PLOS ONE

RESEARCH ARTICLE

Influence of Snowmelt Timing on the Diet Quality of Pyrenean Rock Ptarmigan (*Lagopus muta pyrenaica*): Implications for Reproductive Success

Ricardo García-González[1], **Arantza Aldezabal**[2], **Nere Amaia Laskurain**[2], **Antoni Margalida**[3,4]*, **Claude Novoa**[5]

1 Instituto Pirenaico de Ecología (CSIC), Jaca, Spain, **2** Plant Biology and Ecology Department, Basque Country University, Bilbao, Spain, **3** Department of Animal Production, Division of Wildlife, Faculty of Life Sciences and Engineering, University of Lleida, Lleida, Spain, **4** Division of Conservation Biology, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland, **5** Office National de la Chasse et de la Faune Sauvage, Prades, France

* amargalida@prodan.udl.cat

Abstract

The Pyrenean rock ptarmigan (*Lagopus muta pyrenaica*) is the southernmost subspecies of the species in Europe and is considered threatened as a consequence of changes in landscape, human pressure, climate change, and low genetic diversity. Previous studies have shown a relationship between the date of snowmelt and reproductive success in the Pyrenean ptarmigan. It is well established that birds laying early in the breeding season have higher reproductive success, but the specific mechanism for this relationship is debated. We present an explicative model of the relationship between snowmelt date and breeding success mediated by food quality for grouse in alpine environments. From microhistological analyses of 121 faecal samples collected during three years in the Canigou Massif (Eastern Pyrenees), and the assessment of the chemical composition of the main dietary components, we estimated the potential quality of individual diets. Potential dietary quality was correlated with free-urate faecal N, a proxy of the digestible protein content ingested by ptarmigan, and both were correlated with phenological stage of consumed plants, which in turn depends on snowmelt date. Our findings suggest that the average snowmelt date is subject to a strong interannual variability influencing laying date. In years of early snowmelt, hens benefit from a longer period of high quality food resources potentially leading to a higher breeding success. On the contrary, in years of late snowmelt, hens begin their breeding period in poorer nutrient condition because the peaks of protein content of their main food items are delayed with respect to laying date, hence reducing breeding performance. We discuss the possible mismatch between breeding and snowmelt timing.

Introduction

Optimal selection of breeding onset can be important for reproductive success in birds [1,2]. Earlier laying is often associated with larger clutch sizes and greater production of young [3,4]. Conversely, a delay in laying may result in a reduction of nestling growth and survival of fledged young in migratory [5] and sedentary birds [6]. In arctic-alpine birds, a delay in breeding season caused by a late snowmelt or adverse climatic conditions in spring normally results in lower reproductive success [7–11]. On the contrary, earlier snowmelt facilitates the advancement of laying dates increasing breeding success [11–13] and population growth [14]. In this sense, the reproductive success of Pyrenean rock ptarmigan (*Lagopus muta pyrenaica*) was positively associated with the early appearance of snow-free ground [15].

The Pyrenees are home to a small, southern population of rock ptarmigan (<1000 individuals [16]), considered threatened as a consequence of changes in landscape, habitat fragmentation, human pressure, climate change, and low genetic diversity [17]. This small and isolated population may be more prone to extinction due to demographic and environmental stochasticity [18,19] and global change [20], with juvenile survival being a highly influential parameter in ptarmigan populations [21]. Thus, given that data about their ecology in the Pyrenees is relatively scarce [15], any information provided could be important from a conservation point of view, to improve management and conservation actions based on evidence [22].

Clutch timing depends on intrinsic (age, body condition, endogenous rhythms) and extrinsic (photoperiod, climatic conditions, food availability) factors [11]. Some authors suggest that an increase in temperature exerts a direct effect on the onset of reproduction [23]. Other authors have suggested that, the direct relationship between reproductive success and spring temperature is mediated by food quality available to birds before, and during the breeding season [24–26]. Several factors can affect reproductive success [27], but climatic conditions (and particularly the timing of snowmelt) are one of the main factors determining reproductive output in high mountain environments [6,12,13, 28].

We hypothesised that the availability and quality of diet in spring is associated with the timing of snowmelt conditioning beyond the reproductive success of Pyrenean rock ptarmigan. Late snowmelt during spring reduces the availability of food resources for ptarmigan hens at a time when they must also successively cope with moulting, laying and incubation. Furthermore, adverse climatic conditions during the hatching period can negatively affect chick survival which, in their first weeks, require a quantitatively and qualitatively adequate food supply [6,29].

Relying on previous studies of relationships between climate and reproduction, and a detailed description of food habits of a rock ptarmigan population in the Pyrenees [15,30], we present a possible mechanism to explain the mediating role of food quality in the reproductive success of the Pyrenean rock ptarmigan. Specifically, we aim to show that nitrogen content of woody plants (the main food component of grouse diet at the beginning of the growing season) varies depending on the phenological stage (or phenophase), which in turn is closely linked to snowmelt date. Secondly, we model the relationship between the nutritional quality of the ptarmigan diets (expressed as digestible protein content) and the snowmelt date, and demonstrate that diet quality is more dependent on the phenological stage of food components than their floristic composition. Finally, with the influence of the climatic event (snowmelt) on diet quality during the breeding period established, we propose an explanatory model by which the inter-annual variations in the timing of snowmelt in alpine environments may affect the breeding success of rock ptarmigan.

Materials and Methods

Ethics statement

The study was conducted in full compliance with France and Spanish laws and regulation, including the licence of Office national de la Chasse et de la Faune Sauvage (ONCFS) and Ordesa y Monte Perdido National Park (PNOMP) to sampling Pyrenean rock ptarmigan faeces and plant species forming part of their diet. Radio-tracking monitoring of ptarmigans was not a part of this particular study, rather than the location of bird for droppings collection in the field, confirming that the field studies related with this study did not involve the management endangered or protected species. The radio monitoring was part of a larger study on demography and habitat use of Pyrenean ptarmigans conducted by the ONCFS, for which all permits and ethical and legal requirements were required and enforced. According to instructions of ONCFS (France) and Ethics Committee of CSIC (Spain), when no animal experiments have been done no other licences were needed.

Study area

The study was conducted on the Canigou Massif (42°31' N, 2°29' E), which represents the eastern limit of the distribution of rock ptarmigan in the French Pyrenees and the southern limit in Western Europe. The breeding habitat of the species is mainly located on steep slopes and plateaus, at altitudes ranging from 2200 m to 2700 m. At the lowest elevations, the vegetation is dominated by open woodlands of Mountain Pine *Pinus uncinata* associated with ericaceous shrublands (*Rhododendron ferrugineum*, *Vaccinium myrtillus*, *V. uliginosum*, *Empetrum nigrum*). The upper parts of the habitat are characterised by gneiss screes mixed with sparse alpine vegetation (*Dryas octopetala*, *Salix herbacea*, *Silene acaulis*, *Saxifraga* spp.) [31]. At 2150 m, mean annual temperature is 4.6°C and mean annual precipitation exceeds 1400 mm. The seasonal distribution of rain shows a high interannual variability, but summer is generally the driest season.

The rock ptarmigan in the Pyrenees breeds in alpine habitats at an average elevation of about 2400 m [32]. At those altitudes, the plant vegetative season lasts on average from mid-June to early September [33], but it is only during plant growing period (June—early July) that protein and nutrient content is higher than in the rest of vegetative period in alpine plants [34]. This relatively short period also represents the average breeding-onset window for Pyrenean rock ptarmigan [15].

Diet determination

Fresh droppings were collected during monitoring and population surveys throughout the year in an area of 50 km^2 during three breeding periods (2002 to 2004). In most cases, birds were first located, sex and age class were identified, and then faecal samples were collected. Faecal samples of young were identified by their size. Seasonally, 81 samples (67%) were collected during the spring/summer post-melting period, when the main diet variation took place, and 40 samples (33%) were collected in the autumn/winter period [30]. A total of 121 droppings were collected and analysed by means of microhistological analysis to determine diet composition [35]. This method has been successfully used for investigating the diet of herbivorous birds (Waterfowl: [36]; *Branta bernicla*: [37]; *Perdix perdix*: [38]; *Tetrao urogallus*: [39]). Although we analysed faecal samples separately we did not consider sex and age class categories when analysing diet quality results. This is a study at the population level and we assume diet quality of all sex and age classes (females, males and young) contributed in a less or more degree to the reproductive success of the population. On the other hand, sex/age factor seems to explain only a small proportion of diet variation in this and other rock ptarmigan populations [8,30].

Diet content was ascertained by microscopically identifying leaf, seed, fruit, woody elements and arthropod fragments in the prepared droppings. Usually, only the leaf epidermis has recognisable characters useful in identifying the microscopic fragments at the species or genus level. Flower, seed, fruit or woody elements can normally only be identified at higher taxonomic levels [40]. A plant epidermal collection of the possible grouse food species in the study area was previously assembled. An average of 168 (range 100–364) identifications per individual faecal sample was achieved. Relative frequencies of each dietary item in each faecal sample were calculated.

