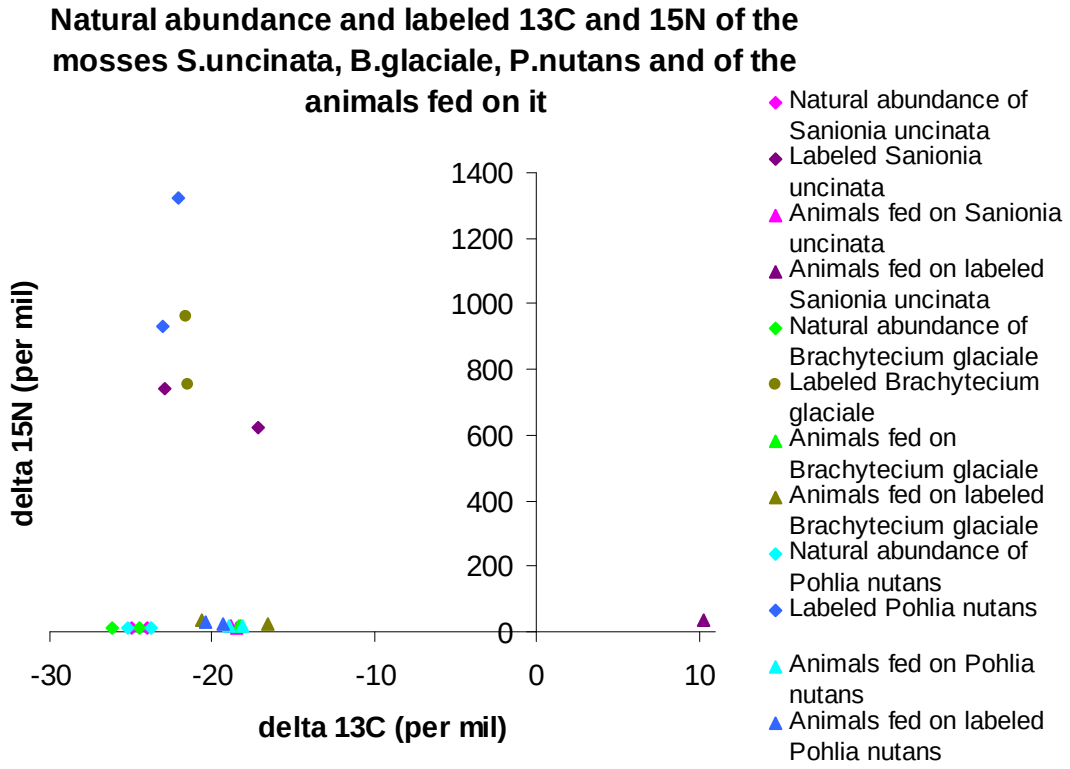


Figure 5.4.8.: values of delta 13C and 15N of the enriched and natural mosses diets and of the animals fed on the natural and enriched mosses diets

Finally, we can then compare the delta values for the animals fed on the mosses; for the carbon values of the animals fed on *S. uncinita*, values shifted from -18,8‰ and –18,5‰ to 10,2‰ and 13,4‰ for ^{13}C delta values, and from 15,5‰ and 14,5‰ to 37,5‰ for ^{15}N delta values. For the moss *B.glaciale*, animals' values shifted from –18,3‰ and –18,2‰ to -16,6‰ and -20,5‰ for ^{13}C values and from 15,1‰ and 16,0‰ to 25,6‰ and 35,7‰ for the ^{15}N delta values. At last, for the animals fed on natural and labeled *P.nutans* moss, values varied from -18,0‰ and -18,9‰ to -20,3‰ and -19,3‰ for delta C values and from 16,7‰ and 16,2‰ to 28,1‰ and 25,5‰ for nitrogen values. Therefore, there is no increase in the ^{13}C delta values of the animals fed on mosses except for the moss *Sanionia uncinita*. For the nitrogen values, there is a small increase in the delta values which is in the same range for the three mosses, with a smaller increase for the animals fed on the moss *Pohlia nutans*.

➢ Feces

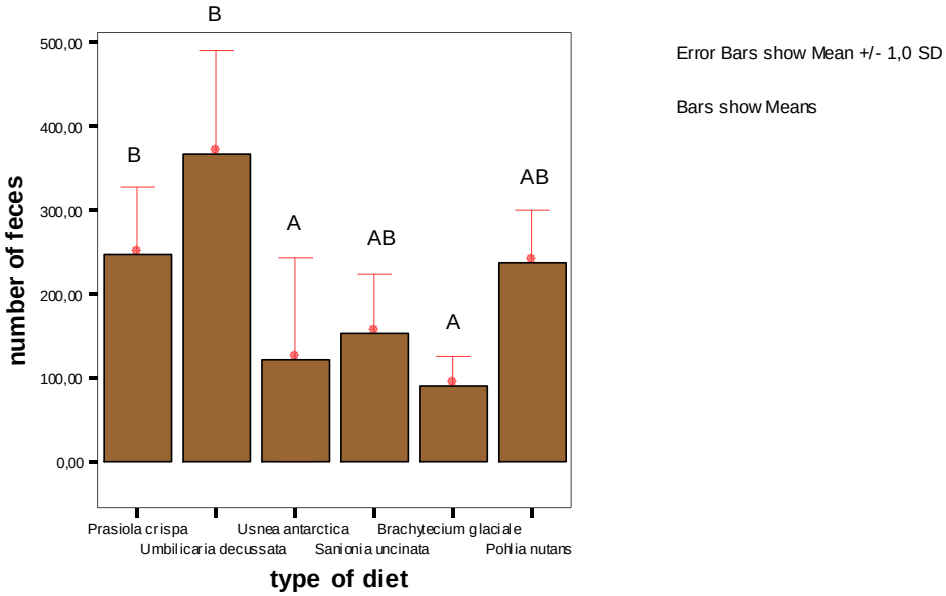


Figure 5.4.9.: Mean number of faecal pellets produced by *C. antarcticus* on different types of diets

