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Authors: Shrestha, Bharat Babu, and Jha, Pramod Kumar

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Life History and Population Status of the Endemic Himalayan *Aconitum naviculare*

Bharat Babu Shrestha* and Pramod Kumar Jha
** Corresponding author: bhabashre@yahoo.com*
Central Department of Botany, Tribhuvan University, Kirtipur, PO Box 5275, Kathmandu, Nepal

Sustainable management of rare medicinal plants is becoming a major conservation issue in the Himalaya, and the need to consider population status and life history strategies for sustainable management of these plants has been expressed. We sampled Aconitum naviculare*, an endemic Himalayan medicinal plant, to study life history strategies and abundance across 6 sampling sites in Manang Valley, central Nepal. The relationship among environmental variables, life history traits, and abundance was analyzed by using regressions. Seed germination, growth characters, reproductive output, and population density varied significantly across the sites; most of these were lowest at Khangsar, a site located at the highest elevation. Growth characters were largely governed by life forms of associated species. Plant height and petiole length were higher in individuals growing within juniper scrub, whereas tuber mass, flowers/plant, and seed/follicle were higher in open areas. Reproductive outputs were determined by the growth vigor of individual plants and associated species, and not by population size. Stem mass and above-ground biomass declined with elevation, whereas density increased with relative radiation index. Soil attributes could not explain the variation in life history traits and abundance. Associated shrubs reduced the pressure of human collection and destructive effects of animal grazing. In conclusion, a plant's life history and responses to different natural environments can explain the variation in abundance of rare species such as* A. naviculare*.*

Keywords: *Alpine; medicinal plants; conservation; environmental variables; facilitation; germination; growth variation; Nepal.*

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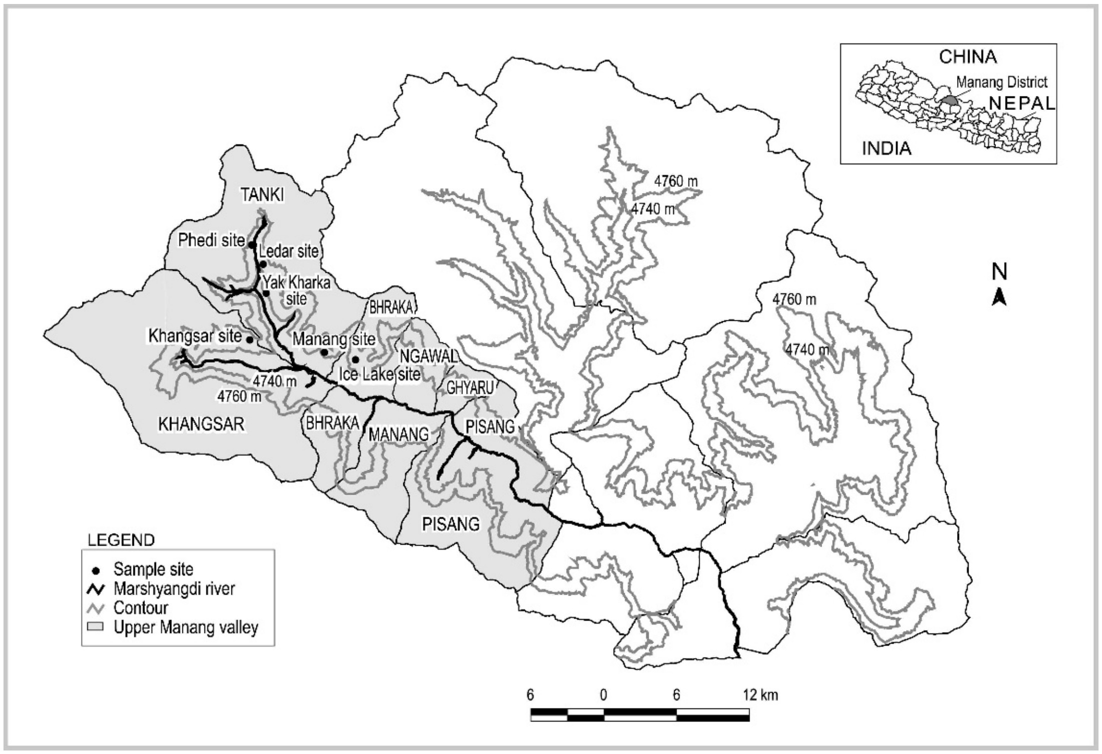
Introduction

Given their minimal access to modern health care systems, people in the high mountains of Nepal have traditionally used >50% of locally available plant species for health care (Kunwar and Bussmann 2008). In addition, trade in medicinal plants collected from the wild has been an integral part of the livelihood of mountain people (Olsen and Larsen 2003), whereas market-driven collection of medicinal plants from the wild is largely unsustainable (Ghimire et al 2005). Although some medicinal plants in the Himalaya have been threatened because of unsustainable harvest from the wild for trade (Kala 2000; Ghimire et al 2005), others are naturally rare, and populations have been declining even when they have been used only for traditional health care (Kala 2005). Understanding life history and demography is essential for developing a scientific management plan (Murray et al 2002). However, except for a few high-profile taxa, population details are largely lacking for most Himalayan medicinal plants (Dhar et al 2000), and life history strategies have been studied only for a few species of Nepal Himalaya (Ghimire et al 2005).

Aconitum spp are among the 10 most traded medicinal plants from the mountain regions of Nepal (Olsen and Larsen 2003). Most of the medicinally important *Aconitum* spp have been categorized as critically endangered in their natural habitats (CAMP 1998), but availability and life history strategies of this taxon have not been studied in Nepal Himalaya. *Aconitum naviculare*, endemic to Himalaya (Stainton 1997), is found from Bhutan to Nepal, including southern Tibet (Grierson and Long 1984; Liangqian and Kodata 2001; Ohba et al 2008). This plant species has been used in Tibetan medicine against colds, fever, and headache (Lama et al 2001; Bhattarai et al 2006; Shrestha et al 2007b); it is the most preferred species in ethnomedicine in Manang, a trans-Himalayan dry valley in central Nepal (Shrestha et al 2007b). Any decline in the abundance of this species could have adverse effects on the local health care system in Manang, where people have little access to modern medicine.

