POPULATION ECOLOGY AND HARVESTING SUSTAINABILITY OF *ACONITUM SPICATUM* AND *DACTYLORHIZA HATAGIREA* IN NEPALESE HIMALAYA

A THESIS SUBMITTED TO

CENTRAL DEPARTMENT OF BOTANY
INSTITUTE OF SCIENCE AND TECHNOLOGY
TRIBHUVAN UNIVERSITY
NEPAL

FOR THE AWARD OF
DOCTOR OF PHILOSOPHY

IN BOTANY

BY

DEEP JYOTI CHAPAGAIN

December 2021

POPULATION ECOLOGY AND HARVESTING SUSTAINABILITY OF *ACONITUM SPICATUM* AND *DACTYLORHIZA HATAGIREA* IN NEPALESE HIMALAYA

A THESIS SUBMITTED TO

CENTRAL DEPARTMENT OF BOTANY
INSTITUTE OF SCIENCE AND TECHNOLOGY
TRIBHUVAN UNIVERSITY
NEPAL

FOR THE AWARD OF
DOCTOR OF PHILOSOPHY
IN BOTANY

BY

DEEP JYOTI CHAPAGAIN

December 2021

DECLARATION

Thesis entitled "**Population Ecology and Harvesting Sustainability of *Aconitum spicatum* and *Dactylorhiza hatagirea* in Nepalese Himalaya**" which is being submitted to the Central Department of Botany, Institute of Science and Technology (IOST), Tribhuvan University, Nepal for the award of the degree of Doctor of Philosophy (Ph. D.), is a research work carried out by me under the supervision of Professor Dr. Suresh Kumar Ghimire, Central Department of Botany, Tribhuvan University and co-supervised by Professor Henrik Meilby, Department of Food and Resource Economics, University of Copenhagen and Associate Professor Dr. Chitra Bahadur Baniya, Central Department of Botany, Tribhuvan University.

This research is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

……………………………….

Deep Jyoti Chapagain

RECOMMENDATION

This is to recommend that **Ms. Deep Jyoti Chapagain** has carried out research entitled "**Population Ecology and Harvesting Sustainability of *Aconitum spicatum* and *Dactylorhiza hatagirea* in Nepalese Himalaya**" for the award of Doctor in Philosophy (Ph. D.) in **Botany** under our supervision. To our knowledge, this work has not been submitted for any other degree.

She has fulfilled all the requirements laid down by the Institute of Science and Technology (IOST), Tribhuvan University, Kirtipur for the submission of the thesis for the award of Ph. D. degree.

……………………………………

Dr. Suresh Kumar Ghimire
Supervisor
(Professor)
Central Department of Botany
Tribhuvan University
Kirtipur, Kathmandu, Nepal

……………………………………………

Dr. Henrik Meilby
Co-Supervisor
(**Professor)**
Department of Food and Resource Economics
University of Copenhagen, Denmark

………………………………………….

Dr. Chitra Bahadur Baniya
Co-Supervisor
(Associate Professor)
Central Department of Botany
Tribhuvan University
Kirtipur, Kathmandu, Nepal

December 2021

LETTER OF APPROVAL

Date: December 2021

On the recommendation of Prof. Dr. **Suresh Kumar Ghimire** (Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu)**,** Prof. Dr. **Henrik Mielby** (Department of Food and Resource Economics, University of Copenhagen, Denmark) and Associate Prof. Dr. **Chitra Bahadur Baniya** (Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu).), this Ph.D. thesis submitted by **Ms. Deep Jyoti Chapagain** entitled "**Population Ecology and Harvesting Sustainability of *Aconitum spicatum* and *Dactylorhiza hatagirea* in Nepalese Himalaya**" is forwarded by Central Department Research Committee (CDRC) to the Dean, IOST, T.U.

....
Sangeeta Rajbhandary
Professor
Head
Central Department of Botany
Tribhuvan University
Kirtipur, Kathmandu
Nepal

ACKNOWLEDGEMENTS

The successful outcome of this study was a result of support and cooperation of many individuals and institutions. It was a pleasure to work with many people from Nepal and Denmark during the PhD journey. Their generous support, advice and encouragement made this research possible. I acknowledge and appreciate the support provided by my supervisors and TGG-N team members for wonderful interaction and critical commemts during number of discussions. I am highly indebted to my supervisors Dr Suresh Kumar Ghimire, Dr Henrik Meilby and Dr Chitra Bahadur Baniya for sharing their vast knowledge on plant science. Simple, always enjoying in teaching, very friendly with the students and high dedication in the conservation of plant science are the synonyms of Dr. Suresh Kumar Ghimire. You have taught me a lot in the field as well as in CDB, TU. You were always motivating and showering me with ideas and suggestions. Thank you very much for being by my side during all the hard times motivating me. Your company during my field work was incredible and a life time memory. This thesis is the output of your continuous help and guidance on project design, data collection, data analysis and writing. I enjoyed thoroughly working with you and will remain indebted always. Dr. Henrik Meilby, you were very generous to accept me in guiding me throughout this research. I would never have imagined publishing such wonderful papers without you. Your patience and great attention to all the details even in the very basic thing is highly appreciable. You were always there when I stuck with analysis and I am very grateful for that. I had found a guardian in you many times. In addition, your advice on scientific research philosophy and art of scientific writing greatly contributed to the production of this thesis, several magnitudes beyond my expectations. The field work in Api- Nampa was actually a life time memory. A big thank you for your support, inspiration and motivation. Dr Chitara Bahadur Baniya, thank you for sharing your positive attitude and wholeheartedness with all the beings surrounding you, and for putting that enthusiasm to your work. Thank you for your continuous encouragement and support. Late Dr. Abohay Kumar Das, your technical support and inspiration was incredible. Thank you, Dr. Carsten Smith Hall, Dr. Marieve Pouliot and Dr. Thorsten Treue for your ideas and support.

I gratefully acknowledge the financial support from many sources. My Dissertation research was funded by the DANIDA and supported by Rufford Small Grant and

Hariyo Ban –WWF. I am very much thankful to the Departmentment of National Park and Wildlife conservation, Government of Nepal for granting me a permission to carry out the field work. I would like to thank ACAP, Dharapani Manang and Ghandruk Kaski for the logistic arrangement during my field work. Special thanks to Shanta Budha-Magar for your wonderful support in my PhD journey. Thank you, Amar Bista, Ramesh Bista, Singh Bahadur Ghale and Buddhi Bahadur Gurung for assisting me in my field work. Your support and guidance were superb. Thank you, Bandana Awastii, Sangram Karki, Neelam Pandey, Pratikshya Chalise and Yagya Raj Paneru for your assistance during the field work. Big thank you to Dr. Babu Ram Lamichhane, for creating the maps of the study area.

Likewise, I owe a very sincere thanks to Prof. Sangeeta Rajbhandary, Head of Central Department of Botany, TU, Prof. Ram Kailash Prasad Yadav, Professor Mohan Siwakoti, and Professor Emeritus Promod Kumar Jha, Former Departments Heads, CDB, TU for providing the necessary laboratory facilities and administrative support during the research work, and all the teaching and non-teaching staff for their support and help. Likewise, I am also grateful to my colleagues and friends who are directly or indirectly involved in this work.

I acknowledge the loving support of my family. The seed of inspiration to carry out the PhD planted inside me by my father late Dinesh Kumar Chapagain who motivated me to persue this challenging PhD and I would like to dedicate this PhD to him. My mother Uma Devi Chapagain, my siblings, Deepen and Deep Tara, also have enthusiastically encouraged and supported me throughout my PhD journey. I would like to thank my brothers in law for their support. I would like to thank my husband, Dr. Naresh Subedi, for his steadfast support with every aspect of this thesis. Huge huge thanks to my elder son Pranjal Subedi for his understanding and encouragement, your words had inspired me a lot and my little one Aashwath Subedi for allowing me to steal your time to write the thesis.

Deep Jyoti Chapagain
December, 2021

ABSTRACT

The harvesting of Medicinal and Aromatic Plants (MAPs) is a customary subsistence activity of forest-dependent communities across the world. However, the harvesting and marketing of MAPs has been highly commercialized in recent years, leading to the colossal pressures on the MAPs resource base. Many MAPs like *Aconitum spicatum* and *Dactylorhiza hatagirea* have been threatened in the Himalaya due to unsustainable harvest. At the same time, scientific knowledge on the demographic properties, magnitude of harvest impact, and the long-term viability of populations of these plant species is largely lacking thus limiting our ability to develop sustainable management strategies. Thus, this research was designed to understand the population ecology and harvesting sustainability of *A. spicatum* and *D. hatagirea* in the Annapurna Conservation Area and Manang in Central Nepal, and Darchula in Western Nepal.

Different levels of tuber harvesting (0%, 25%, 50% and 75%, where 0-25% represents low harvest intensity and 50-75% represents high harvest intensity) were applied directly by the locals for *A. spicatum* among three ecotonal populations (subalpine, lower alpine and alpine) along an elevation gradient (3000–4200 m a.s.l.) in Annapurna Conservation Area, North-Central Nepal. For *D. hatagirea*, two sites were selected: Manang, where harvesting of MAPs was locally regulated (protected); and Darchula, where harvesting was locally unregulated (unprotected) to see the effect of harvesting on its demographic parameters.

Population density and structure of *A. spicatum* was studied and variation in its life history traits among three populations along the elevation gradient in Central Nepal was assessed. The results showed that human disturbances and topographic factors greatly contributed to the variation in density and life history traits. The overall density ranged between 0.56 ± 0.09 (mean ± SE) and 2.48 ± 0.24 plants/m^2 with highest mean density in the lower alpine and lowest in the subalpine population. Harvesting, animal droppings and fire appeared to be the most important factors affecting density of *A. spicatum* at its different life stages.

The demographic data of *A. spicatum* included 4-year observations on growth, fecundity and survival of individuals classified by size. The population dynamics of *A. spicatum* in three different populations along the elevation gradient in Annapurna Base Camp Region, North-Central Nepal was studied. The population growth rate (λ) was >1 in low harvest intensity (0-25%) in all the populations in all the transitions (2015-2018) indicating that the harvesting of this plant at low harvest intensity is sustainable. In the high harvest intensities (50-75%), the lambda value was <1 in lower elevation populations indicating that these populations are sensitive to high harvest. The results showed that the extinction probability derived from the stochastic simulation (1000 runs) in 100 years has extremely low value for all the populations in both the intensities. Transient dynamics revealed that the low harvested alpine population shows gradual increase in its population reaching a size of approximately 4000 individuals during the projection period of 10 years. The deterministic projection revealed that the subalpine population is the most sensitive population. The populations of subalpine (both low harvest and high harvest) can reach the low harvest intensity of alpine in more than 20 years. The high harvest population of subalpine requires 22 years to reach its low harvest intensity in contrast to 7 years of lower alpine. The higher contribution of large size classes to λ indicates that the larger size classes are the critical stages in the life cycle of *A. spicatum* and conservation priority should focus on the protection of theses stages.

Population density, structure, and tuber biomass of *D. hatagirea* were studied and compared in seven different populations. Mean density of *D. hatagirea* was significantly higher in the locally protected (1.31 ± 0.17 plants/m^2) than in the unprotected (0.72 ± 0.06 plants/m^2) site. The protected site showed stable population density with high reproductive fitness and tuber biomass over the three-year period. The densities of different life-stages were highly sensitive to harvesting and livestock grazing. Significant interactions between site and harvesting and grazing indicated particularly strong negative effects of these disturbances on densities of juvenile and adult reproductive stages in the unprotected site.