After a logarithmic transformation of the original data, the Kolmogorov-Smirnov test of normality revealed that the data followed a normal distribution and the variances were assumed to be equal (Leven's test, p=0.299). Therefore, the ANOVA test of variances (see appendix) between groups was used. The analysis of variances (F=6.515, df=5.24, p=0.001) enable us to say that there is significance between the number of feces on each diet type. Furthermore, it is possible to assess the differences among the different diets thanks to the Tukey post hoc test; the mean number of feces on *Prasiola crispa* is significantly different from *Usnea Antarctica* and from *Brachytecium glaciale*, the difference is also significant for *Umbilicaria decussata* and *Usnea Antarctica*, as well as between *Umbilicaria decussata* and *Brachytecium glaciale*. Therefore, the high number of feces found with the lichen Umbilicaria and the alga Prasiola as food sources are significant.

➢ Moults

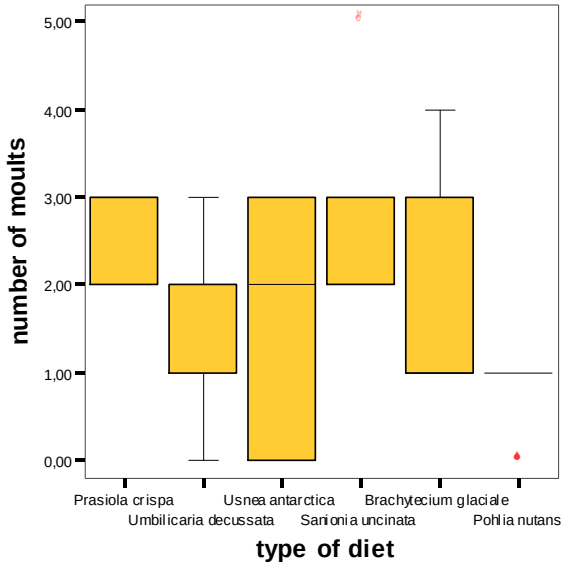


Figure 5.4.10.: Boxplot graph with the medians of moults produced by *C. antarcticus* on different types of diets

According to the Kolmogorov-Smirnov test of normality, it appeared that the data did not follow a normal distribution, and the Leven's test of homogeneity of variances revealed that the variances between the groups were different. Thus, the Kruskal-Wallis non-parametric test (see appendix) was applied with the original data. The test indicates that there is no significant differences in the number of feces between the 6 different diet types (Chi square=9.198, d.f.=5, p=0.101). Therefore, we cannot conclude anything from these observations. Moreover, the Scheffé post hoc test does not enable us to see any significant difference between the different diets.

5. Experiment 5, two-diet choice experiment (labeled experiment)

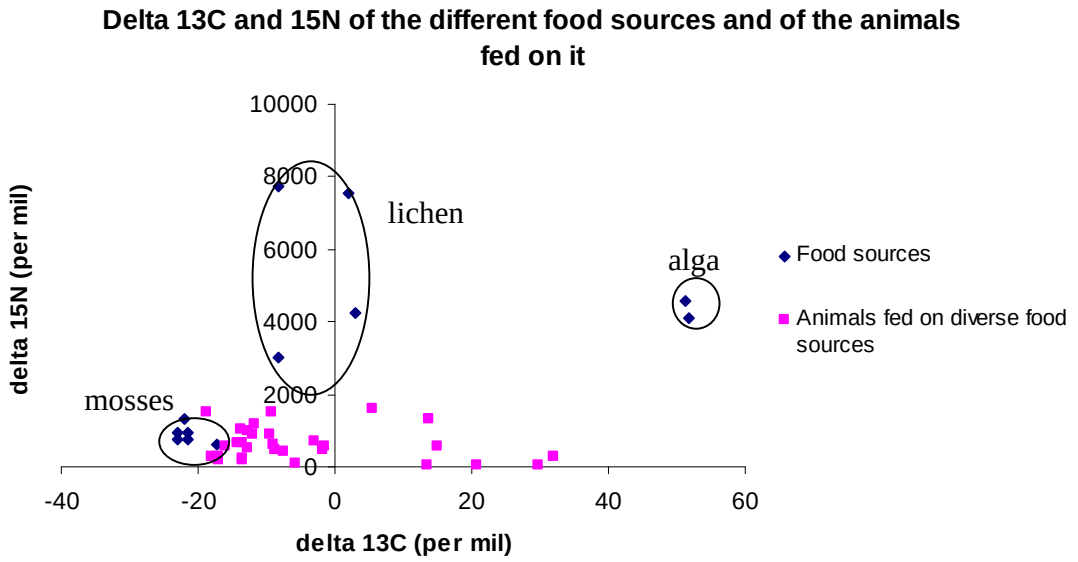


Figure 5.5.1.: Isotopic signatures of the different food sources and of the animals fed on a two-choices diets.

Above is represented graphically, the isotope values of the *Cryptopygus* individuals fed on a two-choice diet and the associated diets. We observe, as in the previous two-choices experiment (experiment 2), that the $\delta^{13}C$ and $\delta^{15}N$ values of the animals are wider spread along the axis and especially the delta ^{13}C axis. If we compare the delta ^{13}C values of the animals fed on different combined food sources, we don't find any significant difference among the values (Kruskall Wallis test, Chi square=22,206, df=14, p=0,074), whereas if we look at the ^{15}N values of the animals fed on different combined diets, we find a significant difference thanks to the Kruskall Wallis test (Chi square=25,665, df=14, p=0,029). Furthermore, we are able to know that the delta ^{15}N values of the animals fed on the combined diets *Umbilicaria decussata/Usnea antarctica* are significantly different from the one fed on the combinations *Brachytecium glaciale/Pohlia nutans* and *Sanionia uncinata/Pohlia nutans* (Scheffé post hoc test).