In this article, we discuss germination, growth characters, reproductive output, and the plant's adaptation to alpine environments to explain the distribution and abundance of this species in Manang Valley. The specific objectives of the study were (1) to understand variation in life history characters of *A. naviculare* among the populations, (2) to understand adaptation of this species to alpine environments, and (3) to explain the abundance of the species based on the life history characters and anthropozoogenic pressure.

FIGURE 1 Map of Manang district showing the sampling sites. (Map by Bharat Babu Shrestha and Prem Sagar Chapagain)



Material and methods

Study site

The study area (28°41″–28°46″N, 83°57″–84°04″E, elevation 4050–4650 m) lies in the upper part of Manang district, central Nepal, which has a glacially formed U-shaped valley (3000–3400 m), traversed by the Marsyangdi River, and surrounded by high mountains (>6000 m) (Figure 1). The valley lies in a rain shadow area of the trans-Annapurna region, with a mean annual precipitation of 444 mm and temperature of 6.2°C (measured at 3420 m, Miehe et al 2001). The area is covered by snow from November to March. Forest is mostly confined to the moist north-facing slope of Annapurna and the valley floor. The lower belt of the north-facing slope (3000–3500 m) has *Pinus wallichiana* and *Juniperus indica* forests, whereas the upper belt (3500–4200 m) has *Abies spectabilis* and *Betula utilis* forests (4200 m) (Shrestha et al 2007a). On the southern slope, there is *Juniperus indica* forest up to 3800 m, whereas the alpine scrub has junipers (*Juniperus indica*, *Juniperus recurva*, *Juniperus squamata*), *Rhododendron lepidotum*, *Rosa* spp, *Berberis* spp, *Caragana gerardiana*, *Ephedra gerardiana*, *Cotoneaster microphyllus*, etc.

The sampling sites represented at least three fourths of the total area where *A. naviculare* occurred in the study area (Table 1). There were a few sites that were not easily accessible. The Ice Lake area, Khangsar, and Manang hill are nearer to the settlements and experience greater pressure from livestock grazing than the other 3 sites. There were no permanent settlement near Yak Kharka, Ledtar, and Phedi, but a few hotels for tourists were present.

Species studied

A. naviculare (Brühl) Stapf. (vernacular name: *ponkar/bongkar*; Fam. Ranunculaceae; Figure 2A–E) is a perennial herb with tuberous white roots; stems ascending, 10–30 cm; leaves mostly basal, palmately divided into 2–5 segments; flowers in loose raceme; sepals tinged dull reddish purple-violet blue, with dark purple veinlets, lower surface pubescent, persistent in fruits, helmet navicular; petals clawed; fruit of 5 follicles, sparsely pubescent (Ohba et al 2008).

Sampling procedure

We sampled 240 quadrats (1 m × 1 m) lying within 30 large plots (10 m × 10 m) during September 2005. Each large plot was divided into 4 quarters (5 m × 5 m), and 2 quadrats were studied in each quarter. In each quadrat, individuals of *A. naviculare* were counted to determine density (pl/m^2). Major associated shrub species were recorded. Because of patchy distribution of *A. naviculare*, sampling plots were subjectively located at localities where the species was relatively common and covered an area >100 m^2. Thus, calculated density should be considered as the maximum density in its habitat. To determine above-ground biomass, all the individuals of *A.*

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TABLE 1 Some features of the sampling sites.[a)]

Sites	WD	Elevation (m)	Slope (°)	Aspect (°)	RRI	Associated shrub species
Ice Lake	3	4400–4550	32 ± 3	193 ± 20 SW	0.980 ± 0.015	*Caragana gerardiana* Royle
Manang	2	4150–4450	30 ± 8	237 ± 37 SW	0.859 ± 0.107	*Spiraea arcuata* Hook. f., *Rosa* spp, *Berberis* spp, *Ephedra gerardiana* Wall. ex. Stapf
Khangsar	2	4400–4650	29 ± 7	208 ± 50 SW	0.908 ± 0.149	*Juniperus squamata* Buch. – Ham. ex. D.Don, *Cotoneaster microphyllus* Wallich ex. Lindley
Yak Kharka	4	4090–4270	36 ± 1	239 ± 21 SW	0.847 ± 0.077	*Spiraea arcuata* Hook. f., *Berberis* spp, *Cotoneaster microphyllus* Wallich ex. Lindley
Ledtar	5	4200–4500	28 ± 5	240 ± 24 SW	0.873 ± 0.093	*Juniperus squamata* Buch. – Ham. ex. D.Don, *Spiraea arcuata* Hook. f., *Berberis* spp
Thorong Phedi	6	4350–4450	38 ± 7	108 ± 13 SE	0.778 ± 0.073	*Spiraea arcuata* Hook. f., *Berberis* spp
Mean*/ range**		4090–4650**	30 ± 6*	227 ± 42 SW*	0.870 ± 0.098*	–

[a)] WD, walking distance in hours from traditional settlements; RRI, relative radiation index.

naviculare (excluding seedlings and saplings) were collected from the single most densely populated quadrat within the large plot. Of 30 large plots, we did not sample 5 plots (2 at Manang hill and 3 at Khangsar) for above-ground biomass because of rarity of the plant at the sampling sites.

The single most vigorous individual was completely uprooted from each quarter to measure tuber and stem mass. About 100 g of soil was collected from the rooting depth (5–10 cm) of the collected plant. The plant and soil samples were air-dried under shade. At each site, the area of occupancy of *A. naviculare* was visually estimated. Because of unequal population size, the number of plots studied was not equal; 3 plots were sampled in the Ice Lake area, 5 each at Manang and Khangsar hills, and 17 at Ledtar. We did not sample the Yak Kharka and Thorong Phedi areas for density and above-ground biomass during the first visit because there had been heavy collection of this plant before we reached the area. During the second visit in October 2006, we sampled individual plants again to measure growth characters (tuber and stem mass, plant height, petiole length) and reproductive outputs (number of flowers borne by each individual plant (flowers/plant) as well as number of seeds in each follicle (seeds/follicle). Mature seeds were collected from each sampling site to determine seed mass and germination behavior.