A matrix population model was prepared to explore variation in demographic parameters in response to different level of disturbances. In case of *D. hatagirea*, demographic data included 2-year observations on growth, fecundity and survival of individuals classified by size. Transition probabilities for stage-based population projection matrices varied between populations and among years. The population growth rate (λ) was less than 1in unprotected (Darchula) and more than 1 in protected (Manang) site. The unprotected populations can resume its sustainable growth at 10-fold increase in its present fecundity while the present protected populations would allow approximately 10% of the populations to be harvested locally maintaining sustainability. The wetland population requires 25-fold increase in its present fecundity to maintain the stable size. Elasticity analysis shows that the juvenile and reproductive adult stages are the critical life stages of *D. hatagirea* in unprotected and protected site respectively and should be conserved with greater priority.

Semi-structured interviews were conducted with informants (n = 65 for *A. spicatum* and n = 186 for *D. hatagirea*) in the villages and at the ecological survey sites. The result shows that the population of *A. spicatum* is decreasing due to indiscriminate harvesting by the outsiders and the locals are highly responsible for the conservation of *A spicatum* of that area. In case of *D. hatagirea*, populations at the protected site are well maintained with almost constant density over years but populations at unprotected sites greatly fluctuated in density.

It is concluded that the long-term viability of *A. spicatum* and *D. hatagirea* largely depends on the magnitude of harvest impact and the level of protection provided directly by local people; and the opportunities of sustainable harvest vary among plant species with different strategies of growth and demographic properties, and these further relate with ecological conditions set by elevation gradient. Sustainability of harvest of *A. spicatum* and *D. hatagirea* can be achieved through integrating species-specific ecological studies, and knowledge and practices of key knowledge holders. The findings of this research suggest that strategies and actions towards reducing human induced disturbances like over-harvesting and grazing would greatly benefit population recovery and productivity of theses plant species.

Key words: Conservation, Extinction Probability, Elasticity, Medicinal and Aromatic Plants, Matrix modelling, Population dynamics, Zero Inflated Poisson

LIST OF ACRONYMS AND ABBREVIATIONS

°C	:	Degree Centigrade/Celsius
ACA	:	Annapurna Conservation Area
AIC	:	Akaike Information Criteria
ANCA	:	Api-Nampa Conservation Area
asl	:	Above Sea Level
CI	:	Confidence Interval
CITES	:	Convention on International Trade in Endangered Species of Wild Fauna and Flora
DNPWC	:	Department of National Parks and Wildlife Conservation
DPR	:	Department of Plant Resources
GPS	:	Global Positioning System
h	:	Hour
KATH	:	National Herbarium and Plant Laboratories
MAPs	:	Medicinal and Aromatic Plants
MuMIN	:	Multi-Model Inference
N	:	North
NTFPs	:	Non-Timber Forest Products
RRI	:	Relative Radiation Index
glmm TMB	:	Generalized Linear Mixed Models Using Template Model Builder
TUCH	:	Tribhuvan University Central Herbarium
TTC	:	2, 3, 5-Triphenyl Tetrazolium Chloride
UV	:	Ultra Violet
WHO	:	World Health Organization
ZIP	:	Zero-Inflated Poisson
λ	:	Lambda (population growth rate)

LIST OF TABLES

Page No.

LIST OF FIGURES

Page No.

TABLE OF CONTENTS

Page No.

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 1

1. INTRODUCTION

1.1 Background

The conservation of plant diversity has received considerably less attention compared to the conservation of animals, as plants lack the popular appeal of many animal groups (Goettsch *et al.*, 2015). As a result, plant conservation is greatly under-prioritized in comparison with animal conservation (Havens *et al.*, 2014). Global estimates on number of species suggest 8.7 million (±1.3 million SE), of which 2.2 million (±0.18 million SE) are marine; however, about 86% of the species on the Earth, and 91% in the ocean, still await description (Mora *et al.*, 2011). On average, over 2,000 plant species are being added to global databases each year as new to science (IPNI, 2022)., The total number of known vascular plant species is estimated to be between 340,000 and 390,000 (Nic Lughadha *et al.*, 2016; Govaerts *et al.*, 2021; Qian *et al.*, 2022). However, despite their importance for life on Earth, the status and trends of global plants relative to vertebrates are poorly understood and usually excluded from global biodiversity analyses (Betts *et al.*, 2017). Geographical, genetic, and characteristic information about plants, including their uses for humans and their function in ecosystems, is even more incomplete (Cornwell *et al.*, 2019). For example, detailed distribution data are lacking for about half of the world's plant species**,** in contrast to the distribution maps available for most vertebrates (Almond *et al.*, 2020).

Human footprints like habitat loss, overexploitation, climate change , pollution and invasion species have threatened global biodiversity towards extinction (IPBES, 2019). On average, about 25 % of species in the assessed flora and fauna groups are threatened, with about 1 million species already risk extinction, many within decades, unless steps are taken to reduce the intensity of drivers of biodiversity loss (IPBES, 2019). Without such actions, global species extinctions would be further accelerated, at least 10- to 100-fold higher than the average over the past 10 million years (IPBES, 2019). The rate of change in nature on a global scale over the past 50 years is unprecedented in human history. The direct drivers of change are changes in land and sea use, direct exploitation of biodiversity, climate change, pollution and invasion of alien species (IPBES, 2019). The amplifying effects of drivers can exacerbate negative impacts on nature, as seen in diverse ecosystems such as coral reefs, Arctic systems and Savannas.

At least 571 plant species have become extinct since 1750s (Humphreys *et al*., 2019), and 40% of plant species known today are at risk of extinction (Antonelli *et al*., 2020). Plant extinction risk is comparable to that of mammals and higher than that of birds (Almond *et al*., 2020).

Non timber forest products (NTFPs), including medicinal and aromatic plants (MAPs) have long been harvested worldwide. Even today, such products contribute substantially to the livelihoods and health care of millions of people (Belcher *et al*., 2005; Shackleton & Shackleton, 2006; Burgener & Walter 2007). MAPs are an important component of health care for most of the world's population and aaproximately 80% of the global population is still dependent on MAPs for primary health care (WHO, 2006; Robinson & Zhang, 2011). Additionally, MAPs are widely used in traditional veterinary medicine (Katerere & Luseba, 2010), in which the improvement of livestock health has obvious benefits for their owners' economic security. Moreover, millions of people make their living as traditional healers or collectors or vendors of MAPs. The harvest and trade of MAPs provide an important source of income to both rural and urban populations, as the global export value for herbal ingredients has reached at over USD 32.6 billion per year (Brinckmann, 2016).

The markets for natural products, especially those used in the commercial production of pharmaceuticals, health foods and nutritional products are growing. MAP products alone are estimated to command a market of more than US $80 billion (Ghimire *et al*., 2016). However, with the rise in demand for natural products, there is an increase in the extraction of environmental products by forest-dependent people, leading to a rise in biodiversity loss. Therefore, there is a need to develop a sustainable use and economic growth strategies that can secure an equitable living standard for forest-dependent people, while conserving ecosystem resources. The World Health Organization (WHO, 2002) estimates that 80% of the world's population relies on herbal medicines for primary healthcare needs. It has been estimated that about 150-300 species of NTFP, including MAP are harvested for commercial purposes in Nepal (Pyakurel *et al*., 2019)

The contributions of MAPs to both traditional and modern medicines have been well documented, but the knowledge about the impacts of MAP and NTFP harvesting on the ecology of plant populations is very scarce. In recent decades, commercialization has enhanced the exploitation of many NTFPs and MAPs (Upreti *et al.*, 2005), raising questions about their sustainability (Ticktin, 2004). A number of studies have shown that NTFP harvesting generally adversely affects ecological processes at individual, population, community and ecosystem levels (Putz *et al.*, 2001; Wolf & Konings, 2001; Peres *et al.*, 2003; Ticktin, 2004). However, a few studies have indicated a limited sensitivity to harvesting for some tropical plant species, like *Aechmea magdalenae*, which can tolerate high levels of harvest (Ticktin, 2004).

Sustainable harvesting of MAPs and other NTFPs is challenged by many factors from both social and ecological perspectives (Ticktin, 2004; Ghimire *et al.*, 2008). The ecological effects of NTFP harvesting are both species- and site-specific and may also vary according to ecosystem, region and management practices (Runk, 1998; Endress *et al.*, 2006). The most direct ecological impacts of NTFP extraction are changes in survival, growth and reproduction rates, affecting the population structure and dynamics. The magnitude of harvest impacts is further dependent on the life-history, parts harvested, intensity and time of harvesting, and the environmental conditions where the species grows (Ticktin, 2004). The harvest of underground parts is found to be much more detrimental compared to the harvest of other parts as it often results in the death of the whole plant (Schmidt *et al.*, 2011; Rokaya *et al.*, 2017). The harvest of NTFPs is usually associated with management practices aimed at increasing yields, and managing other natural resources. However, currently very little is known about how NTFP harvest may interact with other kinds of disturbance or management techniques (Ticktin, 2004).

A growing number of studies are assessing the use of MAPs and harvest impacts; however, there are still major gaps in this field. Importantly, these include a lack of long-term studies on the dynamics of MAP populations, which is critical to assess inter-annual variation in demographic properties and harvest rates. There is also a paucity of information regarding spatial variability in responses to MAP harvesting, as only few studies have investigated and modeled multiple populations (Ticktin, 2004; Schmidt *et al.*, 2011). Spatial and temporal variability data are essential to adequately model and realistically predict MAP population dynamics and to assess long-term harvest effects (Schmidt *et al*., 2011). In addition, ecological studies on various environmental factors that influence MAP population dynamics are also of great importance.

Very limited studies exist in the Himalayas regarding the effects of harvesting and other human-mediated parameters on the demography of MAP populations. Research focusing on the long-term monitoring of Himalayan MAPs is largely lacking. Ghimire *et al*. (2008) examined the demographics of *Nardostachys grandiflora*, a highly threatened species, and found that harvesting adversely affected the population growth rate of this species. Therefore, they recommended controlled harvesting with very low harvest rates and long rotations for long-term population survival. Rokaya *et al*. (2017) studied the population dynamics of two perennial medicinal herbs, *Rheum acuminatum* and *R. australe* and concluded that selective or rotational harvesting strategies could be adopted for the sustainable management of these species. Timsina *et al*. (2021) studied the demography of a medicinal orchid, *Crepidium acuminatum*, and stressed the importance of selective or rotational harvesting for the sustainable development of the orchid population.

The life history of orchids is relatively well understood qualitatively, but quantifying the dynamics and extracting a conservation message remains challenging. Globally there are very few long-term studies quantifying the population dynamics of different terrestrial species of orchids (Shefferson *et al*., 2020). In Nepal, there are around 500 species of orchids belonging to 105 genera (Vaidya, 2019). Most of these species are highly threatened (Raskoti, 2009; Pant & Raskoti, 2013; Subedi *et al*., 2013; Vaidya, 2019), which calls for immediate conservation action (Wraith *et al*., 2020). Further, the high medicinal value of orchids (Singh *et al*., 2009; Teoh, 2019) has spurred indiscriminate collection for local use or trade (Fay, 2018; Hinsley *et al*., 2018; Charitonidou *et al*., 2019).