The graphs below show some results in more details:

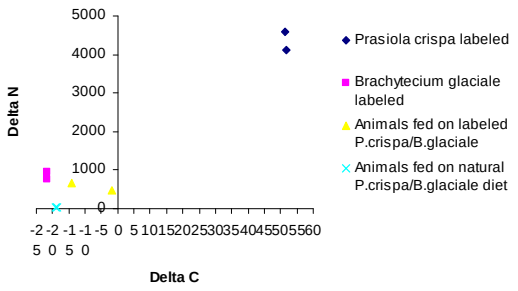


Figure 1

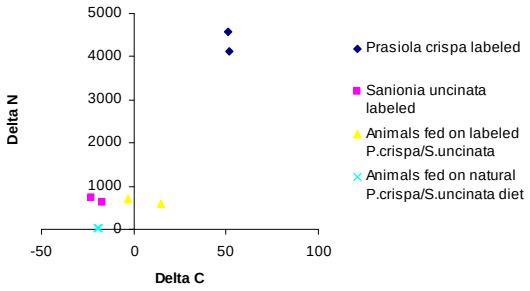


Figure 2

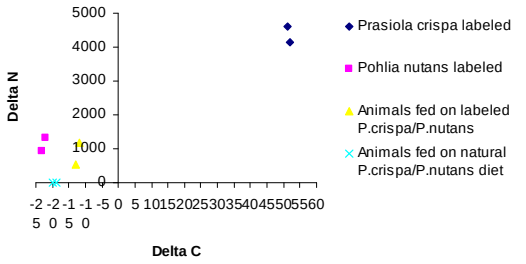


Figure 3

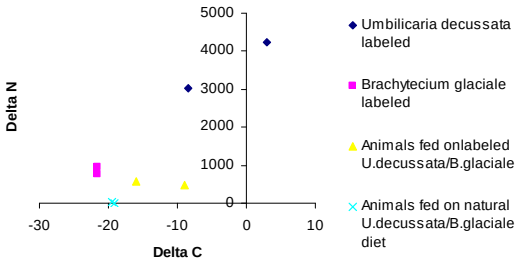


Figure 4

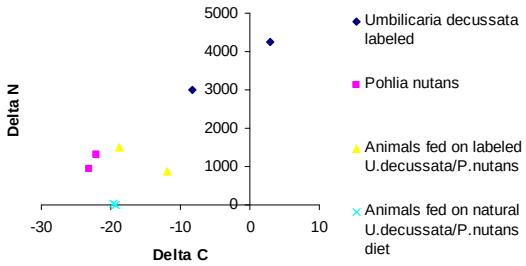


Figure 5

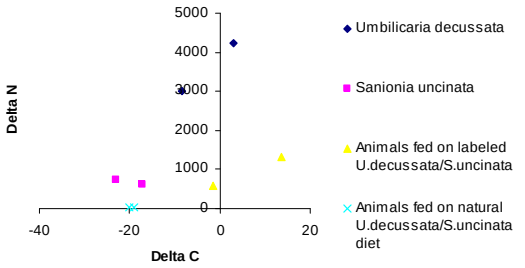


Figure 6

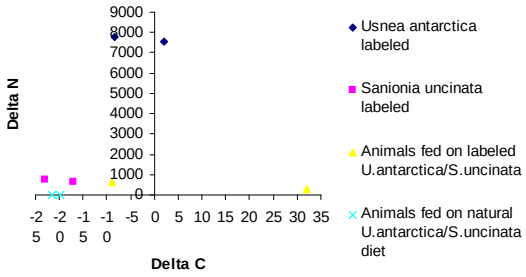


Figure 7

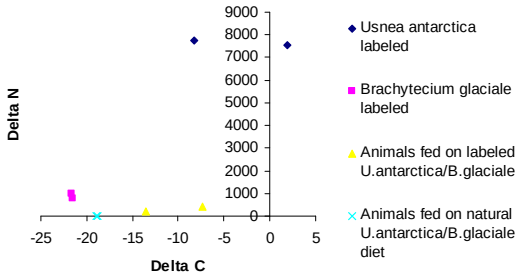


Figure 8

Figure 5.5.2.: Delta ^{13}C and ^{15}N values of the combined labeled food sources and of the animals fed on it, and delta ^{13}C and ^{15}N values of the animals fed on the natural combined food sources

Above are represented graphically, the most relevant results. As we can see from the Figure 1, the ^{13}C values of the animals fed on the combined *P.crispa/B.glaciale* diet shifted (from the natural abundance values) towards the *Prasiola crispa* values; the ^{15}N values increased but in a smaller extent. From the second graph, the ^{13}C values of the animals fed on the combined diet *P.crispa/S.uncinata* shifted towards the delta values of the alga *Prasiola crispa* as well. The graph 3 shows the same pattern, with the ^{13}C values of the animals shifting towards the diet *Prasiola crispa*. The next graph shows that the animals fed on the combined diet *U.decussata/B.glaciale* have ^{13}C delta values close to

the ones of the lichen *Umbilicaria decussata*. Figure 5 shows the same trend that the previous observation, with the ^{13}C values of the animals fed on the combined diet shifting towards the values of the food source *Umbilicaria decussata*. In the Figure 6, we also observe the same feature with the animals ^{13}C values shifting towards the ones of the lichen *Umbilicaria decussata*. In the Figures 7 and 8, the same pattern is observed with in both combinated diets, values of the animals shifting toward the lichen diet *Usnea Antarctica*.