Laboratory analysis

Air-dried plant materials were oven dried (60°C for 72 hours), and the biomass was measured to 0.001 g. Soil samples were air-dried in shade under laboratory conditions, and the dried soil was passed through a fine sieve (0.5 mm). Soil organic carbon (OC; Walkley and Black method) and total nitrogen (N; micro-Kjeldahl method) were determined by following the methods described by Gupta (2000).

Germination experiment: The seeds were stored at 4°C before germination. Germination experiments were carried out 3 times: after 10, 15, and 20 weeks of seed collection. Each time, 100 seeds were selected from each sampling site and divided randomly into 5 groups of 20

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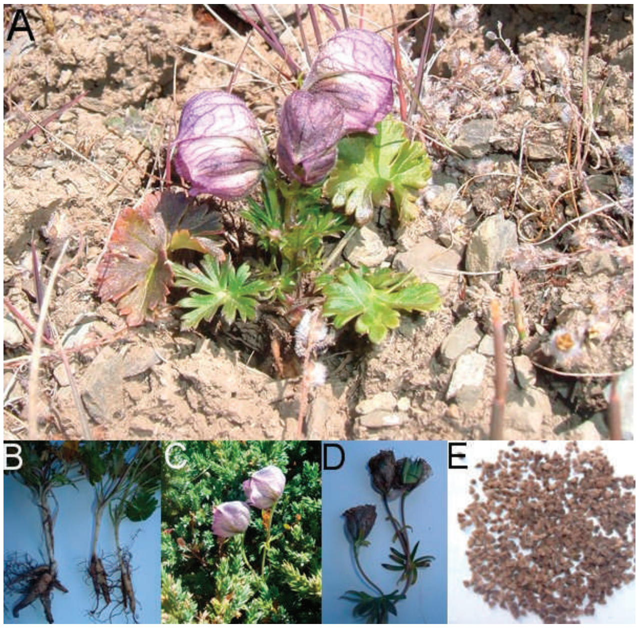
FIGURE 2 *A. naviculare*. (A) Flowering individual; (B) underground tuberous root of previous and current years; (C) flower above the canopy of juniper; (D) fruits covered by persistent sepals; (E) seeds. (Photos by Bharat Babu Shrestha)



seeds each. Because of the large population, 200 seeds were taken from Ledtar for germination. After the mass of each group was taken, the seeds were placed in petri dishes (diameter 10 cm) with double-layered blotting papers saturated with distilled water. Seeds were examined every 4 days over 32 days to analyze germination. The internal environment of petri dishes was kept saturated with moisture by frequently adding distilled water. The experiment was done under laboratory conditions with natural photoperiod (11–12 hours of light/12–13 hours of dark), mid-day temperature from 16 to 22°C, and relative humidity of 36 to 50%. Because most of the alpine plants need light for seed germination (Bliss 1958), the Petri dishes were placed near a window; thus, they received direct sunlight for about 4 hours a day.

Numerical analysis

From the values of aspect (Ω), slope (β), and latitude (Φ), the relative radiation index (RRI; Ôke 1987) of each large plot was calculated by using the following formula:

$$\mathrm{RRI}=\{\mathrm{Cos}(180-\Omega)\ \mathrm{Sin}\beta\ \mathrm{Sin}\Phi\}+\mathrm{Cos}\beta\ \mathrm{Cos}\Phi$$

Population size was estimated as the product of estimated area of occupancy and mean density for each sampling site.

Before comparison of mean of population density, growth characters, and reproductive output among sampling sites, the data were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Levene test). The density was normalized when square root transformed, but variance remained unequal. Therefore, the mean across the sites was compared by analysis of variance, and the pairs of sites were compared by multiple comparison by using the Dunnett *C*-test. Log and square root transformations did not improve normality, and homogeneity of variance in growth and reproductive characters; for these data nonparametric tests (Kruskal–Wallis and Mann–Whitney *U*-tests) were used for comparison of means across the sites. Similarly, morphological characters and reproductive output of individual plants occurring in juniper, other shrubs, and open areas were also compared by Kruskal–Wallis and Mann–Whitney *U*-tests.

Regression analyses were done to understand the relationship among environmental variables (elevation, RRI, soil OC, and soil N), abundance of *A. naviculare* (density and biomass), growth (plant height, petiole length, stem mass, and tuber mass), and reproductive characters (flowers/plant, seeds/follicle, and mass per 100 seeds). The data were checked for normality and were log transformed if necessary. As suggested by Sokal and Rohlf (1995), during log transformation, if the original values were <1, then each of these values was multiplied by 100 to avoid negative values after transformation. All statistical analyses were done by using the Statistical Package for Social Sciences (SPSS 2002, version 11.5).

Results

Life history

A. naviculare is a perennial alpine herb with biennial tuberous roots. *A. naviculare* growing in open areas were much more branched than the individuals growing with junipers and other shrubs. Aerial parts of the plant died at the end of every growing season but it perennated as a tuberous root with a fully developed apical bud buried in the soil at a depth of 2–3 cm (Figure 2B). Thus, *A. naviculare* is a cryptophyte, according to Raunkiaer's classification of life forms. The mother plant produced a fusiform, tuberous white root with apical bud in the first year. After winter perennation, aerial parts developed from the apical bud early in the growing season after snow melt (May–June). The plant flowered from late September to early October. By the time fruit was mature, the development of the new tuber was completed and the old one dried up. Thus, the plant was perennial with biennial tuberous root and annual aerial parts. In general, a single unbranched tuber is produced by each individual plant. There is the presence of meristematic tissue joining tuber and stem; hence, these 2 parts can easily be detached from each other. When aerial parts were pulled during collection, the tuberous root remained in the soil.

A single plant produced 1–17 flowers (Table 2). With the development of fruits, the sepals dried up, becoming papery but remained persistent and covered the developing fruits until dehiscence. Seeds/follicle varied from 4 to 16 (mean, 11). A single plant produced an average of 165 seeds ($3 \times 5 \times 11 = 165$). Seeds were small (mass, 47

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TABLE 2 Mean values of various morphological characters of *A. naviculare* for entire range of sampling sites.[a)]

Morphological traits	Sample size[b)]	Range	Mean ± SD	CV[c)]
Plant height (cm)	373	4–45	19 ± 8	42
Petiole length (cm)	358	2.5–21.0	7.12 ± 3.2	50
Stem mass (g/plant)	297	0.015–1.528	0.164 ± 0.188	115
Tuber mass (g/tuber)	297	0.031–1.221	0.27 ± 0.17	63
No. flowers/plant	395	1–17	3 ± 2.5	83
No. seeds/follicle	262	4–16	11 ± 2.1	19
Seed size (mg/100 seeds)	52	27–75	47 ± 11	23

a) SD, standard deviation; CV, coefficient of variance.

b) Sample size refers to the total number of plants or plant parts measured for the individual trait.

c) CV is the SD expressed as the percentage of mean.