Potential nutritional value of diet

Once the main dietary plant species were identified, we collected samples of them in the field to measure their nutritional value. Samplings were carried out at two localities of similar habitat separated by 200 km: the Canigou Massif at 2250 m (France, Eastern Pyrenees 42° 30'N, 2° 27'E) and Calcilarruego at 2000 m (Spain, Central Pyrenees, 42° 38' N, 0° 3' W). Sampled species were: *Rhododendron ferrugineum* (Rho fer in graphics and tables), *Salix pyrenaica* and *S. herbacea* (hereafter referred as *Salix* spp.), *Vaccinium myrtillus*, *V. uliginosum* (referred as *Vaccinium* spp.), *Calluna vulgaris*, *Dryas octopetala*, *Empetrum nigrum* and *Loiseleuria procumbens*. For *Rhododendron ferrugineum*, buds, old leaves, new leaves, flowers and fruits were clipped separately. For the rest of the species, only old and new leaves (depending on phenology) were taken together with their twigs and buds. Twigs thicker than 2 mm were rejected. A total of 545 samplings were carried out in 2005 at 10-day intervals during the plant growing period, i.e. from late May to early July. In the Spanish locality, samplings were prolonged until autumn to obtain a more extended seasonal variation of chemical composition. At each sampling date, five samples (on average) of each item were clipped in different sampling plots depending on species presence. In each sampling plot plant parts were collected by hand or scissors within a radius of nearly 10 m to obtain a minimum of 30 grams of fresh material. Average phenological stage of each species was also noted during plant samplings.

In the laboratory, samples were cleaned, dried at 60°C and milled to a size of 1 mm. Nitrogen (N) content was determined by means of the Kjeldahl method and expressed as a percentage of dry matter (% DM). N content was used as a proxy of crude protein content and diet quality [41]. Protein content is one of the fundamental components of bird nutrition [42,43], and some authors suggest that it is even more important than metabolisable energy when the ptarmigan selects food during the winter [44,45] or breeding period [46].

Potential N content of each individual diet was estimated from the abundance of each food item in faecal samples (microhistological analysis) multiplied by their N content following the expression, $N_d = \Sigma X_i^* N_x$ where N_d is the potential N content of a faecal sample, X_i the proportion of food item i in the faecal sample and N_x the N content (% of dry matter) of the food item X in the respective month or time period (see S1 Table). Nutritive content estimation of mixed or composite diets from the food nutrient content and the proportion of each food item is a common procedure in herbivorous birds [9,47,48]. Potential N content of faecal samples were calculated by month during the breeding season, and for the whole winter period. Items with unknown N content were estimated from bibliographic data. Food items were grouped in simplified categories. N values for plant species and food categories in each time period are shown in the S1 Table.

Faecal N content

N excretion (total N) is related to N intake [41], but the total N of bird droppings is composed of faecal N and urinary N and they must be separated to obtain a better estimation of the

consumed digestible protein than using total N only. [49,50]. We determined free-urate N content of each faecal sample, and used it as a proxy for protein content of individual diets [49]. Faecal samples were added to 10 ml acetic-formalin and recipients were shaken in an ultrasonic bath for one hour to liberate droppings from the urine portion [50]. N content was estimated by the Kjeldahl method before (total N) and after washing (free-urate faecal N), and we considered N content of droppings after washing (in % DM) as a proxy of digestible protein ingested [51] and hence of diet quality. In samples that could not be washed due to small quantities, we estimated free-urate faecal N from the following linear regression: [free-urate faecal N] = 0.518 [total faecal N]– 0.528 (r^2 = 0.705; n = 74).

Climatic data

Snow depth and daily mean temperatures were obtained from two weather stations: Nivose-Cortalets (2150 m) for the Canigou study area and Góriz station (2250 m) for the Calcilarruego study area, located at 12 and 6 km from sampling areas respectively. While snow depth was measured throughout winter and spring by the meteorological Services, we defined the date of snowmelt as the end of the period of continuous snow cover at weather stations [15, 52]. Dates of snowmelt at Canigou, where both droppings and dietary plants were sampled, were: 28/05/2002, 05/05/2003, 08/06/2004 and 16/05/2005. Date of snowmelt at Calcilarruego, where only dietary plants were sampled, was the 19/05/2005. Degree-days were calculated by summing daily mean temperatures from the date of snowmelt until the plant sampling date. We found a strong positive correlation between sum of degree-days and days from snowmelt (R^2 = 0.99), and a similar level of correlation between these variables and plant phenophases, which is common in alpine environments [53,54]. Thus, we only used the days after snowmelt for relationships to phenophases, or nutrient changes in Ptarmigan faeces and diet.

Statistical analysis

To test the effect of phenophase on N content of woody species, we conducted a Student t-test (or Mann-Whitney U-test, when data did not adjust to normal distribution) for two mean comparisons of plant N content between different phenophases. To model the relationship between faecal N and potential N in diet, we performed an Analysis of Covariance (ANCOVA) with potential N in diet as response variable. This analysis aims to predict potential dietary N from the faecal N of a given sample, and allows us to test whether this relationship is independent of year. Generalised Additive Modelling (GAM regression) was used to model the relationships between potential nitrogen in diet and snowmelt time. Since data for the year 2002 was insufficient, tests were restricted to the data for the years 2003 and 2004. Additive modelling fits smoothing curves through the data and is therefore able to describe a wide variety of relationships [55]). GAM was applied here using R-platform and the function gamm of the package mgcv [56]).

Average botanical composition between and within pre- and post-melting periods were compared by means of Spearman rank correlation tests using the 24 most abundant items in the total diet (which comprise the 80% of total identifications). These tests were performed with SPSS and Statistica packages. We utilised Variance Ratio test to check differences between variability of snowmelt and laying dates [57].

Results

Diet composition and plant N content

Nearly half (52%) of the items found in ptarmigan faeces were leaves of dicotyledons (herbaceous and woody) and along with the stems and buds of woody species (22%), they form the
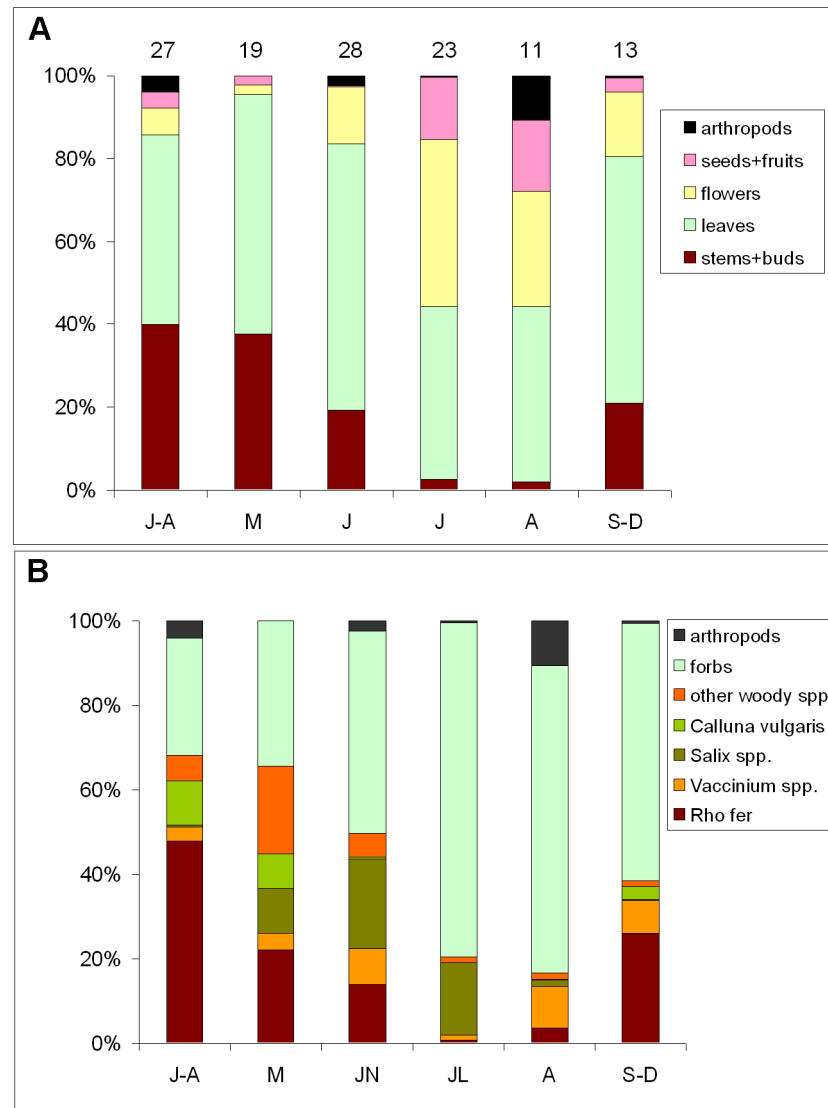
Fig 1. Food types of Rock ptarmigan in Western Pyrenees and their monthly variation. The three samplings years and autumn/winter months have been pooled. Food items are expressed in broad (morphological) (A) and morpho-taxonomical (B) food categories. Numbers above bars indicate sample size.

doi:10.1371/journal.pone.0148632.g001

bulk of the ptarmigan diet in the Eastern Pyrenees on an annual basis. Flowers (17%), arthropods (3%), and seeds and fruits (6%), which are consumed mainly in summer, completed the rest of the ptarmigan diets over the year (Fig 1). Taxonomically, *Rhododendron ferrugineum* (21%), other Ericaceae (*Calluna vulgaris*, *Vaccinium* spp.) (9%), alpine dwarf willows (*Salix* spp.) (9%), Compositae (*Crepis pyrenaica*, *Leucanthemopsis alpina*, *Leontodon* sp.) (16%) and *Jasione crispa* (5%) were the most abundant species in ptarmigan droppings (Fig 1). Graminoids were nearly negligible (1.7%) in ptarmigan diet, regarding their abundance in alpine pastures.