- Feces

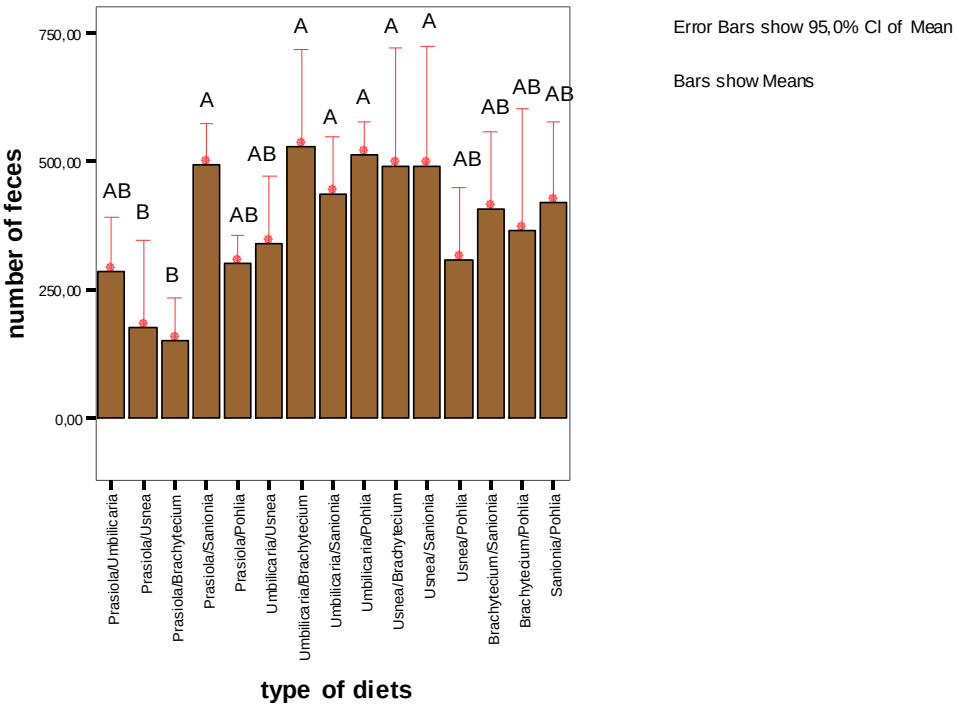


Figure 5.5.3.: Mean number of faecal pellets produced by *C. antarcticus* on different types of diets

The data followed a normal distribution according to the Kolmogorov-Smirnov test and the variances were assumed to be equal (Leven's test, t=0.083, df=14,60, p=0.083). Therefore, the ANOVA test of variances (see appendix) between groups was applied. This analysis enable us to say that there is significance between the number of feces on each diet type (F=4.649, df=14,60, p=0.000). Furthermore, it is possible to assess the differences among the different diets with the Tukey post hoc test; the mean number of feces on *Prasiola/Usnea* is significantly different from *Prasiola/Sanionia, Umbilicaria/Brachytecium, Umbilicaria/Pohlia, Usnea/Brachytecium, Usnea/Sanionia* and the mean number of feces on *Prasiola/Brachytecium* is significantly different from *Prasiola/Sanionia, Umbilicaria/Brachytecium, Umbilicaria/Sanionia, Umbilicaria/Pohlia, Usnea/Brachytecium, Usnea/Sanionia.*

➢ Moults

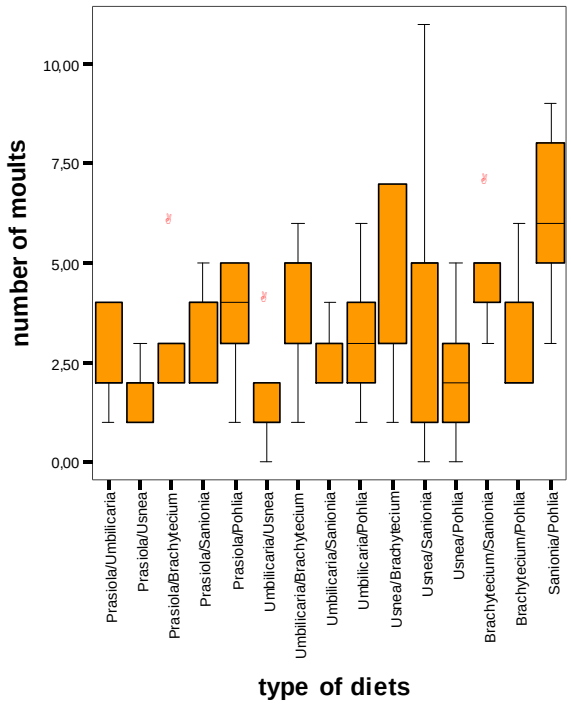


Figure 5.5.4.: Boxplot graph with the medians of moults produced by *C. antarcticus* on different types of diets

According to the Kolmogorov-Smirnov test of normality, it appeared that the data did not follow a normal distribution, but the Leven's test of homogeneity of variances revealed that the variances between the groups were assumed to be equal. Thus, the Kruskal-Wallis non-parametric test (see appendix) was applied with the original data. The test indicates that there is no significant differences in the number of moults between the 15 different diet types (Chi square=21.118, d.f.=14, p=0.099). Therefore, we cannot conclude anything from these observations. Furthermore, the Scheffé post hoc test does not give any significant differences among the different diets.

VI- Discussion

The isotopic analysis of the different vegetation types used as food sources in the experiments enable us to clearly distinguish the signatures of each vegetation type (alga, lichens, mosses) (Figure 5.1. and 5.2.5.).
Our isotope values for the different vegetation types used in this study seem to agree with the figures found in previous studies; for example, in a survey of isotope composition of the Antarctic plants, the Antarctic plants studied have $\delta^{13}C$-values mostly between -25‰ to -21‰ which is the range of our findings. Usually terrestrial plants are characterized by

more negative $\delta^{13}C$-values (Galimov, 1985; Ehleringer et al., 1993 in Galimov, 1999). However the observed impoverishment of the Antarctic plants with the light carbon isotope is appeared to be due to species composition rather than geography.. Terrestrial vegetation in the Antarctic region is represented by the most primitive forms, and lichens are known to be less depleted in ^{13}C than other plants (Galimov, 1999).
It is known that plants possessing the conventional Calvin cycle (C_3 pathway) have ^{13}C values of -20 to – 35‰, plants possessing the Hatch-Slack cycle (C_4 pathway) -7 to -15‰, and plants possessing Crassulacean acid metabolism (CAM) -10 to -22‰ (Ehleringer, 1986).