± 11 mg/100 seeds) and had no active mechanism of dispersal. At the time of seed dispersal, temperature and soil moisture were low, which prevented immediate germination. Germination occurred in the next growing season when the area became snow free in June.

Variation in growth and reproduction

Growth and reproductive characters differed significantly ($P < 0.001$) among the sampling sites (Figure 3). Most of the characters measured were lowest at Khangsar site. The greatest difference among the sites was observed in stem mass, for which the largest value (Manang hill) was 5 times larger than the smallest one (Khangsar and Yak Kharka). *A. naviculare* was generally found in association with thorny shrub species (Table 1). Quantitative measurement at Ledtar showed that life forms of associated species (such as junipers, other shrubs, and herbs between patches of shrubs) had a significant effect on the height of *A. naviculare*, petiole length, and flowers/plant, and a marginal effect on tuber mass and seeds/follicle (Table 3). Average stem mass/plant was not affected by life forms of the associated species. Plant height and petiole length of *A. naviculare* were the highest within juniper patches, whereas tuber mass/plant, flowers/plant, and seeds/follicle were the highest in individuals growing in open areas.

Among the growth and reproductive characters, petiole length and seed mass increased linearly with plant height, but the tuber mass and flowers/plant showed nearly unimodal relations (Figure 4). The relationship was positive in 6 other pairs of characters (Figure 5); it was linear in 3 pairs but, in 3 other pairs, it deviated slightly from linear relationships.

Stem mass/plant decreased with an increase in elevation (linear regression, $P = 0.001$, $R^2 = 0.37$). Plant height, petiole length, tuber mass, flowers/plant, and seeds/follicle did not vary significantly with elevation. We were not able to find any significant relation between growth and reproductive characters of *A. naviculare*, and soil OC and N.

Seed germination

Germination after 10 and 15 weeks of seed collection was 49% (± 35 SD, $n = 35$) and 43% (± 38 SD, $n = 35$), respectively. Most of the seeds germinated between 8 and

TABLE 3 Growth characters and reproductive output of *A. naviculare* in microhabitats at Ledtar.[a)]

Attributes	Juniper	Other shrubs	Open	Test statistics[b)]		
				N	*P* value	χ^2
Plant height (cm)	29.5^c (9.25)	19.5^b (9.75)	18^a (9.5)	186	<0.001	42.7
Petiole length (cm)	13.5^b (3.25)	7.0^a (3.5)	6.5^a (2.0)	188	<0.001	38.3
Stem mass/plant (g)	0.090^a (0.06)	0.096^a (0.112)	0.116^a (0.142)	187	0.312	2.3
Tuber mass/plant (g)	0.194^a (0.168)	0.225^b (0.176)	0.249^b (0.254)	156	0.021	7.7
No. flowers/plant	2.0^a (1.0)	3.0^b (3.0)	4.0^c (5.0)	191	<0.001	18.1
No. seeds/follicle	10.25^a (4.33)	11.14^{ab} (2.58)	11.45^b (2.05)	152	0.039	6.5

a) Main entries are medians, whereas the values in parentheses are interquartile range; values in each row with the same letter in the superscript are not significantly different at $P < 0.05$.

b) Based on Kruskal–Wallis test; *N*, sample size (total number of plants or plant parts measured for each trait).

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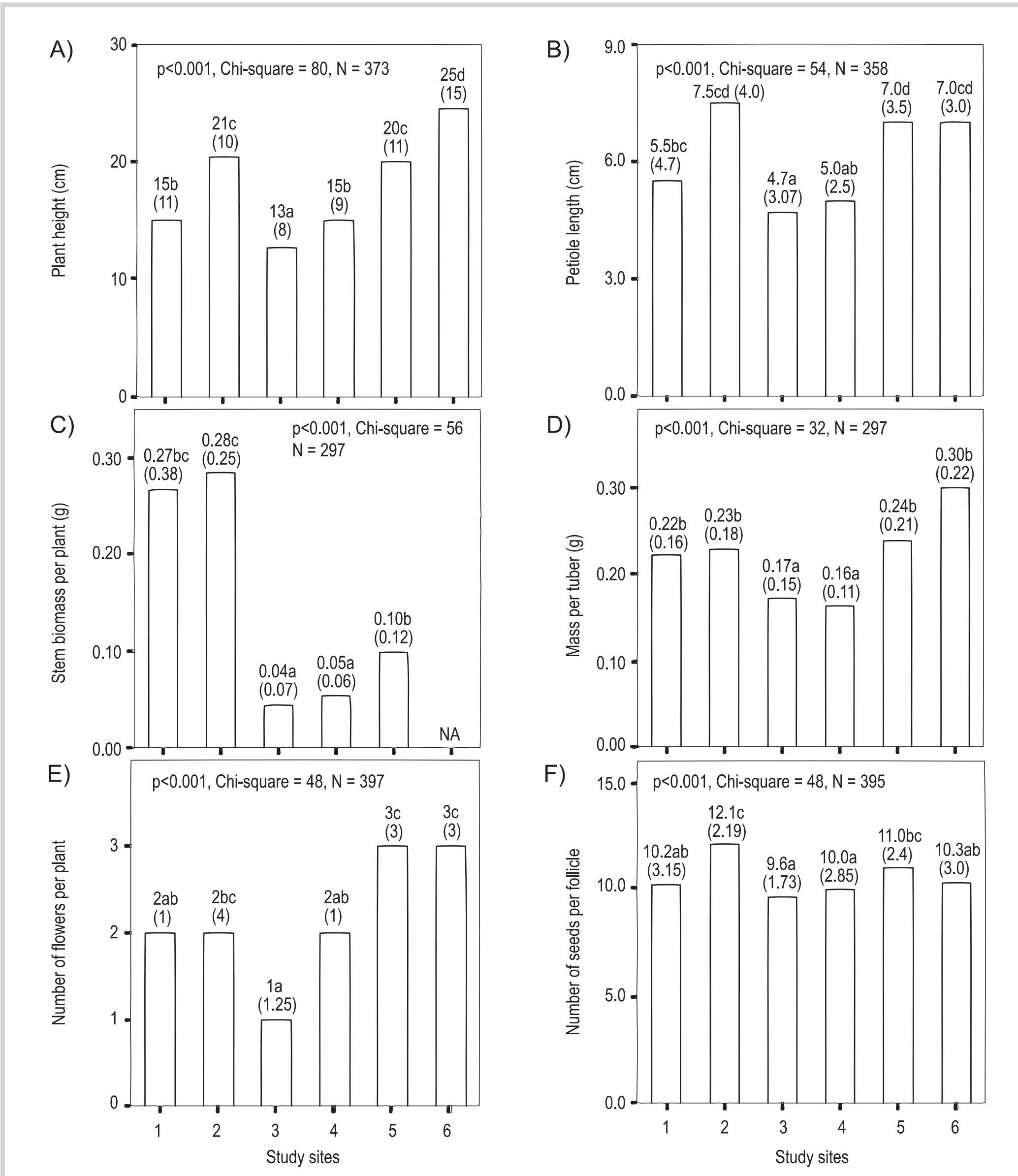
FIGURE 3 (A–F) Morphological characters and reproductive output of *A. naviculare* (1, Ice lake area; 2, Manang hill; 3, Khangsar; 4, Yak Kharka; 5, Ledtar; 6, Phedi). Main entries above bars are medians and values inside parentheses are interquartile range; values with same letter are not different significantly at $P < 0.05$. N = total sample size.