The N content variation of the main species in the ptarmigan diet is shown in Fig 2. In Canigou, where the relationship between rock ptarmigan diet and snowmelt was investigated, only results from the reproductive period are represented (Fig 2 in red). The results of the Calcilarruego sampling show a longer temporal extension of N content variation in order to appraise the total annual variation of protein content (Fig 2 in black). The N content variation of the *R*.
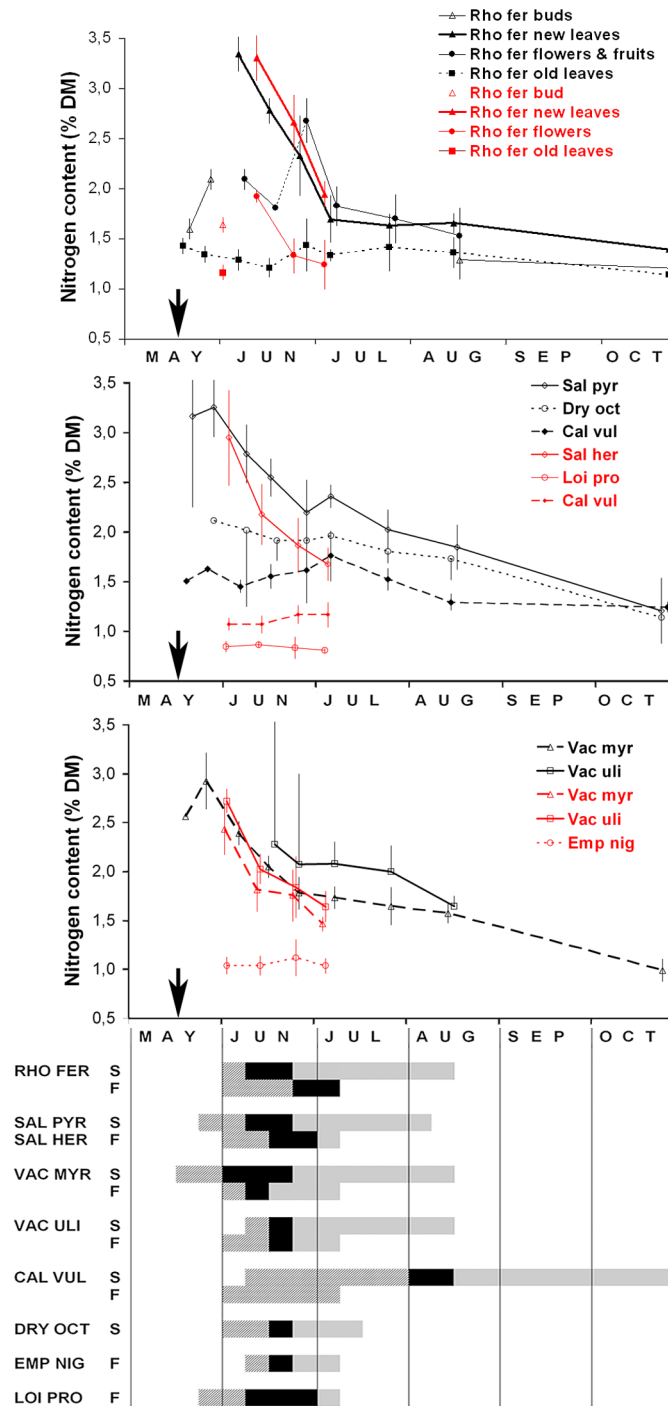
Fig 2. Temporal variation of mean N content (% DM) of the main food species and plant parts of Pyrenean rock ptarmigan in Canigou Massif, France, Eastern Pyrenees (in red) and Calcilarruego, Spain, Middle Pyrenees (in black) during growing and vegetative periods. Species and plant parts are distributed in several figures for clarity reasons. Snowmelt dates are marked by arrows: 16 and 19 May for French and Spanish locations respectively. Plant parts are current year leaves unless otherwise indicated. Vertical bars are 95% confidence limits (only for points with n>3). Below: average phenological stage of sampled species from Spanish (S) and French (F) locations: white, senescence; striped, growing; black, flowering; hatched, fruiting. Species are denoted by the three first letters of genus and species name (see full name in Material and Methods).

doi:10.1371/journal.pone.0148632.g002

ferrugineum parts (stems, buds, flowers, fruits) is shown in the upper part of Fig 2. Old leaves and current-year leaves of *R. ferrugineum* coexisted on the same plant during the growing season. The first had very low and nearly constant N content (1.1%–1.4%). Buds of *R. ferrugineum* also have a low N content, but just one week after snowmelt this increased significantly (Fig 2, $t = 6.4$, $p < 0.001$). If buds gave rise to new leaves, the N content again increased considerably ten days later, and was one of the most N-rich dietary item during the growing season, along with *Salix* spp. and *Vaccinium* spp. leaves. If *R. ferrugineum* buds gave rise to flowers, the N content was maintained, and then increased when they transformed into immature fruits. During ripening, N content drops. The N content of *Salix* spp and *Vaccinium* spp. leaves peaked earlier than *R. ferrugineum*, just seven days after snowmelt, and remained higher over a longer period of time (Fig 2).

The N content depends on the species or plant part, but also on their phenological stage (Fig 2). For woody species in the ptarmigan diet, we found two variation patterns. After snowmelt, most of the species (hump-shaped group) began to grow rapidly and N content increased very quickly, parallel to the sprouting of new leaves (e.g. *R. ferrugineum*, *Salix* spp., *Vaccinium* spp.). A few weeks later, N content decreased significantly, more or less suddenly depending on the species (Mann-Whitney U-test: $U = 321$, $p = 0.02$ for *R. ferrugineum*; $U = 40$, $p < 0.001$ for *Salix* spp and $U = 33$, $p < 0.001$ for *V. myrtillus* between growing and flowering phenophases, respectively). In other species, such as *Empetrum nigrum*, *Dryas octopetala* or *Loiseleuria procumbens* (even group), the N content barely increased during their active period (non-significant differences between growing and flowering phenophases $t = 0.47$ ($p = 0.65$) for *E. nigrum*, $t = 0.56$ ($p = 0.59$) for *Dryas octopetala* and $t = 1.67$ ($p = 0.11$) for *L. procumbens*). The hump-shaped group of species represented 36% of the total ptarmigan diet, while the even group represented only 6%. Leaves of herbaceous dicotyledons, another important component of the ptarmigan diet (30%), also followed the N time variation pattern of the hump-shaped group (S1 Table).

The decline in N content in the shrubs most abundant in the ptarmigan diet (e.g. *R. ferrugineum*, *Salix* spp., *Vaccinium* spp.) was related to the number of days after snowmelt (Fig 3).
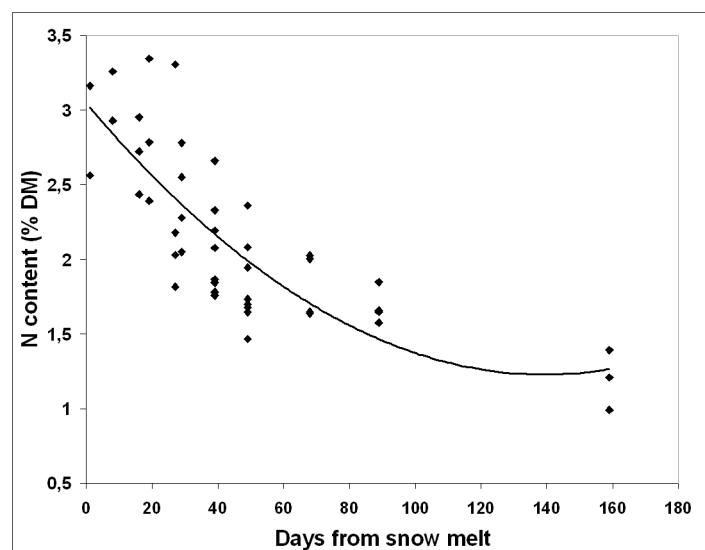
Fig 3. Relationships between days from snowmelt and N content of the main woody species of ptarmigan diet. All species and site samples pooled. Line corresponds to a quadratic adjusted model (see text).

doi:10.1371/journal.pone.0148632.g003

Table 1. ANCOVA results. Observed and fitted relationship between potential dietary N of Pyrenean Rock Ptarmigan (response variable) and the continuous variable free-urate faecal N (Faecal nitrogen) in the years 2002, 2003 and 2004 (factor Year).

| Source	Df	SS	MS	F	p-value
Faecal nitrogen	1	6.12	6.12	30.0	0.0001
Year	2	2.72	1.36	6.7	0.002
Faecal nitrogen×Year	2	5.27	2.64	12.9	0.0001
Residuals	99	20.17	0.20		

Although the fitted relationship between potential nitrogen in diet and faecal nitrogen is positive in all three cases, the slopes vary according to Year. Overall, this model explained Adjusted-R^2 = 0.38 (38%). Df = degrees of freedom; SS = Sum of squares; MS = Mean square.

doi:10.1371/journal.pone.0148632.t001

The general model for N decline for the main dietary woody species as a whole during their vegetative period was: N = $0.00009D^2$–$0.026D$ + 3.04 (R^2 = 0.655; d.f. = 45; $p < 0.001$), with N being nitrogen content (% of dry matter) and D the number of days after snowmelt.