Experiment 1 (single diet experiment)

In the first experiment where animals have been fed with a single diet source, all the ^{13}C and ^{15}N delta values of the animals seem close to each other, the range of values' variation does not exceed 1,2‰ for ^{13}C (from -19,3‰ for the moss *Pohlia nutans* to -18,0‰ for *Prasiola crispa*) and 3,2‰ for ^{15}N (from 13,5‰ for the lichen *Umbilicaria decussata* to 16,7‰ for the moss *Pohlia nutans*). Furthermore, all the individuals' values are close to the *Prasiola crispa* diet values (around -19‰ for $\delta^{13}C$ and 15/18‰ for $\delta^{15}N$). Therefore, it seems that the individuals did not incorporate the isotopic signatures of their respective diet. This observation could be explain in three ways; either the duration of the experiment (6 weeks) was too short for the organisms to incorporate in their tissues the dietary carbon and nitrogen, or the animals fed on diet other than the alga diet did not fed on the food sources proposed, or eventually, the animals fed intensively on the diet *P. crispa* in the field before the beginning of the experiments, and therefore still bear the alga isotope signature; so the "Prasiola signal" is still present in the collembola's body. The second hypothesis seems unlikely as we found feces in all the jars.
From the trophic shift data, we see that animals fed on *P.crispa* present $\Delta\delta^{13}C$ values very low (0,3 and 1,3‰) which correspond to the fact that early studies showed that for C, isotope ratios of consumers usually are similar to isotope ratios of their diets (DeNiro and Epstein, 1978). However, the calculation of the trophic shift values (table) must be handle with care because isotope ratios of C and N for consumers may change gradually in response to changes in diet. For example, Fry and Arnold (1982) found that shrimp approached isotopic equilibrium with a new diet only after their mass had quadrupled. Because the rate of turnover for some tissues is very slow, estimates of trophic shift from diet-switching studies may be influenced by the isotope ratio of the initial diet (in this case *P.crispa*) even after a consumer has been maintained for a long period of time on the same diet.

If we look separately at each diet type and the respective animals' values, some distinctions can be made especially on the $\delta^{15}N$ axis; for instance, the individuals fed on the lichen diets (*Umbilicaria decussata* and *Usnea Antarctica*) present $\delta^{15}N$ values below the ones of individuals fed on alga or moss diets (statistically not significant). Thus, the animals probably fed on these lichens to get a lower $\delta^{15}N$ signature. For the animals fed on the mosses species the $\delta^{15}N$ values do not shift much compared to the values of the animals fed on the alga *Prasiola crispa*. This is not surprising regarding the fact that the $\delta^{15}N$ of *Prasiola crispa* and of the three mosses species are not far apart. At

last, by comparing the nitrogen values of animals fed on *Umbilicaria decussata* and *Usnea Antarctica*, we notice that these values are lower for the animals fed on *U.decussata* than animals fed on *Usnea Antarctica* whereas the natural ^{15}N signature of *Usnea* is lower than the one of *Umbilicaria decussata*. Would it be that the animals ate a larger amount of the lichen *U.decussata* compared to the lichen *Usnea Antarctica*? This hypothesis is reinforced by the feces counts (a higher number of faecal pellets is observed with the presence of *P.crispa* and *U.decussata* as a diet which support the assumption of intensive consumption of the alga and the lichen *U.decussata* by the collembolans). For the $\delta^{13}C$ values, the variation range of animals' values is too narrow to make any distinction between the animals fed on different types of diet.

Experiment 2 (two-diet choice experiment)

In this second experiment, we observe a similar distribution of the $\delta^{13}C$ and $\delta^{15}N$ values of the animals fed on various diet combinations, actually these values are clustered around the carbon and nitrogen values of *Prasiola crispa* diet, as we have already observed in the previous experiment.

It is difficult from this experiment to make any conclusion about possible preferences among two diets types, as the animals barely incorporated the isotopic signatures of their food (as noticed in the first experiment).

Nevertheless, we did observe some animals values' shifts (from the *Prasiola* values), so it seems that animals fed on the diets where a shift is observed, for instance the two lichen species. But this is not surprising as the ^{13}C and ^{15}N values of the lichen species are the lowest of all the food sources.

With the comparisons of two combined diets with a common one (for instance *Umbilicaria decussata/Sanionia uncinata* combination with *Umbilicaria decussata/Pohlia nutans combination*), we can note the occurrence or not of a preference; for example it seems clear that in the two combined diets *Prasiola crispa/Umbilicaria decussata* compared to *Prasiola crispa/Usnea antarctica*, animals fed more intensively on the lichen *U.decussata* than on the *Usnea* species because we observed a larger shift (^{15}N values are on average 13,5‰ for the animals fed on the combination *Prasiola crispa/Umbilicaria decussata* and are 16,6‰ with the *Prasiola crispa/Usnea antarctica* diet) with *Umbilicari*a than with *Usnea* in the diet.

Labeled experiments

In this study, it is essential to note that the reported stable-isotope ratios refer to the animals plus their gut contents. Therefore, the labeling experiments may have been biased because no separation was made between gut contents and animal tissue. Consequently, the label uptake rates reported probably overestimate the amount of label actually incorporated into animal tissue. However, the problem becomes smaller as the bioaccumulation of the compound becomes more important and (for bioaccumulating materials) as the experimental uptake time increases: at a constant gut content, more

bioaccumulating materials have higher tissue concentrations over time. Therefore, for labeled diet carbon and experiments lasting at least a number of hours, the biais due to gut contents is likely to be minor (Herman et al., 2000).

The degree of food sources' labeling was highly variable (uptake much greater for *P. crispa* and the two lichen species for both carbon and nitrogen), factors that contributed to high variability in labeled addition results may have included non-uniform application of label, variation in uptake of label by vegetation. Actually, labeling might be lower in mosses because mosses grow much slowlier than lichen and alga, and therefore need more time to incorporate the same amount of labeled substance than the other food sources. On the other hand, *Prasiola crispa* presents a much greater isotope enrichment due to its very high photosynthetic capacity, therefore we obtained an important ^{13}C enrichment with *P.crispa*.