The increasing trend of overexploitation necessiates developing sustainable harvesting strategies for all commercially important species of MAPs, including orchids (Ghimire *et al*., 2008; Mondragón Chaparro & Ticktin, 2011; Schmidt *et al*., 2011; Klimas *et al*., 2012). This can be achieved through long-term demographic research in partnership with local harvesters, and through the development and evaluation of management practices that can mitigate the negative impacts of harvesting (Ticktin, 2004). Scientific information on how the different disturbance regimes (harvesting, grazing and fire) which shape the life history parameters (survival, growth, reproduction, mortality) of important MAPs is largely lacking or inadequate (Ticktin, 2004; Ghimire *et al*., 2005, 2008). Research on ethnobotanical knowledge, as well as integration of people's perceptions and practices in resource management at the local level is of considerable importance.

In line with this knowledge gap and in line with the objective of this project (Transiting to green growth: natural resources in Nepal (TGG-N) the present PhD study aims to analyze long-term population dynamics and ecological sustainability of harvest of two threatened Himalayan medicinal plant species, *Aconitum spicatum* (hereafter *A. spicatum*) and *Dactylorhiza hatagirea* (hereafter *D. hatagirea*), both harvested for trade. Further conservation status, life form, growth strategy, parts used, habitat specificity and availability in the study area were also considered during the selection of the study species.

A. spicatum is a high-value medicinal plant species traded in huge quantities from Nepal. It's global status is not evaluated. In the context of Nepal, it is vulnerable (CAMP, 2001) and is threatened due to over-harvesting of its tubers (Olsen, 1998). Considering this, the government of Nepal has prioritized it as one of the thirty national priority herbs for development, research and cultivation (DPR, 2012). In recent years increased trans-boundary trade to meet the growing demand from international pharmaceutical companies has led to indiscriminate harvesting of *A. spicatum*. The only way to meet the demand is to collect the plants from the wild as no successful cultivation measures are established for this species. Besides, habitat fragmentation, different disturbance regimes and natural catastrophes are threatening the sustainable development of *A. spicatum* (chapter 2).

Dactylorhiza hatagirea is an endangered orchid in Nepal (CAMP, 2001) with broad spectrum of pharmacological values. Its global status is not evaluated. It has been categorized as critically endangered in Indian Himalaya (Conservation Assesment and Managemenr Plan; CAMP status), critically rare (International Union of Nature and Natural resources; IUCN) and listed under appendix II of Convention on International Trade in Endangered Species (CITES) (Bhatta *et al*., 2005).The tubers of the plant yield a high quality salep (powder made from the orchid tuber) which is used as an aphrodisiac and has attracted the international market (Subedi *et al*., 2013). This has increased the illegal harvest and the populations are deteriorating (Subedi *et al*., 2013; chapter 4). *D. hatagirea* is a habitat-specific and inherently slow growing and poorly regenerating species (Agarwal *et al*., 2008), which is pollinator specific and requires mycorrhizal association for seed germination, seedling establishment, reproduction and survival (Bhatt *et al*., 2005). Habitat loss caused by increasing human population, overexploitation due to high herbal demand, lack of knowledge on sustainable harvesting and climate change are the major challenges for the long-term persistence of this species (Kunwar *et al*., 2021; Shrestha *et al*., 2021). It is protected under Nepal's local laws (Forest Act 1993, Forest Regulations 1995, Amendement in 2001). However, on April 14, 2008, the Nepalese government issued a notice permitting collection of the wild orchid for trade. Poor implementation of these conflicting regulations and guidelines on sustainable harvesting has increased illegal trade in *D. hatagirea* (Subedi *et al*., 2013).

Considering all the constraints related to anthropogenic and ecological factors, recent research has clearly shown the need of further long-term studies focusing on population biology and the impact of different harvesting regimes on population dynamics of commercially threatened Himalayan medicinal plants, including *A. spicatum* and *D. hatagirea* (Ticktin, 2004; Ghimire *et al*., 2008). This thesis attempts to fill the research gap on these two Himalayan medicinal herbs by focusing on their population status, performance and dynamics under different harvesting regimes and environmental conditions in Annapurna Conservation Area, Manang, and Api-Nampa Conservation Area, Nepal.

1. 2 Objectives

The broad objective of this thesis is to study the harvest sustainability and develop management recommendations for *A. spicatum* and *D. hatagirea* across ecological and human use gradients. The specific objectives of this thesis are to:

a. Assess the patterns of distribution (population density and structure) and variation in life history traits of *A. spicatum* and *D. hatagirea* along the elevation gradient and anthropogenic disturbances.

b. Identify the demographic processes that are important in producing the changes in population growth rate caused by harvesting.

c. Assess the major components determining the sustainability of harvest of *A. spicatum* and *D. hatagirea* and make proper recommendations for their management.

d. Assess socio-cultural and economic aspects of *A. spicatum* and *D. hatagirea*.

1.3 Research questions and hypothesis

The main hypothesis of the thesis is "harvesting poses threats to the spatiotemporal resilience and viability of *A. spicatum* and *D. hatagirea* populations". Research questions, methods of data collection and associated chapters are given in Table 1.

Table 1: Specific objectives, research questions and chapter's matrix

Specific objectives	Research questions	Data collection method	Chapters
A	1. How do the studied species differ in terms of spatial distribution and fitness-related traits along the ecological and human use gradients?	*A. spicatum* 66 (3 m × 3 m) temporary plots with 330 (1 m × 1 m) subplots. *D. hatagirea* 144 (3 m × 3 m) temporary plots with 432 (1 m × 1 m) subplots 72 plots and 216 subplots in each site	2, 4
B	2. How do populations in different environmental conditions and harvesting regimes differ in demographic parameters (vital rates: growth, survival and fecundity)?	*A. spicatum* 9 permanent plots of (2 m × 14 m), with 36 (2 m × 2 m) subplots, harvest simulation (0%, 25%, 50% and 75%) was applied by locals in each subplot *D. hatagirea* 21 permanent plots of (20 m × 20 m), with 84 (10 m × 10 m) subplots; 9 plots in each of two grassland sites and 3 in a wetland site	3, 5
C	3. What are the main factors determining the sustainability of harvest?	Same as research question 2	3, 5
D	4. How do the locals use and perceive the values of studied species and what is the contribution of these species to their livelihood?	Interview survey and focus group discussion, 117 informants in Darchula, 69 in Manang and 65 in Upper Modi valley river within Annapurna Base Camp region were interviewed.	2, 4

The thesis is divided into six chapters. Chapter 1 covers a general introduction to the research topic and chapter 6 provides overall conclusions and implications of the research. Research findings are comprehensively covered in chapters 2–5, of which chapters 2 and 4 have been published in peer reviewed scientific journals, and the remaining two (chapters 3 and 5) will be submitted for publication.

The first objective is specifically analyzed in chapters 2 and 4. Chapter 2 describes plant density and life history traits of *A. spicatum* in north-central Nepal focusing on the effect of elevation and anthropogenic disturbances in three different populations throughout its distributional range. Chapter 4 deals with the major threats to *D. hatagirea* in central and far western Nepal. Likewise, it also covers the life history traits and distribution pattern of *D. hatagirea* in Api-Nampa Conservation Area, Darchula and Bhimthang area of Manang.

The second objective is partly analyzed in Chapter 5. This chapter compares the demographic response of *D. hatagirea* to harvesting in a locally unprotected site in Darchula, and a locally protected site in Bhimthang, Manang. This chapter also estimates the effect of habitat, based on comparison between grassland and wetland populations in the unprotected site.

The third objective is addressed in Chapter 3, which describes the effect of different harvest intensities, including low harvest intensity (0-25%) and high harvest intensity (50-75%), on the demography of *A. spicatum*. This chapter also focuses on the extinction probability and future population size of *A. spicatum* under different harvest intensities.

The fourth objective is specifically analyzed in Chapters 2 and 4. These chapters partly assess the local use value, contribution in their livelihood, population abundance and conservation status of the studied species and stresses the need of long-term conservation.

1.4 Limitations of the study

This thesis focuses on the data collected only across three years for *D. hatagirea* and four years for *A. spicatum*, so it was difficult to distinguish between dormant and dead individuals during the final year of the study. Remoteness of the site confined to collect the data for only one season of the year. Observations were made at slightly different times of the season, implying that the risk of overlooking individuals varied from time to time.

Due to restrictions by law, application of experimental harvest in *D. hatagirea* to explore the effect of different harvest intensities was not possible. Instead, observational studies at two sites characterized by different harvest intensities were carried out. This, however, implies that effects of disturbances and site are to some extent confounded.

The disturbances recorded in the study were based on observational approach only. Inability to set up devices to detect the extent of grazing or the exact number of cattle producing the amount of animal droppings recorded was another limitation of the study. Due to extreme human pressure, it was not possible to maintain the permanent plots for more than three years in Darchula (unprotected site). Further, it was not possible to compare the effect of disturbances on wetland populations in Manang (protected site) as such populations were not found in the area.

The limited time period covered by the study made it difficult to distinguish between asexual and sexual reproduction of the studied species. Inability to include the vegetative reproduction during modeling was also a limitation of this research.

In the final year (2018) of the study of *A. spicatum* demography, some individuals infected by pests (fungal or bacterial) were encountered in the permanent plots. It was not possible to identify the disease within the study period and therefore was not able to include in the model, adding one more limitation to this study.

1.5 Key findings of the study

- The long-term viability of *Aconitum spicatum* and *Dactylorhiza hatagirea* largely depends on the magnitude of harvest impact and the level of protection provided directly by the local people.
- The opportunities of sustainable harvest vary among plant species with different strategies of growth and demographic properties and these further relate with ecological conditions set by elevation gradient.
- Sustainability of harvest of medicinal and aromatic plants, like *Aconitum spicatum* and *Dactylorhiza hatagirea* can be achieved through integrating species-specific ecological studies, and knowledge and practices of key knowledge holders.

1.6 Summary of the research chapters

Chapter II

- Human disturbances and topographic factors greatly contribute to the variation in density and life history traits of *A. spicatum.*
- Alpine plants develop adaptive strategy by allocating higher resources in their seeds to cope environmental harshness.
- Persistence and growth of subalpine population depend on the efficiency of the management system to protect younger life stages and control over and pre-mature harvesting, grazing and trampling.
- Outsiders from neighbouring districts (Gorkha and Dadhing) are responsible for the indiscriminate harvesting. Thus, the locals should increase their participation in the protection and conservation of *A. spicatum*

Chapter III

- Populations at different elevations respond differently to harvest.
- At low harvest condition populations show growth rate more than 1 thus probability of extinction is extremely low.
- Subalpine (lowest elevation) population is the most sensitive population to harvest and should be conserved with greater priority.
- Higher contribution of larger size classes (Adult vegetative and reproductive) to λ indicates that these stages are the critical in the life history of *A. spicatum* and the conservation priority should focus on the protection of these stages.

Chapter IV

- Harvesting and grazing impart significant negative effects on plant density at different life stages and play a crucial role in deteriorating plant population through reduction in reproductive outputs.
- Significantly reduced fruit production and low productivity in terms of tuber biomass for plants at the unprotected site may lead to the local extinction of the species.

- The better performance of populations in protected site can be described as a result of the effective conservation efforts of the local community which can be extrapolated to the unprotected site.

- A low level of awareness about population ecology and conservation among local people are the major challenges for the sustainable development of *D. hatagirea* populations at the unprotected site.

Chapter V

- Population growth rate of more than unity allows harvest of 10% of the individuals for local use in the protected populations.

- 10 fold increase in fecundity can maintain steady population in unprotected grassland populations.

- 25 fold increase in fecundity can maintain steady population in unprotected wetland population.