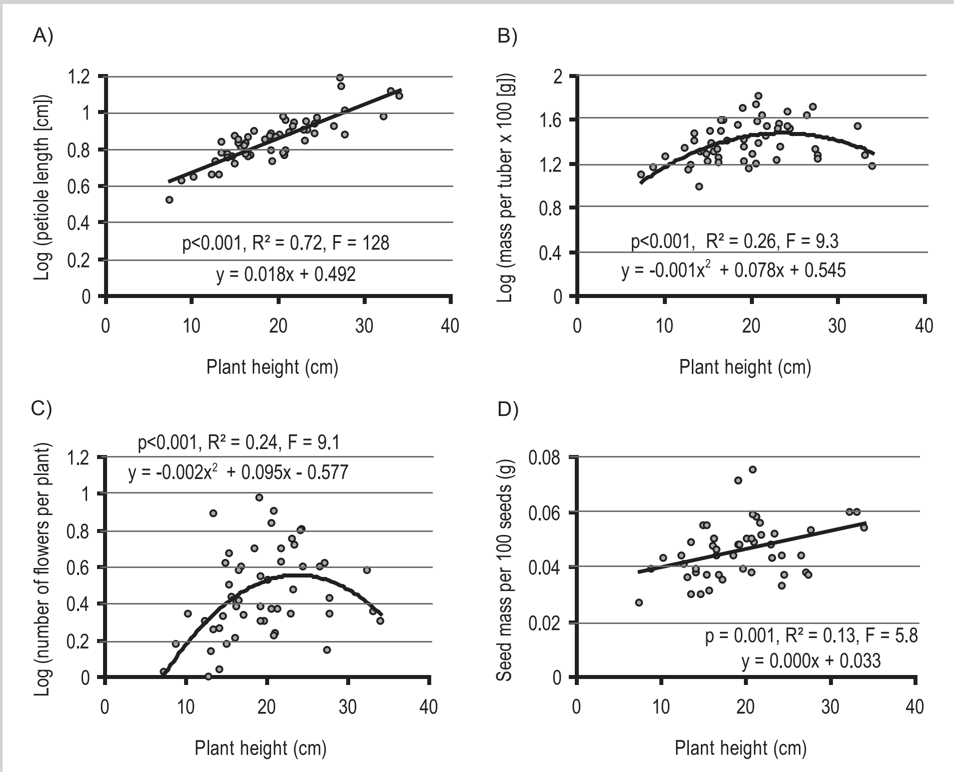


16 days after sowing, and germination of new seeds continued until 24 days (Figure 6A). Seed germination varied across the sampling sites from 17 to 97% after 10 weeks and 6 to 99% after 15 weeks of collection (Figure 6B). After 20 weeks, average seed germination declined to 7% with no germination (Khangsar site) to 24% (Yak Kharka). Germination was epigeal (*sensu* Baskin and Baskin 1998).

Population status and conservation

The total area of occupancy of *A. naviculare* at sampling sites in Manang Valley was estimated to be 40 ha (Table 4). Because the present sampling sites represented nearly three fourths of the total area of occupancy of this plant in Manang Valley, the estimated total area of occupancy could be about 50 ha. In Manang hill, Khangsar, and Phedi, it was found in small and fragmented patches, whereas at the Ice Lake area, it was found only in a single patch. In Ledtar, *A. naviculare* was found in a large area where the population density, estimated population size, and above-ground biomass were also the highest. The density of *A. naviculare* was the highest at the Ice Lake area and the lowest at Manang hill, the latter being at the nearest distance from the densely populated Manang village (Table 4). Average density increased with increasing RRI (Figure 7A). No relationship was detected between population density and

FIGURE 4 (A–D) Variation of growth characters and reproductive output of *A. naviculare* with height of the plant. The fitted lines, equations, and other test statistics were obtained by linear or quadratic regression analyses.



any of the soil variables measured. Above-ground biomass of *A. naviculare* declined with elevation (Figure 7B).