The labeling of the food sources has been efficient and individuals fed on these labeled food have thus incorporated the labeled signatures of their food sources. It is possible to distinguish the three different types of animals fed on the three different types of diets along the delta ^{15}N axis (Figure 5.4.1.): the collembolans fed on the alga *Prasiola* have values situated between 700 and 800‰, the individuals fed on lichen species ^{15}N produce values between 180 and 500‰, and at last animals fed on mosses species have values around 30‰. On the other hand, along the ^{13}C axis, all the animals have the same signatures therefore, it would be less easy to infer any conclusion from the carbon information.

Experiment 3 (cafeteria labeled experiment)

From this experiment, the isotope values of the animals fed on the 6 diet types at the same time, allow us to suppose that the collembolans fed probably more on the alga *Prasiola crispa* than on the other diets, as the delta ^{15}N values of the animals are closer to the animals' values fed either on *Prasiola* or lichens species.

Experiment 4 (single diet labeled experiment)

In this experiment, it seems that animals slightly did incorporate the isotopic signatures of some of the diets, especially the lichen species, but obviously more time would have been needed for the animals to assimilate the ^{13}C and ^{15}N delta values of the various food sources.

Actually, we can calculate the time needed for the animals to reach half the labeling levels in the plant materials using a linear approximation, which is possible because of the low labeling levels in the animals:

	Turnover time or doubling time (days) = ((δ^{13}C labelled plant-δ^{13}C control plant)/2)/(δ^{13}C labelled animal-δ^{13}C control animal)
Prasiola crispa	**79,52**
Umbilicaria decussata	**159,91**
Usnea antarctica	**221,84**
Sanionia uncinata	**389,84**
Brachytecium glaciale	**529,77**
Pohlia nutans	**1130,26**

Table 6.1.: Turnover time of the food sources

The calculation of the turnover time supports the fact that animals incorporate faster the isotope signatures of the green alga *Prasiola crispa* and of the two lichen species compared with mosses which required a much longer time to be incorporated efficiently by the animals' tissues. So, the turnover time for the aga Prasiola used as diet is much smaller than for the rest of the diet types.

If we look at each diet type separately (Figure 5.4.6, 5.4.7., 5.4.8.), we clearly see that when animals have been fed on the labeled alga *Prasiola*, they did took up the labeled signature of this diet, as the animals delta values shifted towards the one of the alga they fed on. If we proceed the same way with the two lichen species, we discern a shift of the animals' values towards the ones of *U. decussata* when fed on this lichen. The shift is not observed or is less apparent for the individuals fed on the lichen *U. antarctica*. At last, for the three mosses species, we do not examine any shift to the labeled values of the diet mosses, values of animals fed on labeled mosses stay close to the ones of animals fed on the non labeled mosses. The delta ^{13}C and ^{15}N slightly changed but we are far from the large shifts observed for the other diets types.
Consequently, it looks as if collembolans fed more intensively (labeled values readily incorporated) in presence of the alga *Prasiola* as diet, compared to the other diets (lichens, mosses). If we compare the animals' delta values within the two lichen species, it also appears that the individuals incorporated more of the labeled signatures of the lichen *U.decussata* than the lichen *U. antarctica*. Within the mosses species, it is complex to assess any preferences among the different species. The only conclusion that we can make concerning the consumption of mosses is that it does not appear that animals took up efficiently the labeled mosses signature.
As a more general deduction, animals did incorporate better alga over lichen's signatures, and lichen over mosses' ones. As a result, we could hypothesize that the springtail could have a probable preference for the alga diet *Prasiola crispa*, as well as a likely preference of the lichens over the mosses in general and *U. decussata* particularly. The faecal pellets data seem to strengthen this feature as the highest number of feces is found with *Prasiola* and the lichen species *U.decussata* as diet types.
It is important to note that these values must be interpreted with great care because as certain types of diets incorporated more labeled ^{13}C and ^{15}N than others (*Prasiola*, lichens), it seems natural that animals fed on a high labeled food source will obtain a

higher delta ^{13}C and ^{15}N values. Therefore, a correction of the values is necessary beforehand before making any definitive conclusion.

Experiment 5 (two-diet choice labeled experiment)

In this experiment, we observe the same kind of animals' values distibution (Figure 5.5.1.) than in the similar experiment 2, which is that animals' values are more spread along both axis (δ ^{13}C and ^{15}N). From the detailed graphs (Figure 5.5.2.), it is possible to assess some favored diet. From the position of the animals delta ^{13}C and ^{15}N values on the graphs compared to the two food sources, we are able to say that the individuals chosen to feed on *Prasiola* rather than the moss *B.glaciale*, the moss *S. uncinata* and the moss *P.nutans*. We can also assess a preference for eating the lichen *U.decussata* rather than the three mosses diets. Eventually, animals fed readily on the lichen *U.antarctica* rather than on the mosses *S.uncinata* or *B.glaciale*. But these suppositions have to be treated with care as even if the animals fed on a specific diet, it does not mean that it can be seen as a food choice; it is also plausible that the animals feed more intensively on a food source because of its low nutrient content for instance.