CHAPTER 2[*]

PLANT DENSITY AND LIFE HISTORY TRAITS OF *ACONITUM SPICATUM* IN NORTH-CENTRAL NEPAL: EFFECTS OF ELEVATION AND ANTHROPOGENIC DISTURBANCES.

Abstract

Increasing cross-border trade of medicinal and aromatic plants (MAPs) has put heavy pressure on a considerable number of species in the Himalayas. One of the threatened species in Nepal is *Aconitum spicatum*. Unfortunately for this species and for many others, our knowledge on population ecology and performance across the distribution range is insufficient, hindering the formulation of species-specific management plans. Thus, density and population structure of *Aconitum spicatum* was studied and variation in its life history traits among three ecotonal populations (subalpine, lower alpine and alpine) along an elevation gradient (3000–4200 m asl) in Annapurna Conservation Area, north-central Nepal was assessed. The results show that human disturbances and topographic factors contributed to the variation in density and life history traits. The overall density ranged between 0.56 ± 0.09 (Mean ± SE) and 2.48 ± 0.24 plants/m^2 with highest mean density in the lower alpine and lowest in the subalpine population. The subalpine population was also characterized by lower investment in reproductive structures with lowest seed mass and low seed viability and fecundity. Among the environmental variables tested, harvesting, animal droppings and fire appeared to be the most important factors affecting density of different life stages of *A. spicatum*. The prevailing harvesting pattern is destructive as it involves uprooting of the whole plant and this appears to be a main reason for low recruitment and reduced density of the subalpine population. The level of disturbance decreased with increasing elevation. In terms of reproductive effort, the alpine population performed best. The present results indicate that the viability of *A. spicatum* populations depends on controlling over harvesting and pre-mature harvesting of tubers and protecting younger life stages from grazing, trampling and

---

[*] **Chapagain, D.J.**, Meilby, H. & Ghimire, S.K. (2019). Plant density and life history traits of *Aconitum spicatum* in North-Central Nepal: Effects of elevation and anthropogenic disturbances. *PeerJ* 7:e7574 http://doi.org/10.7717/peerj.7574

fire. It is therefore recommended that when formulating management guidelines, measures aiming to mitigate such anthropogenic disturbances should be considered.

Keywords: Population density, tuber biomass, sensitivity, Mixed Zero-inflated Poisson

2.1 Introduction

The study of variation in life history traits of a species is crucial to understanding the role of particular types of ecological pressure in shaping population demographics (Roff, 1992; Réale *et al*., 2010, Tonnabel *et al*., 2017). Variation in environmental conditions can affect recruitment dynamics (Franco & Silvertown, 1996) and population growth rates (Caswell, 2001). Himalayan alpine plants respond to environmental and climate change variables including biogeography, elevation and precipitation (Salick *et al*., 2014). Increasing anthropogenic activities have also created and continue to create a range of novel environments in mountain ecosystems (Zhang *et al*., 2019). Along the elevation gradient in mountain ecosystems, large changes in environmental factors occur over short distances, leading to huge variation in the selection pressure imposed on plant life-history strategies and traits (Bresson *et al*., 2011). Particularly, topographic variation affects microclimatic conditions, which may contribute to variation in life history traits. These variations are coupled with disturbance regimes, like harvesting, fire and grazing which themselves are structured along elevation gradients. The concentration of these anthropogenic disturbances, especially in alpine and subalpine ecosystems may put high-altitude plants under stress. To cope with such stressful conditions, high-elevation plants develop self-sustaining adaptation strategies (Körner, 2003), including a long floral axis, more globular flowers (Molau, 1993), persistent sepals, larger root systems (Körner, 1999), vegetative apices or perennating buds at safe depth below ground (Körner, 2003), higher proportion of belowground biomass (Webber & May, 1997) and increased number and size of seeds (Körner, 1999), which contribute to enhance the persistence of their populations.

Population density and individual-level traits, like plant size and reproductive performance (Totland & Nylehn, 1998; Nielsen *et al*., 2007; Fan & Yang, 2009) are considered the most important basic parameters needed to assess the capacity of a plant species to maintain its population. Reproductive traits are especially important for a species to establish and to persist following disturbance. Rates of recruitment vary and depend on, e.g., seed number, seed mass, seed viability, growing season, and disturbance characteristics (Chambers *et al*., 1990). Understanding the variation in such traits is important for the assessment of the long-term persistence of populations growing in habitats disturbed by human activities. Habitat disturbance often has a negative effect on plant reproductive success (Chambers, 1995). Cattle grazing, trampling and fire, for example, may lead to lower fruit set and subsequent recruitment (Mckechnie & Sargent, 2013; Renison *et al*., 2015). However, fire is deemed to be a prerequisite for recruitment, growth and development of plant species in some ecosystems (Bucini & Lambini, 2002) and has in some cases been shown to promote higher species diversity (Hobbs *et al*., 1984).

Harvesting of medicinal and aromatic plants (MAPs) is a customary subsistence practice in the Himalayan region. Over 300 taxa of MAPs are traded from Nepal with a total annual amount of 10,770 tons and a value of USD 60.09 million (Ghimire *et al*., 2016; GoN 2015, 2018). Trans-boundary trade of Himalayan MAPs has increased to meet the growing demand from international pharmaceutical companies and has led to heavy and indiscriminate harvesting of some species. *Aconitum spicatum* (family Ranunculaceae) is among the ten most traded medicinal plants from the Himalayan region of Nepal (Olsen & Larsen, 2003). It is vulnerable in Nepal, mainly due to over-harvesting (CAMP, 2001). Considering the high trade value of *A. spicatum*, the government of Nepal has included it as one of thirty herbs of national priority for development, research and cultivation (DPR, 2012). Despite the commercial interest and high conservation value of *A. spicatum*, detailed studies describing its ecological, morphological and reproductive characteristics are largely lacking within its entire range. Scientific information on how the different disturbance regimes (including harvesting) shape the life history parameters (reproduction and growth) and determine population density and structure of important Himalayan MAPs like *A. spicatum* across ecological gradients is important for the development of management strategies to maintain viable populations (Ticktin, 2004; Ghimire *et al*., 2005). In this chapter I aim to analyze whether impacts of anthropogenic disturbance on population performance of *A. spicatum* depend on ecological factors associated with elevation. Thus, examining populations located within three consecutive elevation ranges, the goal of this study was to answer the following questions: (i) do the impacts of anthropogenic disturbance on population structure and density of *A. spicatum* vary along an elevation gradient? (ii) How do plant size and reproductive performance of *A. spicatum* vary along an elevation gradient? (iii) what are the socio-cultural and economic aspects of *A. spicatum* ?

2.2 Materials and methods

2.2.1 Study area

The study of *A. spicatum* was carried out in upper Modi River Valley (N 28°29.455' and E 083°53.546' to N 28°31.806' and E 083°52.537') within Annapurna Conservation Area (ACA) in north-central Nepal (Figure 1). The study area is characterized by temperate and alpine climate. The vegetation consists of upper temperate coniferous and mixed broad-leaved forests at lower elevations (>3000-3500 m), sub-alpine mixed forests dominated by *Abies spectabilis* and *Betula utilis* at and below the tree line (>3500-4000 m), lower alpine thickets mainly of dwarf bushes of *Rhododendron* spp. at and above the tree line (*ca*. 4000 m), and alpine meadows and grasslands above 4000 m. Snowfall starts in October and the area remains snow-covered for up to six months, until March. High seasonal differences in temperature and rainfall, short growing season and heavy snowfall are the main constraints to plant growth at higher elevations. The study area comprises the most popular trekking routes of Nepal, and each year approximately 30,000 tourists visit the area. Trekking tourism can be considered one of the most important factors affecting the structure of forest ecosystems at high elevation in the Himalayan region (Biønness, 1980; Stevens 2003; Garbarino *et al*., 2014). There are 14 hotels along the trekking route passing through the study area, and the nearest villages (Chhomrong and Sinwa) are inhabited by 62 households. Along the upper Modi Valley there are two cattle sheds ('goths' in Nepali), each with about 500 cattle. The livelihood of local people is based on subsistence agriculture, pastoralism, seasonal trade, and more importantly, tourism. Interviews with local residents indicated that people from the neighboring Gorkha and Dhading districts of Nepal, recently started harvesting highly valued MAPs, including *A. spicatum* from the ACA region.

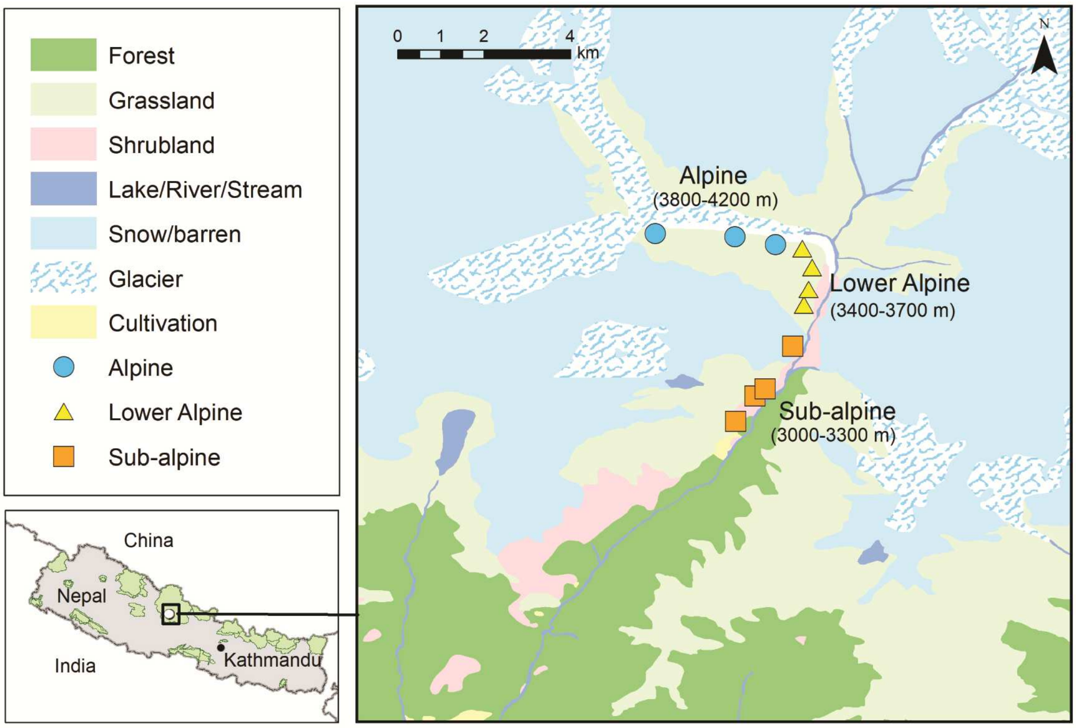


Figure 1: Map showing study area (N28˚29.455' and E083˚53.546' to N28˚31.806' and E083˚52.537', Annapurna Conservation Area) and the locations of the study plots of *Aconitum spicatum*

Source of land cover map: Department of Survey, Government of Nepal

2.2.2 Study species

***Aconitum spicatum* (Briihl Stapf)**

Aconitum species belong to the family Ranunculaceae and are locally known as Bikh and Monk’s hood or aconite in English. The plants of this genus have been used as an arrow poison in prehistoric time. The genus has approximately 300 species. Department of Plant Resources (DPR, 2012) has listed 33 species of *Aconitum* from Nepal. Fifteen species of *Aconitum* are endemic to Nepal and 7 species are included under different threat categories (DRR, 2012). The genus shows temperate to alpine (1,800–4,200 m elevation) distribution ranging from east to west in Nepal.