Relationship between free-urate faecal N and potential dietary N

We found a significant relationship between free-urate faecal N and potential dietary N, but this relationship varied depending on the year (Table 1). The ANCOVA explained nearly 40% of total variance (Adjusted R^2 = 0.382). Year 2004 showed the best fitting line (Fig 4). The highest value of N content corresponded to a sample of high arthropod content (i.e. 58%) while the lowest point (0.6%) corresponded to a sample with a high proportion (i.e. 69%) of *Pinus uncinata* leaves (S1 Fig).
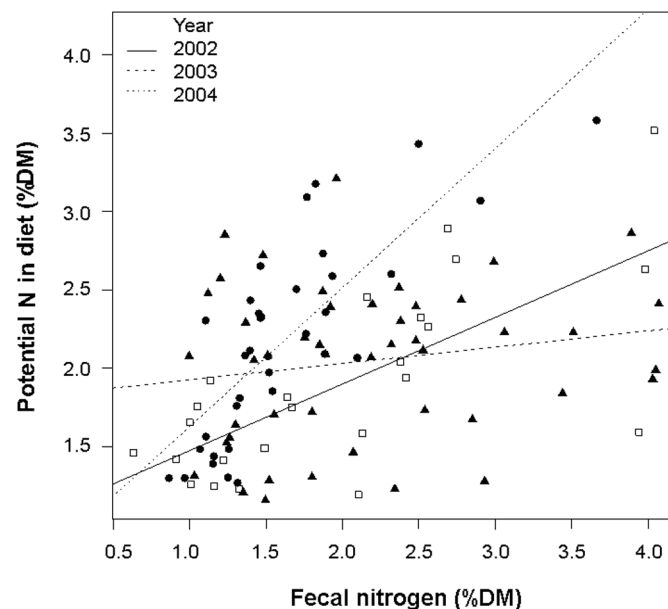
Fig 4. Relationship between potential dietary N and faecal N of Pyrenean Rock Ptarmigan (ANCOVA plot) split by year (2002, 2003 and 2004). Data are expressed in percentage of dry matter (%DM). Empty squares: samples from 2002; black triangles: 2003, and black circles: 2004. See Table 1 for ANCOVA results.

doi:10.1371/journal.pone.0148632.g004

Table 2. Generalised Additive Modelling (GAM) results, where potential dietary N of Pyrenean Rock Ptarmigan recorded in 2003 and 2004 are the response variables and snowmelt time is the predictor.

Response	n	adjusted-R^2	F (edf)	p-value
Potential dietary N (2003)	57	0.32	7.48 (3.75)	0.0001
Potential dietary N (2004)	35	0.57	11.45 (4.04)	0.0001

Adjusted-R^2 = the variance explained by the model; edf = estimated degrees of freedom; n = sample size.

doi:10.1371/journal.pone.0148632.t002

Relationship between potential dietary N and snowmelt time

We rejected in all cases the null hypothesis of no-relationship between potential dietary N and snowmelt time (Table 2). These relationships were hump-shaped and explained R^2 = 0.32–0.57 (32–57% of total variance). Models suggest that the optimum (maximum peak) in nitrogen content occurs at about 50 days after snowmelt (Fig 5).

Comparison of diet composition before and after snowmelt

The botanical composition of diets before the snowmelt period was not associated (Spearman rank correlation) with botanical composition after the snowmelt period in the three years of the study (Tables 3 and 4). Average diet composition before snowmelt was significantly associated between 2002 and 2003, but neither were associated with that of 2004 before the snowmelt period (Table 3). The diets in 2002 and 2003 during the pre-melting period were characterised by a high proportion of buds, stems and old leaves of *Rhododendron ferrugineum*, all of low nutritional value (Table 3 and S1 Table). The pre-melting period of 2004 was characterised by a high consumption of *Calluna vulgaris* and dicotyledon leaves that at this time had very low nutritive value (Table 3 and S1 Table). The composition of diets after snowmelt in 2002 and 2003 were also correlated, and there were also some similarities to that of 2004 (Table 4). Important quantities of leaves and flowers of herbaceous dicotyledons, *Salix* spp leaves and Asteraceae seeds characterised these types of diets (Table 3).

Discussion

Diet composition and plant N content

After spring snowmelt, alpine plants usually experience a rapid growth, which is normally associated with a rise in nutrients (mainly protein content) and an increase in digestibility [58–60]. Plant phenophases and subsequent nutrient variation are generally linked to the increase in temperatures or days from snowmelt, and are species-specific [52,61,62]. *Rhododendron ferrugineum* along with *Salix* spp. and *Vaccinium myrtyllus* are some of the most abundant components of the rock ptarmigan diet in the Pyrenees (Fig 1, [63,64]) as well as in some areas of the Alps [44]. An important N increase in the new leaves of *Rhododendron ferrugineum* occurs 20–25 days after snowmelt. However, in *Salix* spp. and *Vaccinium myrtilus* the peak of N content is only seven days after snowmelt and 15 days earlier than in *Rhododendron ferrugineum* (Fig 2). This also coincides with the sprouting sequence found for the same species in the Alps [52]. Theurillat & Schlüssel [52] found that the sum of degree-days for bud opening in *Vaccinium myrtilus* is -14° ± 24° (beginning before the snow thaw), while for *Salix herbacea* it is 11° (no variability was given), and 194° ± 20° for *Rhododendron ferrugineum*. The concentration of N drops soon after the end of the growth phase and when flowering begins (Fig 2). Adjusting the timing of laying to match the peak of N content is important for reproductive success in birds
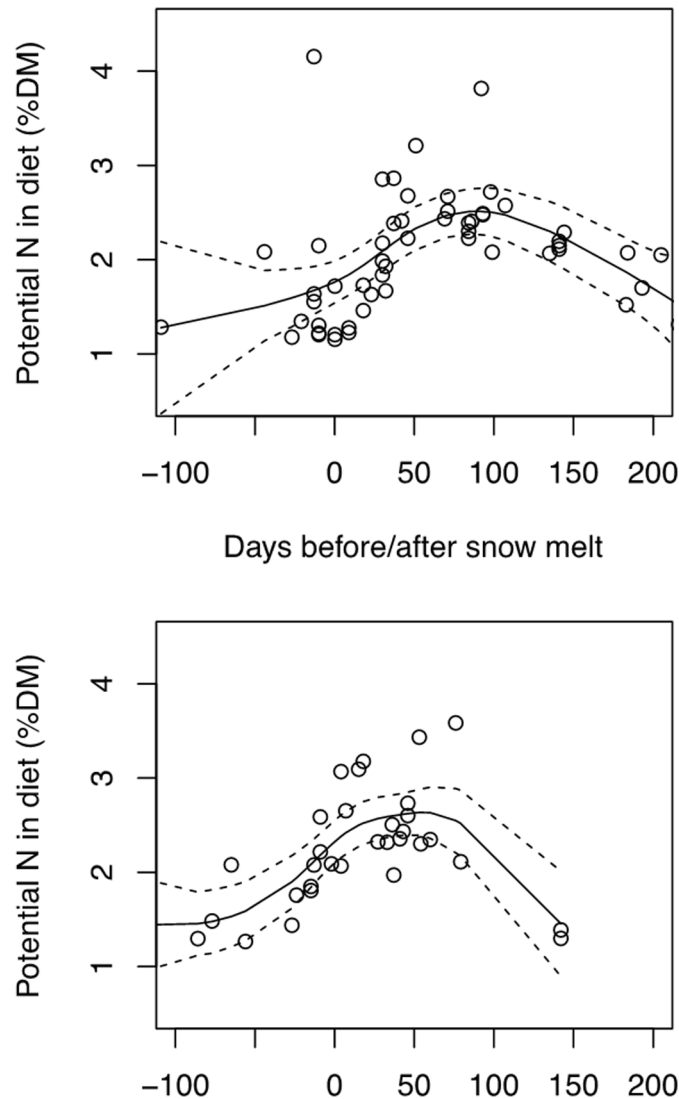
Fig 5. Fitted Generalised Additive Models (GAM plots). The smoothers represent the observed relationship between potential dietary N of Pyrenean Rock Ptarmigan (expressed in percentage of dry matter, %DM) and snowmelt time (indicated in days before/after snowmelt). The upper plot is for 2003 and the lower for 2004. See Table 2 for GAM results.

doi:10.1371/journal.pone.0148632.g005

[2,9] and protein content is a principal component of food quality [45,46]. To use the maximum protein content of the dietary plants for the benefit of reproduction, ptarmigan females should not significantly delay the timing of laying after the snowmelt. The high N content of the dietary plants of ptarmigan lasts barely a month before declining sharply (Fig 2).