Alga consumption

C. antarcticus is known to eat *P. crispa* (Worland and Lukesovà, 2000), and Schofield (1972) found that *Prasiola crispa* accumulated much more nitrogen than any of the blue-green algae, mosses or lichens analyzed from Victoria Land; this alga is typical of moist nitrogen-rich soils associated with penguin rookeries (R.I.Lewis Smith, 1984). Its thalli are abundant over large areas subjected to trampling and manuring by seals and penguins. In areas surrounding elephant seal wallows and penguin rookeries, algal cover may reach 75%. Few other macrophytes can tolerate the conditions of mechanical disturbance, low pH and high concentrations of N, Na and P which characterize such site. Therefore, it seems likely that the collembolans consume this alga because of its high nutrient content (a higher nitrogen content generally is coupled with a higher amount of $\delta^{15}N$ in the diet and subsequently a greater enrichment in the heavier N isotope in the consumer species (Ruess, 2004)). In principle, food resources associated with the high fitness of consumers should be preferentially ingested (Butcher et al., 1971; Pyke et al., 1977 in Scheu, 2004); the results of food preference tests therefore should be consistent with those of fitness parameters (Scheu, 2004).

Lichen consumption

As it appears quite clear that *C.antarcticus* feed on *Prasiola crispa* because of its high nutrient content, the consumption of lichens by these organisms might not be explained the same way. In fact, it is highly probable that the animals fed intensively on the lichens diets (and especially *U.decussata*) because of their poor quality, without rejecting the hypothesis that the animals could also feed on this kind of food because of its good nutrient content or palatability. In order to reject one of the hypothesis, we would need a chemical analysis of the lichen species.

Actually, collembolans compensate for low levels of dietary nitrogen by consuming more food to maintain a relatively constant amount of protein and as a consequence accumulate more fat (Lavy and Verhoef 1996, Haubert et al., in press in Ruess, 2004).

Furthermore, some studies suggested that compared with food of high quality, low-quality food results in an increase in ^{15}N enrichment (Webb et al., 1998). Therefore, food of low quality results in higher stable isotope enrichment compared with high-quality food. The fact that the labeling of the two lichen species resulted in a relatively high enrichment of the animals fed on it (especially for ^{15}N values) would mean that this type of food possess a lower nutrient content compared to the others diets tested. Moreover, despite a high variability in nutrient concentrations reported for different species and growing regions, protein content is usually low among lichen species, along with calcium and potassium concentrations, on the other hand fiber content is generally high (Lawrey, 1987). Additionally, defense compound production by lichens probably also influences their susceptibility to herbivores (Rundel, 1978; Lawrey, 1987; Stephenson and Rundel, 1979; Lawrey, 1980, 1983, 1984 in Lawrey, 1987).

Besides, the enrichment in ^{15}N and ^{13}C in animals may also increase with nutritional stress and starvation (Ambrose and DeNiro, 1986; Hobson et al., 1993; Lajtha and Michener, 1994; Adams and Sterner, 2000 in Oelbermann, 2002), and we observed a net ^{15}N enrichment in the individuals fed on both lichen (between 200‰ and 400‰ for *U.decussata* and between 200‰ and 500‰ for *U. antarctica*, data not shown). In fact, consumption of low-quality food is associated with reduced nitrogen intake (Adams and Sterner, 2000 in Oelbermann, 2002). Therefore, starving animals and those feeding on low-quality food should be enriched in ^{15}N (Oelbermann, 2002).

Consequently, two reasons could be put forward to explain why collembolans consume lichens species; firstly, it could that the animals appreciate this diet and that lichens support biomass increase and reproduction of the animals. Secondly, if we assume that the lichen diet is of much lower nutritive quality (which is very likely as a higher nitrogen content generally is coupled with a higher amount of $\delta^{15}N$ in the diet), either the animals barely feed on the lichen and become starved (as it seems to be the case with *U. antarctica* as the number of feces is very low) or springtails feed intensively on the lichen (here *U.decussata*) in order to be able to sustain themselves with a low quality food source.

Mosses consumption

From the results of the experiments, it look as if the various mosses species are hardly eaten. This can be explain either by the abhorrence of this kind of food by the animals or by a high nutrient content which entail a low but efficient consumption of these mosses.
The principal reserve foods of bryophytes are considered to be starches, lipids, or both (Rastorfer, 1972). Since mosses lack the storage organs of higher plants, a relatively higher lipid content would permit the maximum energy reserves in a limited space (Rastorfer, 1972).
On one hand, mosses could be rejected by the springtails probably because of the low digestibility of most mosses and the production of inhibitory compounds. Compared with tree leaves, mosses generally contain lower concentrations of easily digestible soluble carbohydrates and hemicelluloses, and higher concentrations of structural components less easily digested, such as cellulose and polyphenolic ligninlike compounds, although mosses do not produce true lignin (Lawrey, 1987).

On the other hand, it is important to note that in the Antarctic bryophytic communities there is in a large extent, a microflora composed for instance by algae, bacteria, diatoms, and fungi (Rastorfer, 1972). In addition to algae, mosses have a complex mycobiota that range from specialized ascomycetes (more than 300 species) occurring exclusively on bryophytes (Döbbeler, 1997) to common soil fungi, which colonize and decompose the living or dead plant parts (Varga, 2002). It is possible that collembolans feed selectively on these micro-diets only and avoid the moss itself. Lastly, it is interesting to note that mosses produce a highly unsaturated fatty acid, arachidonic acid, and information available suggests that ingestion of this compound affords protection of cell membranes against very low temperatures. If this is true, moss consumption in cold climate may be adaptive and would enable *C.antarcticus* to avoid cold, despite the low digestibility of mosses generally (Lawrey, 1987).