Aconitum spicatum hereafter *A. spicatum* is a vulnerable (CAMP, 2001) medicinal plant species with a restricted distribution to the Himalayas of Nepal, India, Bhutan and China at elevations between 2900 and 4200 m above sea level (asl) (Ghimire *et al*., 2021). The plant grows in moisture retentive soils of the sub alpine and alpine meadows. In the study area, fragmented populations of *A. spicatum* occur between 3000-4200 m asl. The plant is less exploited by the locals as the area is touristically flourished, however the collectors from other districts are found harvesting the plant for commercial purpose.

A. spicatum is a tall perennial herb with an erect stem reaching upto 2 m in height and bearing crown of large attractive blue or yellow zygomorphic flowers with numerous stamens (Figure 2a). The long floral axis (which facilitates pollination) and large number of blue globular flowers that allows complete development of fruits and seeds (Molau, 1993), persistent sepals, larger root system (Körner & Kèorner, 1999), vegetative apices or perrennating buds at safe depth below the ground that prevents damage from the low temperature (Körner, 2003), higher proportion of belowground biomass (Webber & May, 1997), investment of food reserve of the mother tuber for the growth and development of daughter tuber in the coming year and large seed number are the important adaptive features of *A. spicatum*. All these features are considered as the adaptive features of plant to survive in alpine and subalpine habitats (Bliss, 1962; Körner, 2003).

The plant bears 1-70 flowers (mean ± SE 10.64 ± 1.29) and 1-110 fruits (mean ± SE 14.03 ± 1.53) per plant. Each fruit is an aggregate of 5-6 follicles (Figure 2b). Each fruit produces an average of 41 seeds; thus, an individual plant bears about 574 seeds on average. The plant produces paired dark-brown tubers (Figure 2c), which are best harvested as soon as the above-ground parts die in autumn (Ghimire *et al*., 1999). The aerial part of the plant is annual but the tubers are perennial. The mother tuber dies every year but before this happens it produces a number of sprouting buds, which grow into 1 to 5 daughter tubers (field observation). The plant shows low seed germination potential and the seeds can stay dormant for long time (Srivastava *et al*., 2010) and even tubers show frequent dormancy (Chapter 3).

It is one of the most poisonous plants among 15 plant species reported from Annapurna Himalaya area (Bhandary & Shrestha, 1986). The plant has high medicinal value. Tubers of the plant possess alkaloids (1.21%) like aconitine, hypaconitine and mesaconitine (Srivastav *et al*., 2010) and have been reported to show significant antibacterial, antipyretic, analgesic and enzyme inhibition properties demonstrating its potential use in pharmaceutical companies (Srivastav *et al*., 2010). Tubers of *A. spicatum* are used in the traditional medicinal system after proper detoxification to cure fever, headache, lung and intestine infections and to heal cuts and wounds (Shyaula, 2011). The detoxification techniques however vary according to the traditional practices (Lama *et al*., 2001).

Figure 2 a. Blooming *Aconitm spicatum*

Figure 2 b. Follicles

Figure 2 c. Tubers

Figure 2 d. Mature seeds

2.2.3 Sampling

During the summer of 2015, sampling was carried out in three sites at elevations ranging from 3000 m to 4200 m. Sampling covered the whole elevation range of *A. spicatum* in the study area as the transects were established from the lowest to the highest point of its distribution. In each 100 m elevation band, a transect with six plots

(3 m × 3 m) was established with plots located at a minimum plot-to-plot distance of 10 m. Each plot was divided into nine subplots of 1 m × 1 m, five of which (center and four corner subplots) were systematically selected for detailed biological measurements. In total, 66 (3 m × 3 m) plots, with 330 (1 m × 1 m) sub-plots were sampled. In each plot, latitude and longitude were recorded using an eTrex Garmin GPS device and cross-checked the elevation using a calibrated altimeter. Slope and aspect were measured using clinometer and compass, respectively. In each subplot, the cover (%) of shrubs, herbs, grasses, lichens, bryophytes, litter, bare ground, solid rock and scree were estimated using standard methods (Pauli *et al*., 2015).

Individuals of *A. spicatum* were classified into four stage classes on the basis of their size and reproductive status and were counted. The stage classes were: seedlings (Sd; stem girth ≤ one mm measured at five cm from soil surface, leaf number = 1-2), juveniles (Jv; stem girth = 1-<three mm, leaf number ≥ 2-< 5), vegetative adults (Adv; stem girth ≥ three mm, leaf number ≥2) and reproductive adults (Adr; flowering or fruiting individuals). In each subplot, the count of plants at different stages was used to calculate population structure and density. Animal droppings, trampling, harvesting and fire were considered as anthropogenic disturbances. A five-point ordinal scale (0–4) was applied to describe the level of disturbance. A score of ‘0’ corresponded to no disturbance, while ‘4’ corresponded to heavy disturbance.

Four transects were grouped together and defined three wider ecotone populations, namely subalpine (3000-3300 m), lower alpine (3400-3700 m) and alpine (3800-4200 m). Twenty of the most vigorous plants from each of these populations were selected to record plant biomass and to measure vegetative traits (plant height, stem girth, number of leaves, leaf size and length of floral axis) and reproductive traits (number of buds, flowers and fruits). The fresh weight of aboveground and belowground plant parts was measured in the field. Afterwards, the plants were air dried, packed in paper bags and brought to the laboratory for dry biomass estimation. Seed number and seed mass estimations were based on random selection of 15 mature reproductive individuals from each population during the seed maturation period in October. From each such individual, 5 fruits were collected, recorded their diameter, length and fresh weight in the field, and then packed the fruits separately in paper bags for subsequent dry biomass estimation.

Seed sowing experiments were carried out in October 2015 and 2016. In each year, mature seeds (*n* = 100) were introduced into each of five randomly selected 1 m × 1 m subplots, located in a similar habitat adjacent to the study plots. Dark brown seeds which sank in water were considered mature and were selected for the sowing experiment. The seeds sown during 2015 failed to germinate. However, the seeds sown in 2016 germinated and established in 2017. Seedlings that recruited and survived were recorded in 2017. The data resulting from these experiments along with the information on seed production were used to estimate fecundity. Fecundity was calculated as the percentage of established seedlings from 100 seeds multiplied by the average number of viable seeds per individual in each population.

2.2.4 Laboratory study

The air-dried plant materials were oven dried (at 60° C for 72 hours) and measured their biomass to an accuracy of 0.001 g. Similarly, number of seeds per fruit and per individual plant were counted, and measured the seed mass (expressed as dry mass of 50 seeds) per individual. Seed viability was tested by soaking the seeds in water, cutting them into halves and subsequently dipping them in a 10 percent solution of 2, 3, 5 Triphenyl Tetrazolium Chloride (TTC) for a minimum of 12 hours in total darkness. The pink coloration of the embryo under a microscope, indicated viability of the seed (Beigh *et al*., 2006).

2.2.5 Interview survey

Semi-structured interviews were conducted during 2015-2017 among 65 persons in Annapurna Base Camp region. The informants were local MAP users, leaders, teachers and students in the villages, and hotel owners, local tourist guides and cattle herders working in the sites where the ecological survey was carried out. The goal of this research was explained and obtained the informants' consent before starting the interview. The informants were also informed about their right to withdraw their consent at any stage of the interview. Informants' responses were documented by written notes and in possible cases were also supplemented by voice recording, with the permission of the informants.

The informants were asked to comment on the abundance of *A. spicatum*, collectors (villagers or outsiders), reasons for collecting the plant (local use or commercial purpose), local uses, the collection (areas and time of collection), when they started collecting, indigenous knowledge transfer practices, special tools, prices, qualities, markets (to whom they sold), processing after collection and their other sources of income. Questions concerning nature conservation; changes observed in the biotope, perceived trends in populations of the species, causes of population change, threats, conservation status, protection measurements, practices that could ensure survival and sustainable management were also asked (Appendix I).

2.2.6 Statistical analysis

2.2.6.1 Population structure and density

Given the clustered sampling design with five sub-plots per plot, the variation of *A. spicatum* density using a mixed-modeling approach including a random effect of plot was analyzed. Over-dispersion of the count data was confirmed by using the qcc package (Scrucca, 2004), and the analysis of the counts for different stages was made using the glmmTMB package (Mollie *et al*., 2017) in R, version 3.5.3 (R Development Core Team, 2017).

Direct field observations confirmed the plant as rare and sparsely distributed at the study site. Hence, methods especially designed to cater for count data with excess zeros were considered from the project’s early stages. Simple transformations, such as square root and log, were not capable of making the data appropriate for modeling using standard statistical assumptions of normality. The abundance of zero counts prevented these transformations from having the desired effect of symmetrizing the data and eliminating dependence between the mean and variance. Based on the Akaike Information Criterion (AIC), the best fit among several model alternatives was obtained using Mixed Zero-inflated Poisson (mixed ZIP) models (Hall, 2000). Hence, mixed ZIP models were used to quantify the relationship between environmental factors and the abundance of plants at different stages of development using explanatory variables such as population (a given elevation range), relative radiation index (RRI), cover of shrubs, cover of herbs, harvesting, trampling, animal dropping and fire. All models included a random effect of the plot. Twenty-six sets of candidate models were prepared using the glmmTMB package (see Appendix III) and finally prepared an average model based on set of 16 best candidate models (selected on the basis of delta AIC) using the MuMin package (Barton, 2018) in R. The 16 models with AIC values less than 258.4 for seedling stage, 330.9 for juvenile stage, 583.3 for adult vegetative stage and 777.3 for adult reproductive stage were used to prepare the average model by MuMin (Appendix IV).

The full models were in all cases expressed as:

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b (Population) + c_1 RRI $_{ij}$ + c_2 Herb cover $_{ij}$ + c_3 Shrub cover $_{ij}$ + c_4 Harvesting $_{ij}$ + c_5 Animal dropping $_{ij}$ + c_6 Trampling $_{ij}$ + c_7 Fire $_{ij}$ where, a (intercept), b (population) and $c_1 \ldots c_7$ are fixed model parameters, i = 1…66 is the plot (included as a random effect) and j = 1…5 is the sub-plot. Population had three categories (subalpine, lower alpine and alpine). Density was expressed as the number of individuals in a given stage category counted within a 1 m^2 sub-plot. A relative radiation index (RRI) was used as a proxy of microclimate at the site. RRI is a relative measure of the exposure to solar radiation at noon at a specific location and was calculated for each plot as a function of aspect, latitude, and slope. RRI was calculated as: $RRI = \cos(180° - \Omega) \times \sin(\beta) \times \sin(\Phi) + \cos(\beta) \times \cos(\Phi)$, where Ω = aspect (slope azimuth in degrees), Φ = latitude (degrees) and β = slope inclination (degrees) (Oke, 1987; Vetaas, 1992). The preliminary analysis showed that the shrub, litter, bryophyte and lichen cover were closely correlated. Similarly, herb and grass cover, and scree and bare ground cover were closely correlated. It was therefore decided to exclude litter, bryophyte, lichen, grass and scree cover in subsequent analysis.