Date of snowmelt, diet quality and timing of breeding

ANCOVA analysis suggests that free-urate faecal N is a good estimator of dietary N content and hence of the digestible protein consumed by ptarmigan, but variance explained was quite low and the estimation is not independent of year sampled. Thus we recommend being cautious when using this variable as dietary N estimator. Potential dietary N shows a hump-shaped pattern of temporal variation throughout the years (Fig 5), with a maximum peak coinciding

Table 3. Mean values (%) of diet composition of the top 24 diet items (80% of total diet) before snowmelt (from January to the average day of snowmelt) and after snowmelt (from the day of snowmelt to the end of July).

Simplified food categories	2002		2003		2004	
	Before $n = 13$	After $n = 16$	Before $n = 13$	After $n = 25$	Before $n = 15$	After $n = 13$
R. ferrugineum buds	15.1	1.7	26.5	2.3	11.6	0.0
R. ferrugineum stems	9.9	15.2	19.5	7.8	0.4	0.0
R. ferrugineum leaves	4.7	0.4	5.0	0.0	4.5	0.0
Stem (indeterm.)	7.0	5.4	3.4	2.0	0.5	0.5
Calluna vulgaris	4.9	0.0	3.1	0.1	19.9	0.2
Jasione crispa	2.7	1.4	2.3	7.9	6.5	3.4
Salix pyrenaica	0.5	11.5	0.4	10.6	0.0	0.0
Salix herbacea	0.0	13.3	0.2	8.5	0.7	8.5
Alchemilla catalaunica	6.0	0.2	0.1	0.7	15.4	0.5
Asteraceae (flowers)	0.0	11.0	0.0	6.0	0.1	17.7
Crepis pyrenaica	0.0	9.5	0.0	0.0	0.1	4.2
Forb flower (indet.)	0.1	2.6	0.0	9.4	2.3	5.1
Oxytropis foucadii	3.1	2.1	0.0	1.1	7.6	4.7
Cerastium fontanum	4.6	2.2	3.6	1.4	0.0	1.4
R. ferrugineum flowers	2.0	0.1	3.7	0.4	6.1	0.0
Forb leaf (indeterm)	0.1	0.9	0.2	0.8	2.1	8.4
Arthropods	0.6	1.2	6.7	0.8	1.2	1.4
Campanulaceae	0.0	0.2	0.0	6.0	0.0	5.6
Leontodon sp. seed	0.0	1.8	0.0	4.8	0.0	4.1
Anthyllis vulneraria	10.6	0.0	0.0	0.0	0.0	0.0
Asteraceae seed	0.0	3.5	0.0	0.2	0.4	5.0
Hieracium lactucella	0.2	0.0	4.6	0.1	2.9	1.3
Pinus uncinata	6.8	0.0	1.5	0.0	0.5	0.0
Vaccinium myrtillus stem	5.8	0.0	0.4	0.6	0.7	0.0

Diet items are leaves unless otherwise indicated.

doi:10.1371/journal.pone.0148632.t003

Table 4. Values of Spearman correlations (Rho) between the top 24 diet items (Table 3) of the ptarmigan diet before and after snowmelt.

	2002 after	2003 before	2003 after	2004 before	2004 after
2002 before		0.45*			-0.76**
2002 after	-		0.64**		0.39[a]
2003 before	-	-			-0.57**
2003 after	-	-	-		
2004 before	-	-	-	-	

Diet items are leaves unless otherwise indicated.

Only significant results are shown.

* $p < 0.05$;

** $p < 0.01$;

[a] $p = 0.056$.

doi:10.1371/journal.pone.0148632.t004

with the increase in diet quality during the breeding season and minimum values coinciding with winter shortages. The analysis of nutrient content of rock ptarmigan crops in Alaska provided a similar pattern of seasonal variation [65]. The rise in N content in the ptarmigan diet is linked to the increase in N content of plants consumed, and this depends on the timing of snowmelt and temperatures rising [52]. Until plants begin to grow, they do not seem to have sufficient N content to allow the breeding onset of ptarmigan hens. According to Moss [66], domestic hens need more than 2.8% of N content in the diet to breed (see also [8]). A delay in the growth of key dietary species may negatively affect the nutrition level of ptarmigan females at the beginning of the breeding season, and beyond their reproductive success [12,25,26]. In willow grouse (*Lagopus lagopus*), females produce fewer young when they are in poor physical condition [67].

Diet quality is influenced by the type of food consumed [30] but also by phenological stage. Although botanical diet composition before snowmelt is different from that of the post-melting period [44] and varied between years (Table 4), potential dietary N content did not differ significantly. Until snowmelt, diet quality is very low. Dietary N contents rise significantly after snowmelt, regardless of species or items consumed (Fig 5). What seems to matter for food quality is the N content of dietary plants, less than the species consumed, although there are species such as *Calluna vulgaris*, *Empetrum nigrum* or *Loiseleuria procumbens* that always present a low N content ([68] and Fig 2). In fact, the consumption of some species such as *Rhododendron ferrugineum* or *Calluna vulgaris* on an annual basis is inversely correlated to N content in the Pyrenean ptarmigan diet [30].

Two important variables determining reproductive success are the age of breeding birds and the availability of food, the latter being an important determinant (among other factors) of the quality of the habitat [69–71]. In general, older individuals nest earlier, produce larger clutches and have greater breeding success [69,72,73]. Late laying dates are often associated with a low reproductive success. For example, in Barnacle goose (*Branta leucopsis*), a delay in the incubation period due to a late snowmelt negatively affected the proportion of pairs starting to breed, the nest success and the brood size at hatch [9]. Late springs can reduce the size and number of eggs, or the chick weight at birth of White-tailed ptarmigan (*Lagopus leucura*), decreasing their probability of survival [6]. Chicks that are born later in the breeding season do not have enough time to develop or to build fat reserves for the winter, and their mortality probabilities increase [7]. The studied population of Pyrenean ptarmigan revealed a significant inverse relationship between the date of snowmelt and reproductive success defined as the ratio of young to adult in August [15].

Evolutionarily, it is accepted that birds try to match laying dates with the maximum abundance of food resources [1,26]. In arctic-alpine environments, these maximum values of energy and nutrients are associated with the timing of snowmelt [28,58,59], but this is normally subject to a high temporal variability [54]. When the date of snowmelt is delayed (late springs), Pyrenean ptarmigan females also delay the timing of laying [15]. If they do not, laying coincides with the beginning of an ascending phase of the plant nutrient cycle with low nutrient values (Fig 6 middle), resulting in the malnutrition of hens. Nevertheless, the range of variation of laying date seems to be narrower than that of snowmelt (ILDV < ISDV, Fig 6) and this imposes a limit to the delay in laying. From the data collected by Novoa et al. [15], it can be inferred that an inverse relationship exists between the date of snowmelt and the interval between snowmelt and hatching date from 1997 to 2006, in the same studied population ($R^2 = 0.84$, $p < 0.01$, $n = 10$). Thus, as snowmelt is delayed, the interval between snowmelt and hatching is reduced (and hence laying [74]). In late springs, ptarmigan hens could be "forced" to lay without having reached an optimal level of protein intake.
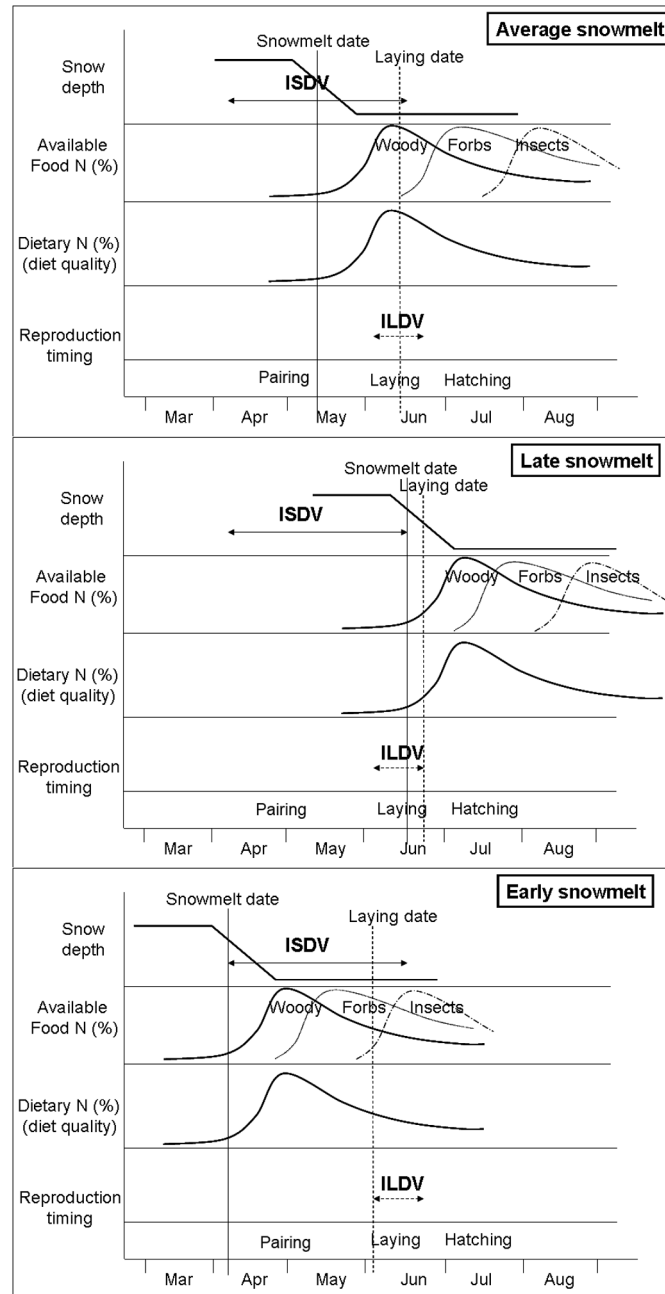
Fig 6. Explanatory model showing possible relationships between date of snowmelt, diet quality and reproductive success in Pyrenean ptarmigan. Average and both extremes of interannual snowmelt variation are shown. Snowmelt determines the beginning of growth and N content in the plants fed on by ptarmigan, which in turn regulates the N content of hen diets and protein increases during reproduction period. Previous data (Novoa et al. 2008) show that interannual variability of laying date (ILDV) are significantly less than interannual variability of snowmelt date (ISDV). Thus, in years of late snowmelt hens have less time to obtain good-quality food, resulting in poor body condition for laying and incubation, and diminishing breeding success. In years of early snowmelt, there is no loss of protein gain for ptarmigan hens, because of the asymmetric size of N content curve in plants and the appearance of new N peaks of major summer foods for ptarmigan (forbs and insects).