Discussion

Variation in growth and reproductive characters across the sites

Growth and reproductive characters varied significantly across the sites, which could be attributed to large microhabitat differences in alpine regions (Bliss 1956). The lowest value of most of the characters measured (Figure 3), together with the lowest seed mass (Figure 6B) and the smallest population size (Table 4) at Khangsar, could be an indication of harsh environmental conditions, including low temperature (because of upper elevation limit of *A. naviculare* in Manang) and high anthropogenic pressure (grazing and collection). Plant height and petiole length were determined mainly by height and canopy structure of associated species. Lower height and petiole length at Khangsar, Ice Lake area, and Yak Kharka were caused by the dominance of dwarf shrub species, such as *C. microphyllus* and *C. gerardiana* as associated species (Table 1). Highest flowers/plant at Ledtar (Figure 3) were caused by dominance of individuals in open areas in the population of *A. naviculare* because individuals growing in open areas produced more flowers/plant than individuals growing within patches of shrubs (Table 3).

Seeds/follicle was the highest at Manang hill (Figure 3) where density of *A. naviculare* was the lowest (Table 4). Bosch and Waser (1999) reported a positive correlation between density and seed set in *A. columbianum* and *Delphinium nuttallianum*, and the decline in seed set in sparse populations was attributed to receipt of inbreed pollens or harsh environmental conditions. But at Manang hill, despite low population density, growth vigor was high as evident from high biomass (Figure 3, Table 4). This indicates favorable physical environmental conditions, and the low density could be attributed to high anthropogenic pressure. Thus, it appears that seed set was determined by growth vigor of individual plants, and population density was not low enough to prevent effective pollination, fertilization, and seed set at Manang hill.

Variation in growth and reproductive output with life forms of associated species

Variation in growth characters and reproductive outputs among the individuals of *A. naviculare* growing in juniper, other shrubs, and open areas (Table 3) represent growth responses to resource availability and can be considered as phenotypic plasticity. The variation in plant height and petiole length was an adaptive response to light. Plasticity of shoot helps to place the leaves in areas of high light (de Kroon and Hutchings 1995). Herbaceous dicot plants

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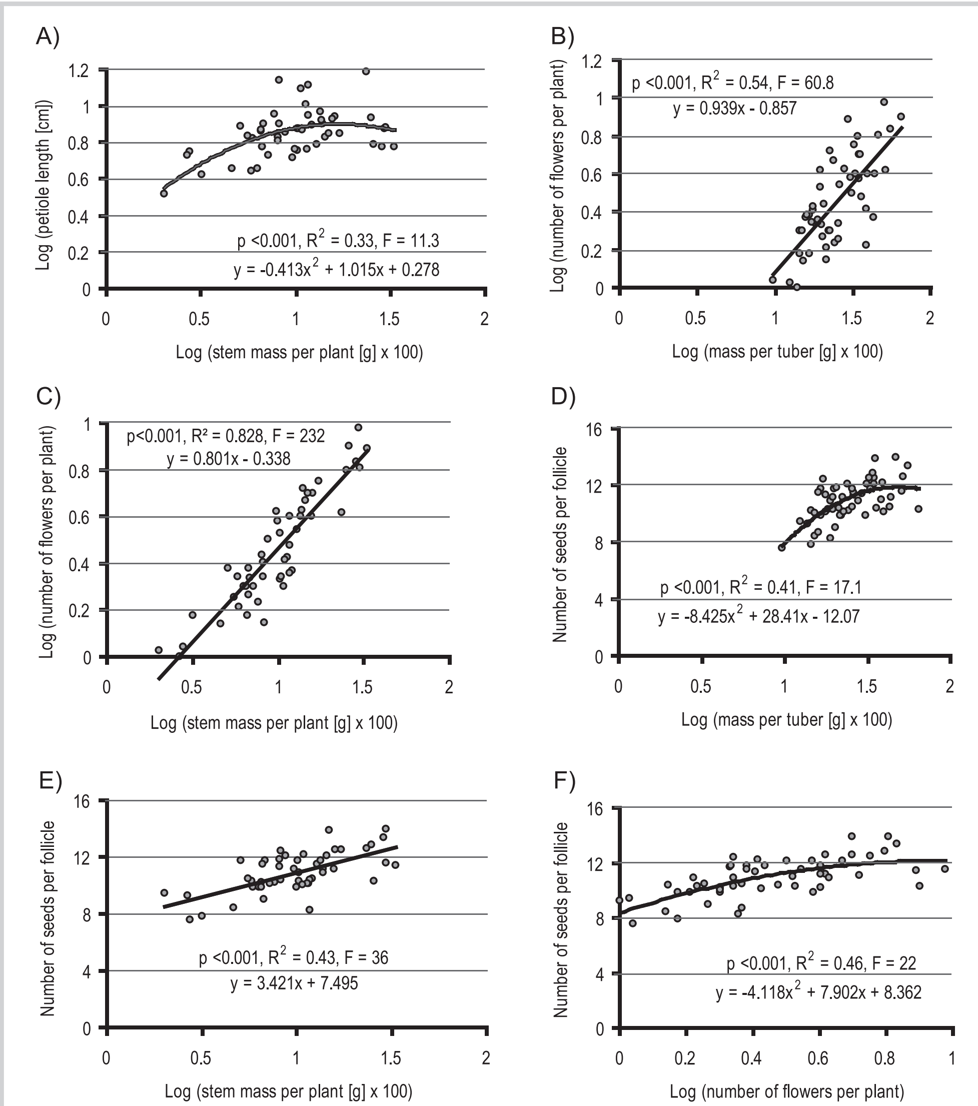
FIGURE 5 (A–F) Relationship among growth characters and reproductive output of *A. naviculare*. The fitted lines, equations, and other test statistics were obtained by linear or quadratic regression analyses.



growing in open habitats often respond to shading by elongating internodes and petiole length (Huber 1996), and improved access to light (Falster and Westoby 2003). Because of the long stem and petiole of *A. naviculare* growing with shrubs, the leaf lamina were exposed to full sunlight and flowers to pollinators. This variation allowed *A. naviculare* to grow and reproduce in open areas as well as with dense shrubs.

Stem mass of *A. naviculare* did not vary significantly among the 3 habitats (Table 3). There appeared to be a trade-off between height and thickness of stem. In shaded conditions, the stems are thinner and longer than in full sunlight (Huber 1996). The lowest tuber biomass of this plant growing within juniper scrubs could be the result of the "inevitable effect of resource limits" (*sensu* Sultan 2003) rather than adaptive response. To position the leaves and flower in the upper layer of canopy, relative allocation of biomass to stem increased in shade conditions (Huber 1996), which could have reduced tuber biomass of *A. naviuclare* growing in juniper scrubs. Relatively low stem and tuber biomass of *A. naviculare* growing in juniper scrub was also indicative of a stressful environment, which was expressed as the lowest flowers/plant of *A. naviculare* growing in juniper scrub (Table 3).