2.2.6.2 Plant size and reproductive performance

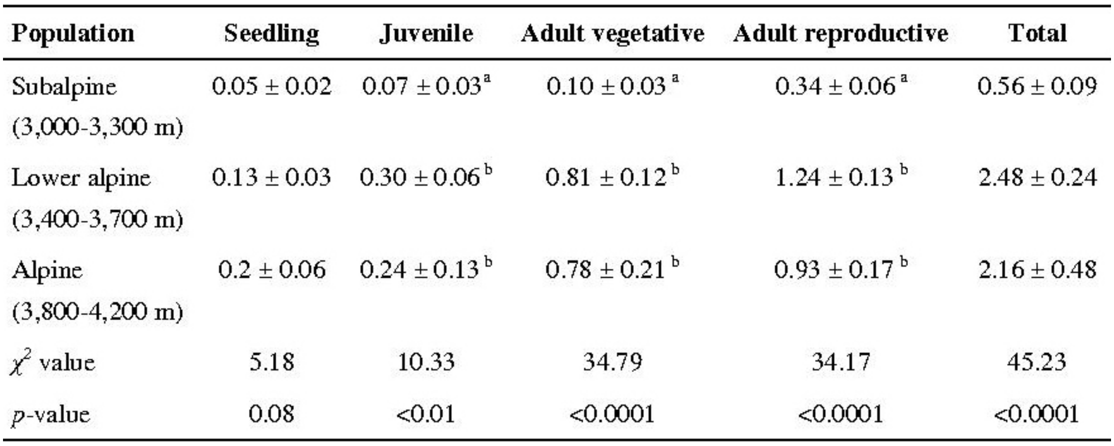
Unlike plant density and structure, other life history traits were studied using sample individuals selected at the population level (see above). As the measurements were done on individual plants, 20 replicates were sampled for each of the size- based and reproductive trait variables. Before doing the analysis, data for each of these variables were tested for the assumptions of parametric tests. Except for plant height and biomass, all other variables that relate to plant size and reproductive performance did not meet the standard assumptions of normality and homogeneity of variance. Therefore, Kruskal-Wallis tests was applied to examine the variation of these traits among the three populations. In the case of plant height and biomass, logarithmic transformation of the data gave statistical normality, so in these cases one-way ANOVA was applied. A double logarithmic allometric model was prepared to describe the relationship between plant height and the biomass of tubers. Multiple comparison tests were performed after ANOVA and Kruskal Wallis to compare the significant differences among the populations. All tests were conducted using the R version 3.5.3 (R Development Core Team, 2017).

2.3 Results

2.3.1 Population structure and density

Significant differences were found in the densities of juvenile, and vegetative and reproductive adults among the three populations (Table 2). Similarly, differences in the observed stage distributions were recorded among them (Figure 3). All the populations revealed lower proportions of plants in smaller size classes (seedlings and juveniles) than in larger size classes (vegetative and reproductive adults). The variation in density of *A. spicatum* across elevations was hump-shaped and density reached its maximum in the lower alpine population with a mean density of 2.48 ± 0.24 plants/m^2. The lowest density (0.56 ± 0.09 plants/m^2) was found in the subalpine population.

Table 2: Population density (m^{-2}) for different life stages of *A. spicatum* in three populations in Annapurna Conservation Area, Central Nepal. Densities are stated as mean ±SE. χ^2 and *p*-values were based on Kruskal-Wallis test, *df* = 2, n = 66. Values for each stage class among the populations with same superscript letter do not vary significantly at *p* = 0.05 level based on multiple comparison test after Kruskal Wallis



Population	Seedling	Juvenile	Adult vegetative	Adult reproductive	Total
Subalpine (3,000-3,300 m)	0.05 ± 0.02	0.07 ± 0.03^{a}	0.10 ± 0.03^{a}	0.34 ± 0.06^{a}	0.56 ± 0.09
Lower alpine (3,400-3,700 m)	0.13 ± 0.03	0.30 ± 0.06^{b}	0.81 ± 0.12^{b}	1.24 ± 0.13^{b}	2.48 ± 0.24
Alpine (3,800-4,200 m)	0.2 ± 0.06	0.24 ± 0.13^{b}	0.78 ± 0.21^{b}	0.93 ± 0.17^{b}	2.16 ± 0.48
χ^2 value	5.18	10.33	34.79	34.17	45.23
p-value	0.08	<0.01	<0.0001	<0.0001	<0.0001

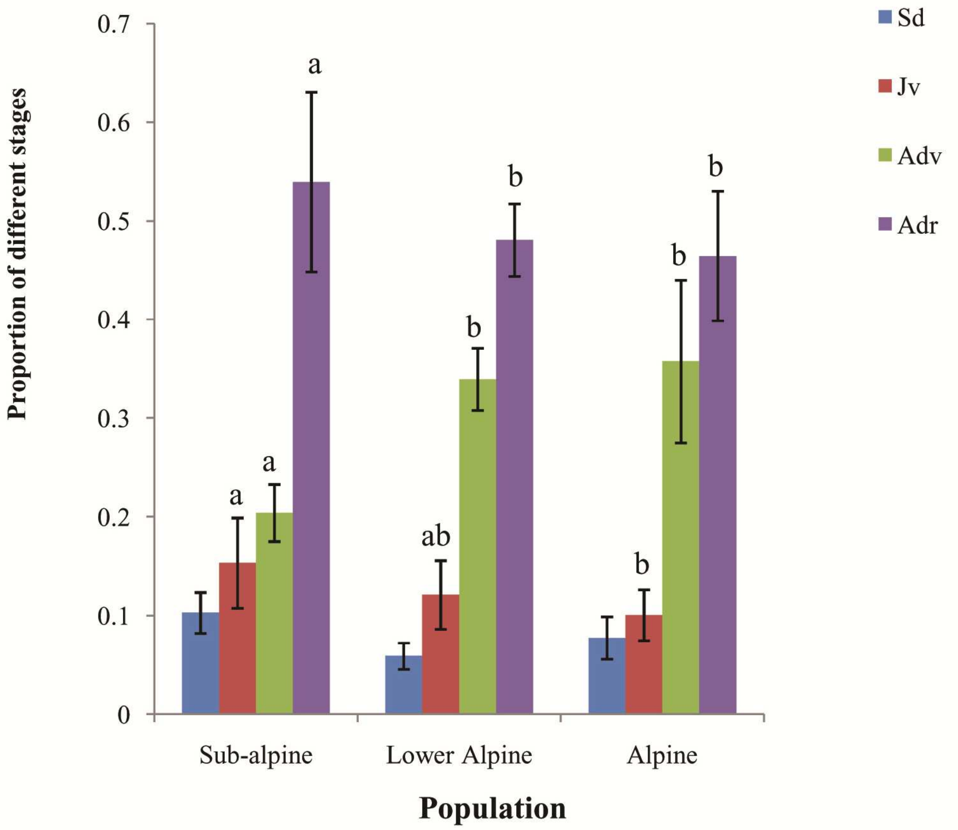


Figure 3: Population structure of *A. spicatum* in Annapurna Conservation Area, Central Nepal (Sd, seedling; Jv, juvenile; Adv, adult vegetative; Adr, adult reproductive). Bars with same letter for each stage class among populations do not vary significantly at $p = 0.05$. Sd (ns)

2.3.2 Variation in plant size and reproductive performance

The plants showed the greatest vegetative vigor in terms of plant height and stem girth in the subalpine population (Table 3). Similarly, the subalpine population showed the highest total numbers of flowers and fruits per plant (Table 3).

The number of daughter tubers per individual tended to be higher in higher-elevation populations though the difference was not statistically significant (Table 3). The dry mass of daughter tubers, and that of below- and aboveground plant parts were significantly lower in the alpine than in the other populations (Table 3). In lower alpine and subalpine populations, the average biomass of belowground plant parts exceeded that of the aboveground parts. The dry biomass ratios for plant parts below and above ground, and for aerial vegetative and reproductive parts varied significantly among the studied populations (Table 3). The dry biomass ratios for plant parts below and above ground were highest for plants from the subalpine population, and the ratio of dry biomass of reproductive and aerial vegetative parts was highest in the alpine population (Table 3).

Table 3: Variation in life history traits (vegetative traits and reproductive outputs) of *A. spicatum* among three populations in Annapurna Conservation Area, Central Nepal. Values are mean ± SE (n = 60). *Population comparisons were based on either ^{1}Kruskal-Wallis tests or ^{2}one-way ANOVA. Values for each trait among the populations with same superscript letter do not vary significantly at p = 0.05 level based on multiple comparison tests after ^{1}Kruskal Wallis tests and ^{2}one-way ANOVA

Traits	**Subalpine (3,000-3,300 m)**	**Lower alpine (3,400-3,700 m)**	**Alpine (3,800-4,200 m)**	***χ2/F* value***	***P*-value**
Leaf number^{1}	12.05 ± 0.33^{a}	10.9 ± 0.46^{a}	6.35 ± 0.18^{b}	40.22	<0.001
Plant height (cm)^{2}	156.43 ± 5.62^{a}	151.28 ± 5.29^{a}	81.77 ± 4.20^{b}	67.47	<0.001
Stem girth (mm)^{1}	10.04 ± 0.95^{a}	9.60 ± 0.68^{a}	6.62 ± 0.25^{b}	12.51	0.001
Length of floral axis (cm)^{1}	19.67 ± 0.86^{a}	19.13 ± 1.42^{a}	10.01 ± 0.60^{b}	33.18	<0.001
Average leaf area (cm^{2})^{1}	175.36 ± 14.21^{a}	190.98 ± 10.98^{a}	91.57 ± 4.95^{b}	31.26	<0.001
No. of buds per plant^{1}	29.77 ± 10.03^{a}	9.14 ± 2.89^{ab}	6.00 ± 1.19^{b}	12.48	0.001
No of flowers per plant^{1}	15.85 ± 4.44	10.61 ± 2.03	6.20 ± 0.71	5.29	0.070
No of fruits per plant^{1}	22.15 ± 6.80^{a}	17.5 ± 4.56^{a}	4.12 ± 0.67^{b}	10.72	0.004
Total reproductive parts per plant^{1}	39.85 ± 7.31	26.75 ± 5.07	16.45 ± 1.71	4.89	0.086
Number of daughter tuber per plant^{1}	1.6 ± 0.19	1.85 ± 0.25	1.80 ± 0.27	0.45	0.799
Volume of daughter tuber (mm^{3})^{1}	272.66 ± 25.97^{a}	246.41 ± 41.94^{a}	109.67 ± 12.29^{b}	25.88	<0.001
Dry biomass of a daughter tuber (g)^{2}	17.88 ± 2.83^{a}	12.18 ± 0.82^{a}	4.58 ± 0.55^{b}	32.58	<0.001
Total dry biomass of daughter tubers per plant (g)^{2}	21.92 ± 3.47^{a}	16.09 ± 1.32^{a}	5.60 ± 0.86^{b}	27.02	<0.001
Total dry biomass of mother tuber per plant (g)^{2}	8.04 ± 1.03^{a}	7.85 ± 0.79^{a}	1.87 ± 0.21^{b}	40.29	<0.001
Total belowground dry biomass (including daughter and mother tubers) per plant (g)^{2}	29.97 ± 4.39^{a}	23.95 ± 1.83^{a}	7.86 ± 0.95^{b}	36.14	<0.001
Total above ground dry biomass per plant (g)^{2}	23.61 ± 2. 90^{a}	21.95 ± 1.46^{a}	9.95 ± 1.05^{b}	16.22	<0.001
Ratio of dry biomass of below-ground and aboveground parts^{2}	1.34 ± 0.12^{a}	1.13 ± 0.07^{a}	0.86 ± 0.07^{b}	7.00	<0.01
Ratio of dry biomass of reproductive and aerial vegetative parts^{2}	0.21 ± 0.03^{a}	0.27 ± 0.04^{a}	0.46 ± 0.05^{b}	9.61	<0.001

The double-logarithmic allometric model revealed a significant relationship between plant height and total dry mass of tubers (both daughter and mother tubers) (*P* = <0.0001, Figure 4). Overall, compared to the subalpine and lower alpine populations, the alpine population was generally characterized by stunted growth, low biomass, fewer and smaller leaves and fewer and smaller reproductive structures.