doi:10.1371/journal.pone.0148632.g006

Conversely, early spring (Fig 6 below) normally leads to a higher reproductive success [15], probably as a result of improved nutrition in females [2, 25]. It could be argued that, as in the previous case, an advance of the spring could also produce a mismatch between the peak abundance of plant nutrients and laying date [75,76]. If laying coincides with the decline of the hump-shaped curve (Fig 4), a malnutrition of females could take place, lowering reproductive success [7]. The main difference with late snowmelt is that the sinusoidal curve of nutrient plant variation is leftward skewed, with the increase being very fast and the decline in N content very slow (Fig 4). Several studies in arctic-alpine environments confirm this pattern of temporal variation of N content and other nutrients in plants [58,77,78]. In addition, once the peak of highest N content in the woody species of the ptarmigan diet has been surpassed (continuous line of second row in Fig 6), peaks of other basic foods in the ptarmigan diet successively appear, i.e. dicotyledonous forbs and insects, which have later abundance peaks. The proportion of forbs and arthropods in the ptarmigan diet grew in July and August, respectively [30], coinciding with their greater abundance in Pyrenean alpine habitats [34,79]. In this way, the protein provision in the right side of the sinusoidal curve is ensured. Thus, females that lay early obtain a better nutrient supply and in general increase their chances of reproductive success [25].

The interannual variability of laying date (ILDV) seems to be significantly smaller than the interannual variability of snowmelt date (ISDV) (Fig 6). In the case of the Pyrenean ptarmigan, the date of snowmelt varied by 79 days in Canigou between 1997 and 2006, whereas the median date of laying varied by only 30 days [15]. Snowmelt variation was 63 days in the Calcilarruego sampling area between 1993 and 2012 [54]. The date of snowmelt (linked to the snow pack), tends to experience large annual fluctuations associated with the North Atlantic Oscillation index [80]. In the Mountain White-crowned Sparrow (*Zonotrichia leucophrys*) [81], the date of 25% snow cover varied by 48 days in four years, while the average date of laying ranged only 17 days. The interannual variability of the average date of laying in other species of grouse was also quite reduced, i.e. 16 days in 8 years for *Lagopus lagopus* [7] and 17 days in 16 years for *Dendragapus canadiensis* [82]. In the ptarmigan population studied, the variance of the snowmelt date was significantly greater than that of laying (ISDV > ILDV in Fig 6; test of homogeneity of variance $F = 5.3$, $p < 0.01$) and this supports the initial hypothesis of the relationship between snowmelt and reproductive success mediated by food quality.

Climate change and breeding success in Pyrenean ptarmigan

The Pyrenean ptarmigan occupies one of the southernmost ranges of the species worldwide distribution. It has been suggested that populations near the limit of range distribution may be more vulnerable to the effects of climate change [83,84]. One of these effects is that the alpine species adapted to a cold environment may be faced with an unfavourable habitat if weather becomes warmer. In fact, some models predict the disappearance of Pyrenean ptarmigan in a future scenario of temperature increases [85,86]. Nevertheless, the increase in temperatures could also have a favourable effect on the reproductive success of the Pyrenean ptarmigan. If temperatures rise there could be an advance of plant growing season, improved nutrition level of hens, and a higher reproductive success, providing Pyrenean ptarmigan have the adaptive flexibility to sufficiently advance their timing of laying (i.e., phenotypic plasticity, [3,87]).

Various studies demonstrate an advance in the phenology of plants and animals as a result of climate change [3,88–90]. In some migratory insectivorous birds (e.g. European pied flycatcher [*Ficedula hypoleuca*]) an advancement of the peak abundance of their prey (caterpillars) in relation to laying date, caused a decrease in their reproductive success [5,91]. In the case of the Pyrenean ptarmigan the effect could be the opposite. First, the basic foods of

herbivorous birds are not subject to such narrow pulses of abundance as that of insectivorous birds, occurring more gradually over time (Fig 6). In addition, because of the nature of the nutrient availability curve, the low level of resources is not as important on the right side of the curve (early snowmelt), as on the left side (late snowmelt).

A decline in snow pack and an advance in snowmelt date have been observed over the last 50 years in the Pyrenees [80]. According to the predictions of recent models on climate change, winter precipitations may decrease in the Pyrenees over the course of this century, with a greater incidence in the Eastern sector [92]. However, there are still no conclusive studies of how this will affect the reproductive performance in birds [89,93]. Although earlier laying of clutches is often associated with larger clutch sizes and greater production of young, the effects of earlier laying on reproductive performance (i.e. population dynamics, recruitment) are less clear [3].

In temperate ecosystems, temperature increase tends to have a direct influence on the start of the growing season [94]. In alpine environments the advancement or delay of the plant growing season depends on the disappearance of snow cover [52,95], which in turn depends on temperature, but also on other factors. High altitude, northern exposure or late spring snowfall can cause the presence of a thick snowpack and a delay in the snowmelt in alpine environments [80]. Variability and unpredictability of rainfall in alpine ecosystems are higher than in areas of low altitude [15,96] and climate change may increase this variability [97]. As we have previously shown, the date of laying could depend on less variable factors (such as photoperiod) than climate variability [98]. As in other birds, the impact of climate change on Pyrenean ptarmigan reproduction may depend on their adaptive capacity to adjust their timing of laying [87].

In this study, we found that free-urate faecal N content was related to the protein content of the ptarmigan diet. Therefore, the number of breeding periods analysed (only three years) is insufficient to find conclusive relationships between the N content of the ptarmigan diet during the post-melting period and their reproductive success. We need to further investigate the relationships between diet, plant phenology, weather and reproductive success of ptarmigan to consolidate the assumptions proposed in this work. An individual survey of diet quality of females during the breeding season related to reproductive success would likely be required. The understanding of such relationships will allow the development of models that predict the potential impact of climate change on the population dynamics of the Pyrenean rock ptarmigan and other alpine birds.

Supporting Information

S1 Fig. Distribution of free-urate faecal N (circles) and potential dietary N estimated from microhistological composition (crosses) throughout sampling period (2002–2004). Each point corresponds to a single faecal sample. Arrows indicate the date of snowmelt at nearest weather station.
(TIF)

S1 Table. Mean values of N content (% of dry matter) of different food items utilised to estimate the potential N content of each individual diet determined by microhistological analysis. Winter period comprises from September to date of snowmelt. Diet items are leaves unless otherwise indicated.
(PDF)

Acknowledgments

Author Contributions

Conceived and designed the experiments: CN RGG. Performed the experiments: RGG CN NAL AA. Analyzed the data: NAL AA RGG CN AM. Contributed reagents/materials/analysis tools: RGG NAL AA CN AM. Wrote the paper: RGG CN AA AM.

References

1. Bêty J, Gauthier G, Giroux J-F. Body condition, migration, and timing of reproduction in snow geese: a test of the condition-dependent model of optimal clutch size. The American Naturalist 2003; 162(1): 110–121. PMID: 12856240

2. Davies S, Deviche P. At the crossroads of physiology and ecology: Food supply and the timing of avian reproduction. Hormones and Behavior 2014; 66(1): 41–55. doi: 10.1016/j.yhbeh.2014.04.003 PMID: 24727023

3. Dunn P. Breeding Dates and Reproductive Performance. In: Møller AP, Fiedler W, Berthold P, editors. Birds and Climate Change. London: Academic Press; 2004. p. 69–87.

4. Møller AP. Climate change and micro-geographic variation in laying date. Oecologia 2008; 155(4): 845–857. doi: 10.1007/s00442-007-0944-3 PMID: 18224340

5. Sanz JJ, Potti J, Moreno J, Merino S, Frias O. Climate change and fitness components of a migratory bird breeding in the Mediterranean region. Global Change Biology 2003; 9(3): 461–472.

6. Clarke JA, Johnson RE. The influence of spring snow depth on White-tailed Ptarmigan breeding success in the Sierra Nevada. The Condor 1992; 94: 622–627.

7. Hannon SJ, Martin K, Schieck JO. Timing of reproduction in two populations of Willow Ptarmigan in northern Canada. The Auk 1988; 105: 330–338.