Adaptation to the alpine environment

A. naviculare showed a number of adaptive features to alpine environments with strong wind. The flower did not open completely and was globular, with a small opening on the lateral side. A globular shape has been suggested as

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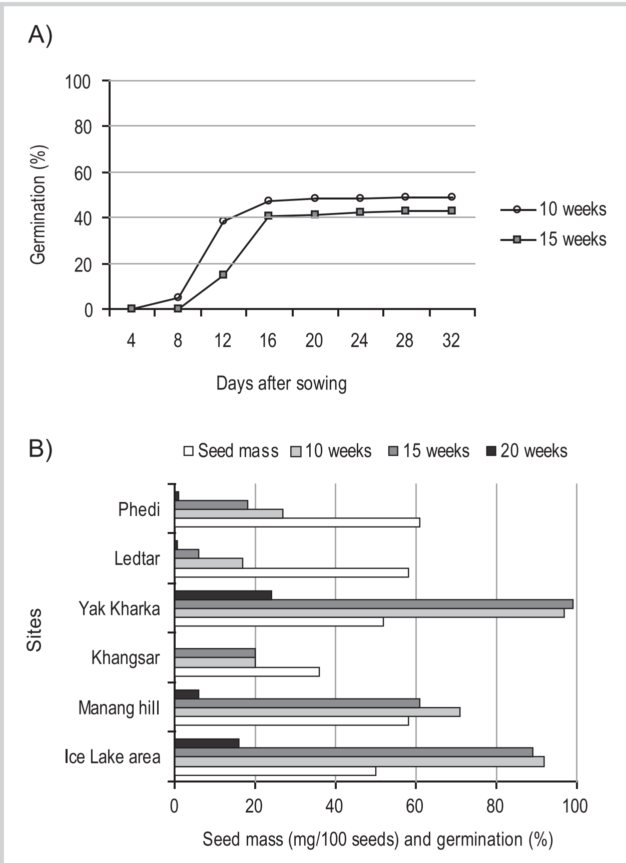
FIGURE 6 Seed germination pattern of *A. naviculare*. (A) Mean seed germination after 10 and 15 weeks of collection; (B) seed mass and germination. Seed mass is the mean value of 3 experiments.



an adaptation to minimize mechanical damage by strong wind in alpine environments (Garg and Husain 2003). The temperature inside the blue flower is higher than in flowers of other colors (Bliss 1962). This could, to some extent, compensate the problem of the short time span with favorable air temperature, enabling late flowering plants to develop seeds and fruits completely (Molau 1993). Persistent sepals could help to maintain relatively high temperature inside for rapid development of fruits and protect developing fruits from the desiccating effects of strong wind and solar radiation. Burial of perennating bud and meristem below the soil surface may prevent damage from low temperature (Körner 2003). *A. naviculare* also used food reserves from the previous year for early growth during the growing season. All these characters have been considered important adaptive features of plants to alpine environments (Bliss 1962; Körner 2003).

Seed size and germination

Seed size of *A. naviculare* (mean, 47 mg/100 seeds = 470 μg/seed) was very close to mean seed size (467 μg/seed) of alpine forbs from various latitudes compiled by Körner (2003). There was wide variation in germination of seed across the sampling sites. However, we could not detect any consistent pattern in the relationship between population size and seed germinability of *A. naviculare*. Some earlier studies also failed to detect the effect of population size on germination (eg Oostermeijer et al 1994; Morgan 1999).

Seeds collected from Khangsar had the smallest size and lost germination after 20 weeks of storage (Figure 6B), which could be an indication of harsh environmental conditions at that site. Low seed germination at the Khangsar site, at the upper elevation limit, could be attributed to low temperature. In late flowering alpine plants like *A. naviculare*, low temperature may result in underdeveloped seeds with low seed mass, poor germination, and short viability (Molau 1993). Relatively high temperature at the Ice Lake area because of the highest RRI among the sampling sites (Table 1) allowed complete seed and fruit development in a short period before the plant died. The habitat of *A. naviculare* at Yak Kharka represented the lower elevation limit (4090–4270 m) of distribution in upper Manang valley,

TABLE 4 Estimated area of occurrence, population density, estimated population size (number of individuals), and aboveground biomass of *A. naviculare* at the 6 sampling sites.[a)]

Sites	Area of occupancy (ha)	Population density (pl/m^2)[b)]	Estimated population size	Above-ground biomass (g/m^2)
Ice lake	2.0	$8.67^{c} \pm 3.37$	173,200	2.67 ± 0.24
Manang Hill	5.5	$4.08^{a} \pm 2.45$	223,850	5.02 ± 1.32
Khangsar	1.3	$6.43^{ab} \pm 6.35$	80,250	3.06 ± 1.4
Yak Kharka	7.3	na	na	na
Ledtar	21.0	$8.35^{bc} \pm 4.47$	1,751,400	5.73 ± 2.71
Phedi	3.0	na	na	na
Total*/mean**	40.1*	7.35 ± 4.75**	2,228,700*	4.99 ± 2.58**

[a)] na, data not available.

[b)] Values were square root transformed before performing ANOVA; values with same letter in superscript are not significantly different at $P < 0.05$.