The volume of fruits varied significantly among the populations (χ^2 = 51.58, *P* = <0.0001) with highest mean value (19.19 ± 0.49 cm^3) for the lower alpine population and lowest mean (14.41 ± 0.33 cm^3) for the alpine population (Table 4). Similar result was recorded for the number of seeds per fruit (χ^2 = 17.85, *P* = 0.0001). The number of seeds per unit volume of fruit was found to vary significantly among the three populations with highest mean value for the alpine population ($\chi^{2\,=}$ 7.74, *P* = 0.0208). Similarly, higher seed mass per 50 seeds was found in the alpine than in the lower alpine and subalpine populations (*F* = 5.69, *P* = 0.006) (Table 4). Seed viability and fecundity were highest in the alpine and lowest in the subalpine population (Table 4). The seed viability showed statistically significant differences among the populations (*F* = 10.69, *P* < 0.0001).

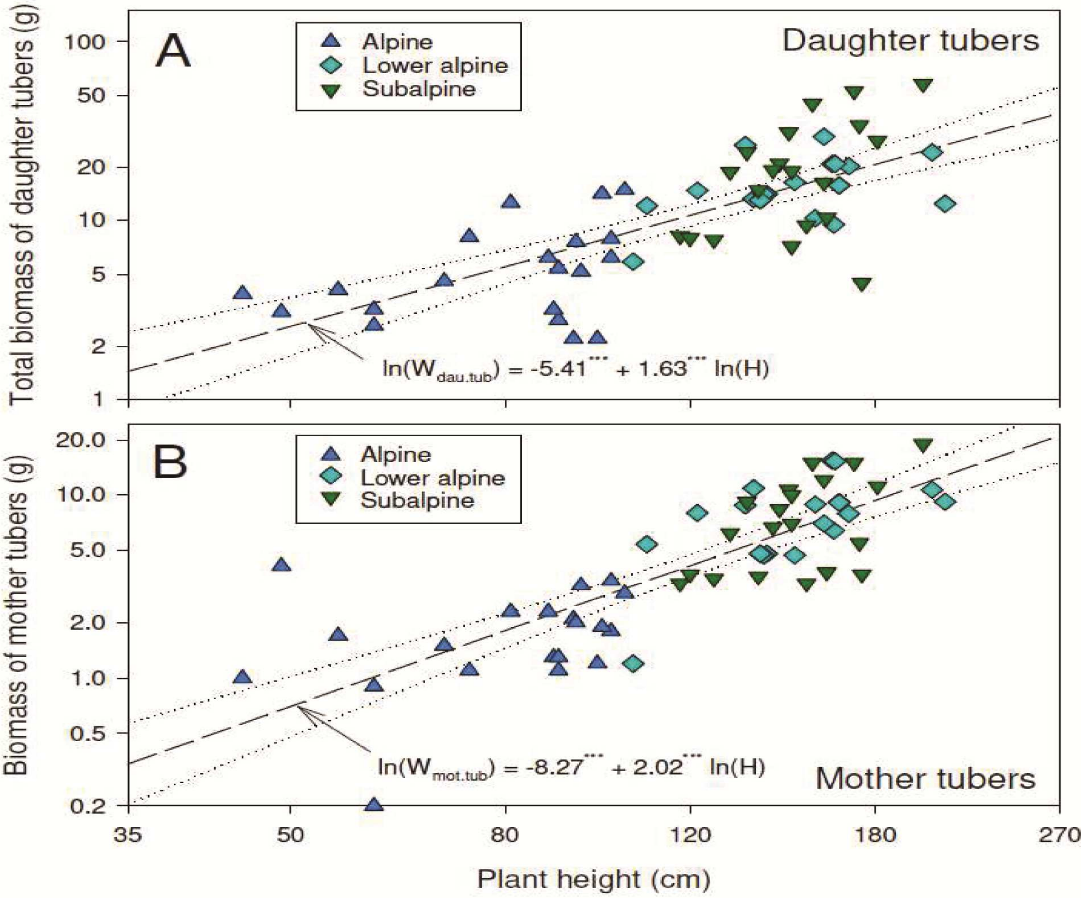


Figure 4: Relationship between plant height and dry biomass of daughter tubers (A) and mother tubers (B) of *Aconitum spicatum*

Table 4: Variation in reproductive performance of *A. spicatum* among three populations in Annapurna Conservation Area, Central Nepal. Values are mean ± SE (n = 225). *Population comparisons were based on either ^{1}Kruskal-Wallis tests or ^{2}one-way ANOVA. Values for each trait among the populations with same superscript letter do not vary significantly at p = 0.05 level based on multiple comparison tests after ^{1}Kruskal-Wallis and ^{2}ANOVA

Reproductive performance traits	**Subalpine (3,000-3,300 m)**	**Lower alpine (3,400-3,700 m)**	**Alpine (3,800-4,200 m)**	**$\chi 2/F$ value***	***P*-value**
Dry weight of fruit and seed (g)^{1}	0.08 ± 0.004 ^{a}	0.09 ± 0.004 ^{ab}	0.1 ± 0.003 ^{b}	9.64	0.008
Volume of fruit (cm^{3})^{1}	18.91 ± 0.75 ^{a}	19.19 ± 0.49 ^{a}	14.41 ± 0.33 ^{b}	51.58	<0.0001
No. of seeds per fruit^{1}	37.21 ± 1.76 ^{a}	47.16 ± 1.95 ^{b}	36.63 ± 2.59 ^{a}	17.85	<0.0001
No. of seeds per unit volume of fruit^{1}	2.08 ± 0.10 ^{a}	2.44 ± 0.07 ^{b}	2.60 ± 0.18 ^{ab}	7.74	0.0208
Dry weight of seeds per fruit (g)^{1}	0.03 ± 0.002	0.03 ± 0.003	0.04 ± 0.003	1.34	0.511
Seed mass of 50 seeds (g)^{2}	0.04 ± 0.004 ^{a}	0.04 ± 0.004 ^{a}	0.06 ± 0.002 ^{b}	5.69	0.006
Seed viability (%)^{2}	16.07 ± 0.99 ^{a}	17.14 ± 1.05 ^{a}	22.5 ± 0.64 ^{b}	10.69	<0.0001
Fecundity	2.59	2.71	2.9		

Table 5: Summary of environmental variables (substrate and topographic) characterizing the three study populations of *A. spicatum* in Annapurna Conservation Area, central Nepal. Estimates for substrate and topographic variables are specified as mean ± SE; for topographic variables ranges are also stated

Substrate variables	**Subalpine (3,000–3,300 m)**	**Lower alpine (3,400–3,700 m)**	**Alpine (3,800–4,200 m)**
Shrub cover (%)	7.74 ± 1.23	11.28 ± 2.02	0.78 ± 0.51
Herb cover (%)	62.4 ± 2.51	60.2 ± 1.96	49.44 ± 3.21
Grass cover (%)	6.92 ± 1.04	8.43 ± 1.19	30.5 ± 2.89
Bryophyte cover on soil (%)	6.02 ± 0.41	7.96 ± 0.76	5.1 ± 0.46
Lichen cover on soil (%)	1.18 ± 0.10	0.89 ± 0.10	1.06 ± 0.11
Litter cover (%)	6.07 ± 0.45	11.45 ± 1.15	7.56 ± 0.80
Solid rock cover (%)	16.62 ± 2.59	7.65 ± 1.10	5.34 ± 1.15
Scree cover (%)	0.00 ± 0.00	0.63±0.20	0.00 ± 0.00
Bare ground cover (%)	0.80 ± 0.28	2.78 ± 0.41	1.00 ± 0.27
Lichen cover under vascular plants (%)	1.05 ± 0.10	0.88 ± 0.13	1.04 ± 0.14
Bryophyte cover under vascular plants (%)	9.63 ± 0.74	11.72 ± 1.01	5.92 ± 0.65
Bryophyte cover on rock (%)	24.93 ± 3.34	17.92 ± 2.67	7.49 ± 1.66
Lichen cover on rock (%)	2.37 ± 0.50	1.06 ± 0.15	4.73 ± 1.18
Bare rock cover (%)	20.88 ± 3.15	41.86 ± 3.84	22.22 ± 3.70
Topographic variables			
Elevation (m asl)	3204.16 ± 9.74 (3000–3300)	3584.16 ± 11.15 (3400–3700)	3968.66 ± 11.80 (3800–4200)
Slope (degrees)	24.5 ± 1.37 (3–55)	19.25 ± 1.03 (3–39)	11.94 ± 0.94 (3-40)
Relative radiation index (RRI)	0.85 ± 0.01 (0.71–0.99)	0.88 ± 0.01 (0.78–0.99)	0.85 ± 0.01 (0.59–0.96)

2.3.3 Response of density of different life stages to environmental variables (mixed ZIP models)

The three studied populations varied in substrate and topographic conditions (Table 5). The subalpine population was located in herb-dominated pasture with loamy soil. The lower alpine population was in a shrub dominated pasture with high bryophyte, litter and scree cover. It had sandy loam soil. The alpine population was found in a grass-dominated pasture with silty soil. The subalpine population was affected more by anthropogenic pressure compared to the other two populations (Figure 5).

Mixed Zero-inflated Poisson (mixed ZIP) regression models revealed that harvesting and herb cover had significant negative effects on seedling density. For juvenile density, harvesting had a significant negative effect. The other variables tested did not show significant effects on seedling and juvenile density. In the case of adult stages (both vegetative and reproductive adults) population showed a significant effect on density. The mixed ZIP model analysis further showed that, compared to the alpine population and taking cover and disturbance variables into account, the subalpine population exhibited a significantly lower density of vegetative and reproductive adult stages. Interestingly, animal droppings and fire had a significant positive effect on adult density (Table 6). The zero–inflation model component was significant for juvenile and adult reproductive stages but no covariates showed any significant effect on this component.