8. Pulliainen E, Tunkkari PS. Responses by the capercaillie *Tetrao urogallus*, and the willow grouse *Lagopus lagopus*, to the green matter available in early spring. Holartic Ecology 1991; 14: 156–160.

9. Prop J, de Vries J. Impact of snow and food conditions on the reproductive performance of Barnacle Geese Branta leucopsis. Ornis Scandinavica 1993; 24: 110–121.

10. Scherini GC, Tosi G, Wauters LA. Social behaviour, reproductive biology and breeding success of Alpine Rock Ptarmigan Lagopus mutus helveticus in northern Italy. Ardea 2003; 91(1): 11–23.

11. Wilson S, Martin K. Variable reproductive effort for two ptarmigan species in response to spring weather in a northern alpine ecosystem. Journal of Avian Biology 2010; 41(3): 319–326.

12. Martin K, Wiebe KL. Coping mechanisms of alpine and arctic breeding birds: Extreme weather and limitations to reproductive resilience. Integrative and Comparative Biology 2004; 44(2): 177–185. doi: 10.1093/icb/44.2.177 PMID: 21680497

13. Jensen GH, Madsen J, Johnson FA, Tamstorf MP. Snow conditions as an estimator of the breeding output in high-Arctic pink-footed geese *Anser brachyrhynchus*. Polar Biology 2014; 37(1): 1–14.

14. Imperio S, Bionda R, Viterbi R, Provenzale A. Climate change and human disturbance can lead to local extinction of Alpine rock ptarmigan: New insight from the Western Italian Alps. PLoS ONE 2013; 8(11): e81598. doi: 10.1371/journal.pone.0081598 PMID: 24260581

15. Novoa C, Besnard A, Brenot JF, Ellison LN. Effect of weather on the reproductive rate of Rock Ptarmigan *Lagopus muta* in the Eastern Pyrenees. Ibis 2008; 150: 270–278.

16. Figueroa I, López BC, López A, Potrony D. What happens to ptarmigan when marmots arrive? Ethology Ecology and Evolution 2009; 21(3–4): 251–260.

17. Bech N, Boissier J, Drovetski S, Novoa C. Population genetic structure of rock ptarmigan in the 'sky islands' of French Pyrenees: Implications for conservation. Animal Conservation 2009; 12(2): 138–146.

18. Lawton JH. Range, population abundance and conservation. Trends in Ecology & Evolution 1993; 8: 409–413.

19. Pulliam HR. Sources, sinks, and population regulation. American Naturalist 1988; 132: 652–661.

20. Visser ME, Both C, Lambrechts MM. Global Climate Change Leads to Mistimed Avian Reproduction. In: Møller AP, Fiedler W, Berthold P, editors. Birds and Climate Change. London: Academic Press; 2004. p. 89–110.

21. Wilson S, Martin K. Influence of life history strategies on sensitivity, population growth and response to climate for sympatric alpine birds. BMC Ecology 2012; 12: 9. doi: 10.1186/1472-6785-12-9 PMID: 22747571

22. Arlettaz R, Schaub M, Fournier J, Reichlin TS, Sierro A, Watson JEM, et al. From publications to public actions: When conservation biologists bridge the gap between research and Implementation. BioScience 2010; 60: 835–842.

23. Visser ME, Holleman LJM, Caro SP. Temperature has a causal effect on avian timing of reproduction. Proceedings of the Royal Society B: Biological Sciences 2009; 276(1665): 2323–2331. doi: 10.1098/rspb.2009.0213 PMID: 19324731

24. Drent RH, Daan S. The prudent parent: energetic adjustments in avian breeding. Ardea 1980; 68: 225–252.

25. Moss R, Watson A. Maternal nutrition, egg quality and breeding success of Scottish Ptarmigan *Lagopus mutus*. Ibis 1984; 126: 212–220.

26. Carey C. Female Reproductive Energetics. In: Carey C, editor. Avian Energetics and Nutritional Ecology. New York: Champman & Hall; 1996. p. 324–374.

27. Sawa Y, Takeuchi Y, Nakamura H. Nest site selection and nesting biology of Rock Ptarmigan Lagopus muta japonicus in Japan. Bird Study 2011; 58(2): 200–207.

28. Liebezeit JR, Gurney KEB, Budde M, Zack S, Ward D. Phenological advancement in arctic bird species: Relative importance of snow melt and ecological factors. Polar Biology 2014; 37(9): 1309–1320.

29. Erikstad KE, Andersen R. The effect of weather on survival, growth rate and feeding time in different sized Willow Grouse broods. Ornis scandinavica 1983; 14: 249–252.

30. García-González R, Aldezabal A, Laskurain N, Margalida A, Novoa C. Factors Affecting Diet Variation in the Pyrenean Rock Ptarmigan (Lagopus muta pyrenaica): Conservation Implications. Plos One (accepted).

31. Bolòs O, Vigo J, Masalles R, Ninot J. Flora Manual dels Països Catalans. Barcelona: Ed. Portic; 1990.

32. Gonzalez G, Novoa C. The partitioning of space between sympatric Pyrenean Ptarmigan (Lagopus mutus pyrenaicus) and the Partridge (Perdix perdix hispaniensis) in the Carlit mountain range, Eastern Pyrénées. The respective roles of elevation and exposure. Revue d'Ecologie (Terre & Vie) 1989; 44(4): 347–360.

33. Del Barrio G, Creus J, Puigdefabregas J. Thermal seasonality of the high mountain belts of the Pyrenees. Mountain Research and Development 1990; 10(3): 227–233.

34. Marinas M, García González R. Preliminary data on nutritional value of abundant species in supraforestal Pyrenean pastures. Pirineos 2006; 161: 85–109.

35. García-González R, Boudarel P, Dendaletche C. Premiers donnés sur l'alimentation hivernale du lagopède alpin (Lagopus mutus) dans le massif d'Ossau (Pyrénées Atlantiques). Acta biol. montana 1987; 7: 35–50.

36. Owen M. An assessment of fecal analysis technique in waterfowl feeding studies. Journal of Wildlife Management 1975; 39(2): 271–279.

37. Prop J, Deerenberg C. Spring staging in Brent Geese *Branta bernicla*: feeding constraints and the impact of diet on the accumulation of body reserves. Oecologia 1991; 87: 19–28.

38. Novoa C, García-González R, Aldezabal A. Le régime alimentaire automnal de la perdrix grise (Perdix perdix hispaniensis) dans Pyrénées-Orientales. Revue d´Ecologie (Terre et Vie) 1999; 54: 149–166.

39. Blanco-Fontao B, Fernández-Gil A, Obeso JR, Quevedo M. Diet and habitat selection in Cantabrian Capercaillie (Tetrao urogallus cantabricus): ecological differentiation of a rear-edge population. J. Ornithol 2009; 151(4): 901–906.

40. Metcalfe CR, Chalk L. Anatomy of the Dicotyledons. Oxford: Clarendon Press; 1965.

41. Robbins CT. Wildlife feeding and nutrition. New York & London: Academic Press. 2nd ed.; 1993.

42. Jones PJ, Ward P. The level of reserve protein as the proximate factor controlling the timing of breeding and clutch-size in the Red-billed *Quelea Quelea quelea*. Ibis 1976; 118(4): 547–574.

43. Sedinger JS. Adaptations to and Consequences of an Herbivorous Diet in Grouse and Waterfowl. The Condor 1997; 99(2): 314–326.

44. Bossert A. Winter nutrition of Alpine Rock Ptarmigan Lagopus mutus and the influence of snow-cover and the melting period. Ornithologische Beobachter 1980; 77(3): 121–166.

45. Andreev AV. Ecological energetics of Palaearctic Tetraonidae in relation to chemical composition and digestibility of their winter diets. Canadian Journal of Zoology 1988; 66(6): 1382–1388.

46. Moss R. Food selection by red grouse (Lagopus lagopus scoticus (Lath.)) in relation to chemical composition. Journal of Animal Ecology 1972; 4(2): 411–428.

47. Barnett JK, Crawford JA. Pre-laying nutrition of sage grouse hens in Oregon. Journal of Range Management 1994; 47(2): 114–118.

48. Filacorda S, Sepulcri A, Piasentier E, de Franceschi PF. Estimation of the chemical composition of black grouse Tetrao tetrix diets in the eastern Italian Alps. Wildlife Biology 1997; 3: 187–194.

49. Moss R, Parkinson JA. The digestion of heather (Calluna vulgaris) by red grouse (Lagopus lagopus scoticus). British Journal of Nutrition 1972; 27: 285–298. PMID: 5015248

50. Terpstra K, Hart N. The estimation of urinary nitrogen and faecal nitrogen in poultry excreta. Z. Tierphysiol., Tierernährg. u. Futtermittelkde 1974; 32: 306–320.

51. Moss R, Trenholm IB, Watson A, Parr R. Plant growth and nitrogen metabolism of Red Grouse Lagopus lagopus scoticus in spring. Ornis scandinavica 1990; 21: 115–121.

52. Theurillat J-P, Schlüssel A. Phenology and distribution strategy of key plant species within the subalpine-alpine ecocline in the Valaisan Alps (Switzerland). Phytocoenologia 2000; 30(3): 439–457.