VII- Conclusion

The results of this research do agree with earlier previous studies on *C. antarcticus* on the feeding using microscopic examination of gut and faecal pellet contents (Broady, 1979), culture techniques for detection of viable micro-organisms in guts and faeces (Broady, 1979). From Broady's study, it results that *C. antarcticus* ingests a wide range of materials (melanized fungal hyphae, hyaline fungal hyphae, septate fungal spores, aseptate fungal spores, yeasts cells, diatoms, *Prasiola crispa*, green and yellow-green algal filaments, green and yellow-green algal unicells, blue green algae, moss protonemata, dead moss and liverwort tissue, micro-arthropod remains…), hence the food consists largely of decaying plant material, fungi and microscopic algae (Broady, 1979). Despite the wide variety of gut contents, there is evidence that *C. antarcticus* feeds selectively. The most obvious absence from the guts is green living bryophyte tissue (Broady, 1979). However, there was a preference for dead and decaying bryophyte tissue (Broady, 1979). Christiansen (1964) considered fungal hyphae, dead or decaying plants and algae were the two most frequent dietary groups of Collembola. *C. antarcticus* is thus similar in its diets to other soil Collembola (Broady, 1979).
Our study completes those studies by using stable isotope methods which are among the most powerful tools for the study of trophic relationships. The ratio of naturally occurring stable isotopes in the consumer tissue reflects the entire feeding history of the organism. We show that the springtail *C.antarcticus* presents a preference in its feeding among at least three different types of diets (alga, lichen, moss), with a predilection for the alga *Prasiola crispa* amongst all, followed by the lichens and mosses. This categorization is made on a consumption basis but further investigations are needed in order to determine whether collembolans ingest more lichen because of its potentially low nutrient content, and less mosses fragments for their nutritive effect or randomly as ballast carrying the searched fungal propagules.

Although it may be a simple matter to show that a particular food is eaten under laboratory conditions, it is more difficult to show that it is preferred under field conditions. Verhoef et al. (1988) found that most suitable diets were not necessarily those that were used by field animals (Ponge, 1991). In laboratory experiments, Ponge and Charpentié concluded that spores were always preferred to hyphae in cultural tests of

P. alba fed with known species of fungi, although spores were rarely found in the gut contents of field animals (Ponge, 1991). Since choices change with the food resources available to the animals, no model can explain changes in the present state of our knowledge. Thus, *C. antarcticus* occupies different habitats because of its ability to feed opportunistically on a variety of readily available material (Broady, 1979).

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Appendix

Experiment 1

Feces:

Kruskal-Wallis non-parametric test:

Ranks

	type of diet	N	Mean Rank
number of feces	prasiola	5	22.30
	lichen	5	27.40
	moss HL	5	7.70
	moss SDG	5	18.60
	moss SLG	5	9.90
	6.00	5	7.10
	Total	30	

Test Statistics(a,b)

	number of feces
Chi-Square	23.250
df	5
Asymp. Sig.	.000

a Kruskal Wallis Test
b Grouping Variable: type of diet

Moults:

Kruskal-Wallis non-parametric test:

Ranks

	type of diet	N	Mean Rank
number of moults	Prasiola crispa	5	9.00
	umbilicaria decussata	5	16.40
	Usnea antarctica	5	17.40
	Sanionia uncinata	5	21.70
	Brachytecium glaciale	5	22.10
	Pohlia nutans	5	6.40
	Total	30	

Test Statistics(a,b)

	number of moults
Chi-Square	14.197
df	5
Asymp. Sig.	.014

a Kruskal Wallis Test
b Grouping Variable: type of diet

Experiment 2

Feces

ANOVA

number of feces

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	204886,587	14	14634,756	2,513	,007
Within Groups	349386,400	60	5823,107		
Total	554272,987	74			

Moults

Kruskal Wallis test

Ranks

	type of diets	N	Mean Rank
number of moults	Prasiola/Umbilicaria	5	46,60
	Prasiola/Usnea	5	25,70
	Prasiola/Brachytecium	5	49,80
	Prasiola/Sanionia	5	34,00
	Prasiola/Pohlia	5	23,10
	Umbilicaria/Usnea	5	31,50
	Umbilicaria/Brachytecium	5	24,00
	Umbilicaria/Sanionia	5	21,20
	Umbilicaria/Pohlia	5	58,50
	Usnea/Brachytecium	5	42,90
	Usnea/Sanionia	5	67,00
	Usnea/Pohlia	5	37,10
	Brachytecium/Sanionia	5	32,10
	Brachytecium/Pohlia	5	30,10
	Sanionia/Pohlia	5	46,40
	Total	75	

Test Statistics(a,b)

	number of moults
Chi-Square	27,584
df	14
Asymp. Sig.	,016

a Kruskal Wallis Test
b Grouping Variable: type of diets

Experiment 4

Feces:

ANOVA

LOGFECES

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.630	5	.326	6.515	.001
Within Groups	1.201	24	.050		
Total	2.832	29			

Moults:

Kruskal-Wallis non-parametric test:

Ranks

	type of diet	N	Mean Rank
number of moults	Prasiola crispa	5	21.30
	Umbilicaria decussata	5	13.70
	Usnea antarctica	5	14.10
	Sanionia uncinata	5	20.80
	Brachytecium glaciale	5	15.80
	Pohlia nutans	5	7.30
	Total	30	

Test Statistics(a,b)

	number of moults
Chi-Square	9.198
df	5
Asymp. Sig.	.101

a Kruskal Wallis Test
b Grouping Variable: type of diet

Experiment 5

Feces

ANOVA

number of feces

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1002266,587	14	71590,470	4,649	,000
Within Groups	923921,200	60	15398,687		
Total	1926187,787	74			

Moults

Kruskal Wallis test

Ranks

	type of diets	N	Mean Rank
number of moults	Prasiola/Umbilicaria	5	30,20
	Prasiola/Usnea	5	20,00
	Prasiola/Brachytecium	5	37,20
	Prasiola/Sanionia	5	40,20
	Prasiola/Pohlia	5	42,50
	Umbilicaria/Usnea	5	18,20
	Umbilicaria/Brachytecium	5	46,20
	Umbilicaria/Sanionia	5	33,50
	Umbilicaria/Pohlia	5	36,80
	Usnea/Brachytecium	5	45,00
	Usnea/Sanionia	5	40,80
	Usnea/Pohlia	5	25,90
	Brachytecium/Sanionia	5	54,90
	Brachytecium/Pohlia	5	36,60
	Sanionia/Pohlia	5	62,00
	Total	75	

Test Statistics(a,b)

	number of moults
Chi-Square	21,118
df	14
Asymp. Sig.	,099

a Kruskal Wallis Test
b Grouping Variable: type of diets