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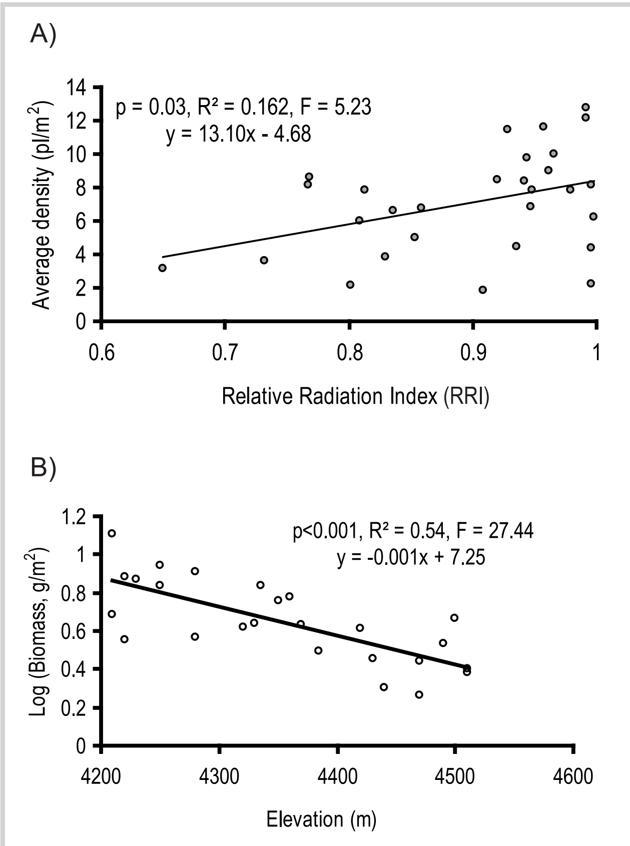
FIGURE 7 (A, B) Relationship between mean density of *A. naviculare* and relative radiation index (A), and biomass and elevation (B). Fitted line, equation, and other test statistics were based on linear regression analysis.



which could be relatively warm. Seed development could be complete there too, leading to the highest seed germination (approximately 98%) (Figure 6B).

Population status, harvesting pattern, and conservation prospects

Population density of *Aconitum* spp found in the Himalaya was relatively low (<1 to 8 pl/m^2) (Ghimire et al 1999; Kala 2000, 2005; Nautiyal et al 2002). The mean density (7.3 pl/m^2) of *A. naviculare* in the present study area was comparable with the mean density (6.7 pl/m^2) of *A. violaceum* in the Garhwal Himalaya, India (Nautiyal et al 2002). Among 3 *Aconitum* spp (*Aconitum balfourii*, *Aconitum heterophyllum*, and *Aconitum violaceum*) found in Garhwal Himalaya, the highest density was recorded for *A. violaceum*, which was attributed to habitat inaccessibility and less exploitation by humans (Nautiyal et al 2002). The smaller size of *Aconitum* spp found in the alpine zone could also have contributed to their higher density than other *Aconitum* spp found at lower elevation. The mean density of *A. naviculare* in Manang was relatively high in comparison to reported values for other *Aconitum* spp. However, the present data represented the maximum density of this species because the sampling plots were selected subjectively. By using a similar sampling strategy, Kala (2000) reported density up to 7.7 pl/m^2 for *A. violaceum*. *A. naviculare* was found only in small areas in 3 of the 6 sampling sites (Table 4), and the population was either much fragmented (Yak Kharka) or confined to a single patch (Ice Lake area).

Population density of *A. naviculre* increased with increasing RRI (Figure 7A); it was highest at Ice Lake area (Table 4) where RRI was the highest (Table 1). The highest density might also be because of the high seed germination at Ice Lake area (Figure 6B). Population density was the lowest in Manang hill (Table 4), which is close to the densely populated villages of Manang and Tanki Manang. The lowest density at Manang hill could be attributed to the high pressure of human collection and animal grazing. At Ledtar, *A. naviculare* was more commonly found in open areas than within patches of shrubs, which may indicate low animal damage. This was the least disturbed site because of its location far from traditional settlements. The great abundance (high density, above-ground biomass, and the largest population) of *A. naviculare* at Ledtar might be attributed to the lowest pressure of human collection and animal damage.

The destructive effect of cattle grazing, and collection of aerial parts by local people before seed set, appeared to be responsible for declining abundance of *A. naviculare* and the very low density at some sites. The natural habitats of *A. naviculare* are near to human settlements, with high pressure of livestock grazing (Shrestha and Jha 2009). Large animals with wide mouths often avoid thorny shrub species (Chandrashekhar et al 2007), which are the most common associates of *A. naviculare* (Table 1). The population density of this species was the highest at Ice Lake area (Table 4), but it was only found in a single patch among a thorny shrub *C. gerardiana*, which could protect *A. naviculare* from damage by livestock and collection by humans. The protective role of shrubs is often important for the persistence of rare species (Facelli and Temby 2002). Because *A. naviculare* was found only within alpine scrubs at the localities with high grazing pressure (Ice Lake area, Manang hill, Khangsar, Yak Kharka, and Phedi), the importance of protective roles, as hypothesized by Bertness and Callaway (1994), was high at these localities. The protective role of associated scrubs against animal damage and human collection is a kind of facilitation, as proposed by Callaway (2007), and it is important for the persistence of this species in the study area.

For sustainable management of alpine medicinal plants in their natural habitats, the harvesting pattern and life history characters should be considered together (Ghimire et al 2005). The entire plant is used in the case of alpine species of *Aconitum* (eg *A. violaceum*, *A. naviculare*, *Aconitum gammiei*) (Baral and Kurmi 2006). Although whole plant of *A. naviculare* is medicinally important, people in the study area have been collecting only aerial parts at the flowering stage (Shrestha et al 2007b). This method is less destructive in terms of parts removed, but collection of the plant at flowering stage did not allow regeneration by seeds. This might have a negative impact on increasing or maintaining population size, as has been also reported by Ghimire et al (1999). The negative impact could be more critical for areas with a small populations and/or low density, such as Ice Lake area, Manang hill, and Khangsar.

Conclusions

The growth characters and reproductive outputs of *A. naviculare* varied significantly across the sites; most of them were lowest at Khangsar, a site at the highest elevation, which indicates harsh environmental conditions for this plant at Manang. Variation in plant height, petiole length, and flowers/plant were largely governed by life forms of associated species. Reproductive outputs were determined by growth vigor of individual plants and associated species. Seed germination varied across the sites. Temperature and anthropozoogenic pressure appeared to be the most important environmental factors that determine distribution and abundance of *A. naviculare*. The presence of tuberous root with well-developed winter perennating bud, shoot, and leaf meristem buried inside soil, globular blue flower, and persistent sepals, and rapid germination of seed after moistening are likely adaptive features of this plant in alpine environments. Variation in plant height and petiole length enabled this plant to grow from open areas to dense juniper scrubs. Alpine scrub seems to have protected this plant from livestock damage and human collection.

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