53. Price MV, Waser NM. Effects of experimental warming on plant reproductive phenology in a subalpine meadow. Ecology 1998; 79: 1261–1271.

54. García-González R, Gómez-García D. Relaciones entre integral térmica y fenología en especies de pastos de puerto del Pirineo Central. In: Olea L, Poblaciones MJ, Rodrigo SM, Santamaría O, editors. Los pastos: nuevos retos, nuevas oportunidades. Badajoz: SEEP; 2013. p. 41–48.

55. Wood SN. Generalized Additive Models: An Introduction with R: Chapman and Hall / CRC; 2006.

56. Wood SN. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. Journal of the Royal Statistical Society (B) 2011; 73(1): 3–36.

57. Fisher RA, Yeates F. Statistical Tables for Biological, Agricultural and Medical Research. Edinburg: Oliver & Boys; 1963.

58. Chapin FS III, Cleve KV, Tieszen L. Seasonal nutrient dynamics of tundra vegetation at barrow, Alaska. Arctic and Alpine Research 1975; 7(3): 209–226.

59. Körner C. Alpine Plant Life. Functional Plant Ecology of High Mountain Ecosystems. Berlin: Springer-Verlag; 1999.

60. Van der Wal R, Madan N, van Lieshout S, Dormann C, Langvatn R, Albon SD. Trading forage quality for quantity? Plant phenology and patch choice by Svalbard reindeer. Oecologia 2000; 123(1): 108–115.

61. Jouglet JP, Bernard-Brunet J, Dubost M. Phénologie de quelques espèces de pelouses subalpines du Briançonnais. Fourrages 1982; 92: 67–90.

62. Laine KM, Henttonen H. Phenolics/nitrogen ratios in the blueberry Vaccinium myrtillus in relation to temperature and microtine density in Finnish Lapland. Oikos 1987; 50: 389–395.

63. Martínez C. Autumn food of the Ptarmigan (Lagopus mutus, Martin 1776) in the Spanish Central Pyrenees. Doñana Acta Vertebrata 1985; 12(1): 174–181.

64. Boudarel P, García-González R. Approche du régime alimentaire du lagopède alpin (Lagopus mutus pyrenaicus) dans les Pyrénées occidentales-2- Printems-été-automne. Acta biologica montana 1991; 10: 11–23.

65. Gasaway WC. Seasonal variations in diet, volatile fatty acid production and size of the cecum of rock ptarmigan. Comp. Biochem. physiol. 1976; 53A: 109–114.

66. Moss R. Food selection and nutrition in Ptarmigan (Lagopus mutus). In: Crawford MA, editor. Comparative Nutrition of Wild Animals. London & New York: Academic Press; 1968. p. 207–216.

67. Brittas R. Nutrition and reproduction of the willow grouse Lagopus lagopus in Central Sweden. Ornis scandinavica 1988; 19: 49–57.

68. Gardarsson A, Moss R. Selection of food by Icelandic Ptarmigan in relation to its availability and nutritive value. In: Watson A, editor. Animal populations in relation to their food resources. Oxford & Edinburgh: Blaskwell Sci. Publ.; 1970. p. 47–71.

69. Newton I. Lifetime reproduction in birds. London: Academic Press; 1989.

70. Kadmon R. Population dynamic consequences of habitat heterogeneity: an experimental study. Ecology 1993; 74: 816–825.

71. Penteriani V, Balbontin J, Ferrer M. Simultaneous effects of age and territory quality on fecundity in Bonelli's eagle Hieraaetus fasciatus. Ibis 2003; 145: E77–E82.

72. Nielsen T, Drachmann J. Age-dependent reproductive performance in Northern Goshawks *Accipiter gentilis*. Ibis 2003; 145: 1–8.

73. Margalida A, González LM, Sánchez R, Oria J, Prada L, Caldera J, et al. A long-term large-scale study of the breeding biology of Spanish Imperial eagle (Aquila adalberti). Journal of Ornithology 2007; 148: 309–322.

74. Grabowski MM, Doyle FI, Reid DG, Mossop D, Talarico D. Do Arctic-nesting birds respond to earlier snowmelt? A multispecies study in north Yukon, Canada. Polar Biology 2013; 36: 1097–1105.

75. Coppack T, Pulido F. Photoperiodic Response and the Adaptability of Avian Life Cycles to Environmental Change. In: Møller AP, Fiedler W, Berthold P, editors. Birds and Climate Change. London: Academic Press; 2004. p. 131–150.

76. Ludwig GX, Alatalo RV, Helle P, Lindén H, Lindström J, Siitari H. Short- and long-term population dynamical consequences of asymmetric climate change in black grouse. Proceedings of the Royal Society Series B 2006; 273: 2009–2016.

77. Van Rees H, Beard JA. Seasonal variation in vitro digestibility and chemical composition of a range of alpine plants, from Victoria, Australia. Australian Rangeland Journal 1984; 6(2): 86–91.

78. Albon SD, Langvatn R. Plant phenology and the benefits of migration in a temperate ungulate. Oikos 1992; 65: 502–513.

79. Isern-Vallverdú J. A quantitative ecology of Orthopteran communities on Pyrenean grasslands above timberline (Huesca, Spain). Boletín de Sanidad Animal "Plagas" 1990; 20: 311–320.

80. López-Moreno JI. Recent Variations of Snowpack Depth in the Central Spanish Pyrenees. Arctic, Antarctic, and Alpine Research 2005; 37(2): 253–260.

81. Morton ML. Snow conditions and the onset of breeding in the Mountain White-crowned Sparrow. Condor 1978; 80: 285–289.

82. Smyth KE, Boag DA. Production in spruce grouse and its relationships to environmental factors and population parameters. Canadian Journal of Zoology 1984; 62(11): 2250–2257.

83. Cattadori I, Hudson PJ. Are grouse populations unstable at the southern end of their range? Wildlife Biology 2000; 6: 213–218.

84. Bech N, Barbu CM, Quéméré E, Novoa C, Allienne JF, Boissier J. Pyrenean ptarmigans decline under climatic and human influences through the Holocene. Heredity 2013; 111(5): 402–409. doi: 10.1038/hdy.2013.62 PMID: 23838689

85. Storch I. Conservation status and threats to grouse worldwide: an overview. Wildlife Biology 2000; 6(4): 195–204.

86. Huntley B, Green R, Collingham Y, Willis S. A Climatic Atlas of European Breeding Birds. Barcelona: Lynx Edicions—RSPB—Durham Univ.; 2007.

87. Charmantier A, McCleery RH, Cole LR, Perrins C, Kruuk LEB, Sheldon BC. Adaptive phenotypic plasticity in response to climate change in a wild bird population. Science 2008; 320(5877): 800–803. doi: 10.1126/science.1157174 PMID: 18467590

88. Peñuelas J, Filella I, Comas P. Changed plant and animal life cycles from 1952 to 2000 in the Mediterranean region. Global Change Biology 2002; 8(6): 531–544.

89. Crick HQP. The impact of climate change on birds. Ibis 2004; 146(s1): 48–56.

90. Oberbauer SF, Elmendorf SC, Troxler TG, Hollister RD, Rocha AV, Bret-Harte MS, et al. Phenological response of tundra plants to background climate variation tested using the International Tundra Experiment. Philosophical Transactions of the Royal Society B 2013; 368: 20120481.

91. Both C, Artemyev AV, Blaauw B, Cowie RJ, Dekhuijzen AJ, Eeva T, et al. Large-scale geographical variation confirms that climate change causes birds to lay earlier. Proceedings of the Royal Society B: Biological Sciences 2004; 271(1549): 1657–1662. PMID: 15306284

92. López-Moreno JI, Beniston M, García-Ruiz JM. Environmental change and water management in the Pyrenees: Facts and future perspectives for Mediterranean mountains. Global and Planetary Change 2008; 61(3–4): 300–312.

93. Revermann R, Schmid H, Zbinden N, Spaar R, Schröder B. Habitat at the mountain tops: How long can Rock Ptarmigan (Lagopus muta helvetica) survive rapid climate change in the Swiss Alps? A multi-scale approach. Journal of Ornithology 2012; 153(3): 891–905.

94. Haferkamp MR, MacNeil MD, Grings EE. Predicting Nitrogen content in the Northern Mixed-Grass Prairie. Rangeland Ecology & Management 2005; 58(2): 155–160.

95. Molau U, Nordenhall U, Eriksen B. Onset of flowering and climate variability in an alpine landscape: a 10-year study from Swedish Lapland. American Journal of Botany 2005; 92(3): 422–431. doi: 10.3732/ajb.92.3.422 PMID: 21652418

96. Afonso I. Síntesis del Medio. In: Afonso I et al. editors. El lagópodo alpino (Lagopus muta pyrenaica) en el Parque Nacional de Aigüestortes i Estany de Sant Maurici. Madrid: Organismo Autónomo de Parques Nacionales. Ministerio de Medio Ambiente; 2006. p. 11–15.

97. Pendlebury CJ, MacLeod MG, Bryant DM. Variation in temperature increases the cost of living in birds. Journal Experimental Biology 2004; 207(12): 2065–2070.

98. Dawson A. Control of the annual cycle in birds: Endocrine constraints and plasticity in response to ecological variability. Philosophical Transactions of the Royal Society B: Biological Sciences 2008; 363(1497): 1621–1633. PMID: 18048294