Table 6: Mixed Zero-inflated Poisson regression models for the density (m^{-2}) of seedling, juvenile, adult vegetative and adult reproductive stages *of A. spicatum*. Densities of different stages were modeled as functions of population, relative radiation index (RRI), shrub cover (%), herb cover (%) and disturbance (animal droppings, trampling, harvesting and fire, which were assessed using an ordinal 0-4 scale). Zi is the intercept in the zero-inflation component; all other parameters refer to the count component of the model. Parameter estimates with standard errors in brackets for full average and conditional average models

		ZI component	**Count component**									
	Average model	**Zi**	**Intercept**	**Lower alpine**	**Subalpine**	**Shrub cover**	**Herb cover**	**RRI**	**Harvesting**	**Trampling**	**Animal droppings**	**Fire**
Seedling	**Full**	-0.382	-1.703	-0.185	-0.303	0.003	-0.008	1.247	-0.955**	0.011	0.022	0.007
		(1.003)	(2.109)	(0.410)	(0.555)	(0.006)	(0.007)	(2.349)	(0.332)	(0.131)	(0.072)	(0.063)
	Conditional	-0.382	-1.703	-0.185	-0.303	0.010	-0.012*	3.491	-0.955**	0.053	0.185	0.081
		(1.003)	(2.109)	(0.411)	(0.555)	(0.008)	(0.006)	(2.759)	(0.332)	(0.281)	(0.116)	(0.191)
Juvenile	**Full**	0.941***	-0.131	0.083	-0.620	0.001	-0.000	0.248	-0.536*	-0.187	0.044	0.001
		(0.280)	(1.110)	(0.380)	(0.573)	(0.005)	(0.003)	(1.188)	(0.252)	(0.269)	(0.093)	(0.086)
	Conditional	0.941***	-0.131	0.083	-0.620	0.011	0.000	1.390	-0.561*	-0.369	0.182	0.007
		(0.280)	(1.109)	(0.380)	(0.573)	(0.009)	(0.005)	(2.510)	(0.229)	(0.276)	(0.102)	(0.189)
Adult Vegetative	**Full**	0.121	0.206	0.152	-1.685***	-0.000	0.000	0.138	-0.097	-0.104	0.123	-0.029
		(0.198)	(0.589)	(0.215)	(0.375)	(0.001)	(0.002)	(0.630)	(0.125	(0.155)	(0.088)	(0.085)
	Conditional	0.121	0.206	0.152	-1.685***	-0.002	0.003	1.749	-0.173	-0.183	0.165**	-0.047
		(0.198)	(0.589)	(0.215)	(0.375)	(0.007)	(0.003)	(1.478)	(0.121)	(0.167)	(0.060)	(0.104)
Adult reproductive	**Full**	-1.007**	0.035	0.171	-0.826***	0.000	-0.000	0.044	-0.025	-0.046	0.107	0.178*
		(0.312)	(0.329)	(0.181)	(0.235)	(0.00)	(0.000)	(0.334)	(0.059)	(0.096)	(0.064)	(0.082)
	Conditional	-1.007**	-0.035	0.171	-0.826***	0.004	-0.000	1.485	-0.088	-0.099	0.124*	0.186*
		(0.312)	(0.329)	(0.181)	(0.235)	(0.004)	(0.002)	(1.255)	(0.083)	(0.121)	(0.052)	(0.074)

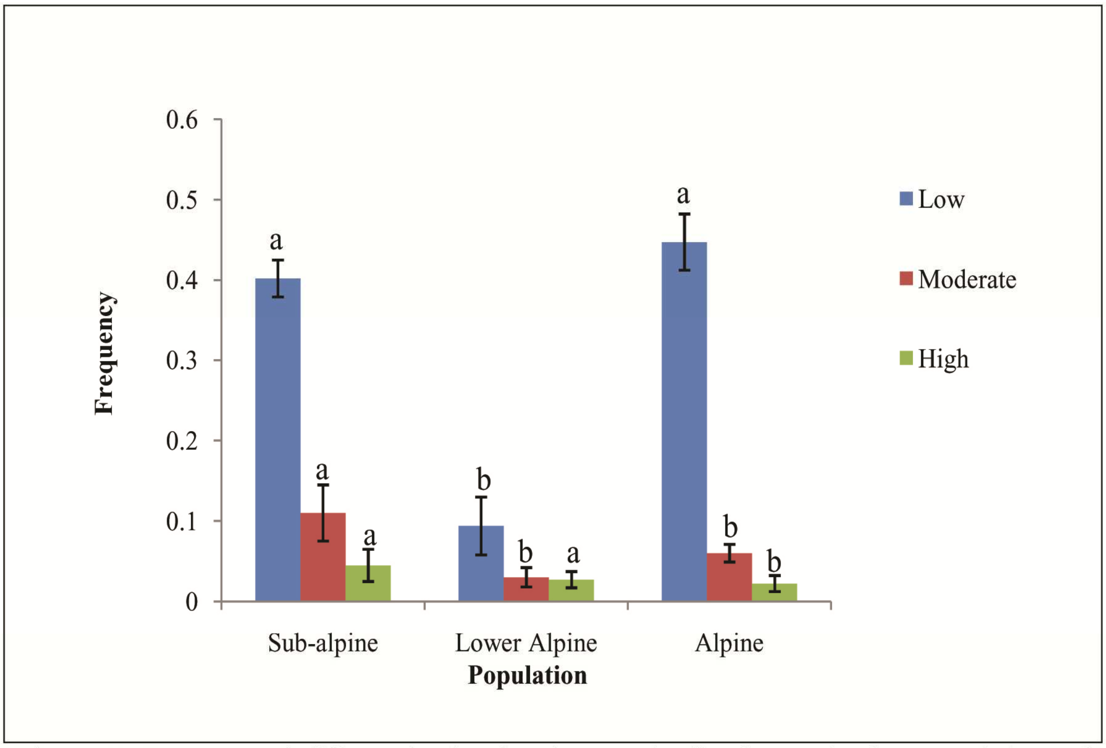


Figure 5: Frequency of different levels of anthropogenic disturbances in three populations of *A. spicatum* in Annapurna Conservation Area, Central Nepal. Mean of animal droppings, trampling, harvesting and fire intensity. Scale: 0-1 = low, 2 = moderate, 3-4 = high. Bars with same letter for each disturbance class among populations do not vary significantly at p = 0.05 level based on multiple comparison test after Kruskal Wallis

2.3.4 Socio-cultural and economic aspects of *A. spicatum*

Ethnoecological survey revealed that only one percent of the harvester use it locally (all the local healers) while ninety nine percent of the harvesters harvest it for trade due to its poisonous nature. Locally the tuber is given orally (after proper detoxification) only by the healers to induce abortion and to treat chronic diseases (long period of sickness). Detoxification is done by prolonged boiling in water, milk or alcohol. Forty- five percent of the informants are of the opinion that the seedlings with the single leaf possess high medical efficacy so collectors even harvest the seedlings. Commercial collectors are the outsiders (Dhadhing and gorkha districts) as ninety percent of the informants are of the opinion that the population is decreasing due to the harvesters from other parts of the country. Eighteen percent of the informants were aware of the population ecology of *A. spicatum*. Twenty six percent of the informants are of the opinion that the collectors harvest the plant irrespective of the plant size and season (seed dispersal and tuber maturity) and they also believe that wild bear, pika, insects and pathogents are destroying the populations of *A spicatum*.

Each harvester harvest 0.2 kg of dried tuber per year, thus a total of 13-15 kg dried tuber was harvested from that region annually. A total of 800-900 individuals were harvested per year from the region as 1 kg of dried tuber consists of 60-80 individual tubers. A kg of fresh tuber was sold in NRS 500 and dried tuber in NRS 1000.

2.4 Discussion

2.4.1 Variation in population density and structure

The mean density of *A. spicatum* in the populations examined is comparable with previous findings. For example, densities of 0.34 (Kala, 2005) to 3.71 (Nautiyal *et al*., 2002) plants/m^2 in *A. balfourii*, 2.57 (Nautiyal *et al*., 2002) to 3.1 (Semwal *et al*., 2007) plants/m^2 in *A. heterophyllum*, 2.7 plants/m^2 in *A. rotundifolium* (Kala, 2005), and 2 plants/m^2 in *A. violaceum* (Kala, 2000, 2005) have been reported from the Indian Himalayas. However, much higher densities have also occasionally been observed. For example, Nutiyal *et al*. (2002) recorded a density of 6.7 plants/m^2 in *A. violaceum* in Gharwal Himalaya, India, which was attributed to limited human exploitation due to habitat inaccessibility. Similarly, Shrestha and Jha (2010) reported a mean density of 7.3 plants/m^2 in *A. naviculare* in Manang, Nepal, which was described as a result of subjective sampling due to the patchy distribution of the species.

In the high mountain ecosystem, elevation represents a complex gradient closely correlated with numerous other environmental variables, such as temperature, precipitation, soil texture and nutrients, substrate stability, and disturbance (Auerbach & Shmida, 1993; Ramsay & Oxley, 1997). All these factors affect plant life in a number of ways. The mixed ZIP model showed that the subalpine population exhibited a significantly lower density of adult vegetative and adult reproductive plants than did the lower alpine and alpine populations. The higher plant density in lower alpine and alpine populations might partly be due to lower human pressure (Figure 5) in these habitats. In addition, the smaller size of individuals in higher-elevation populations also makes it possible for a higher number of individuals to share a given amount of space. With increasing elevation, the number of competing species of similar or larger size decreases. This causes a small area to hold a large number of individuals, thus increasing the density. The shift from an environment characterized by high degree of competition to a more facilitative environment favors this higher density (Liancourt *et al*., 2017). Bosch and Waser (1999) found that the pollinator visitation is higher in dense populations of plants than in sparse populations, which may further increase seed set and ultimately the population density.

The low proportions of younger life stages (seedlings and juveniles) and correspondingly high proportion of adults (vegetative and reproductive) in the studied populations clearly indicate that the recruitment potential of *A. spicatum* is generally rather low. The recruitment was further reduced in the subalpine population. The small seed size, and low seed viability and fecundity recorded in the subalpine population compared to those of the lower alpine and alpine population support that the subalpine population is suffering from lack of sufficient natural regeneration, consequently lowering the population density. Studies have shown that disturbance characteristics determine the success of different reproductive strategies (Chambers, 1995). Anthropogenic disturbance, like harvesting and trampling can reduce seed viability and fecundity in plants from mountain ecosystems (Tonnabel *et al*., 2017) and thereby reduce the population density.

2.4.2 Response of density of different life stages to environmental variables

The mixed ZIP model showed significant negative impact of harvesting on both seedling and juvenile plant density, and population (elevation) had a significant negative effect on adult density. Based on conversations with collectors and key informants, it appears that traditional healers prefer to harvest *A. spicatum* at younger stages, believing that relatively young tubers have high medicinal efficacy. However, commercial collectors prefer mostly the large adult plants. Removal of the entire plant before seed maturation reduces the possibility of seed development for future regeneration (Sheldon *et al*., 1998). The negative effects of such damages caused by collectors may be further enhanced by infrequent sexual regeneration and possibly also by limited availability of suitable sites for regeneration in subalpine and alpine grasslands (Austhreim & Eriksson, 2001). The mixed ZIP model analysis further showed that herb cover had a significant negative impact (at the 10% level) on the density of seedlings, which may be a consequence of herb cover reducing the space available for seedling establishment, increasing the competition and thereby affecting growth and development.

Although frequent trampling by sheep, wild animals and humans damage plants and prevents them from flowering, developing seeds to maturity, and dispersing their seeds (Chardon *et al*., 2018), and seedlings of *A. spicatum* may be particularly sensitive in this regard, as they have soft stems with only 1-2 leaves, positive effects of some disturbances on density of adults was found. Mixed ZIP model revealed positive significance of animal droppings and fire on adult (both vegetative and reproductive) density. Direct observation also supports the result as the *A. spicatum* individuals seemed to show preference for growing in the proximity of cattle sheds where the soil had higher concentration of animal droppings. Ohlson and Gronli, 2006 also reported a significant positive effect on life history parameters in *A. septentrionale* growing in experimental plot supplied with higher concentrations of nutrients. The tolerance of adult stages of *A. spicatum* to fire in the present study is comparable with the findings of Uys *et al*. (2004) who showed the tolerance of many forb species to fire.

2.4.3 Variation in plant size and reproductive performance

Aconitum spicatum has developed an astonishing range of life history traits in response to highly specific ecological environments in subalpine, lower alpine and alpine habitats. A high degree of variation with respect to different traits was found among the populations (Table 3). The decreasing trend of plant height, stem girth, leaf area, length of floral axis and plant biomass observed in plants from subalpine to alpine populations (Table 3) showed that the size of individuals generally tends to decrease with increasing elevation. *A. spicatum* in the subalpine (lower elevation) population exhibited better performance in terms of high vegetative vigor and production of higher number of flowers and fruits per individual. Shrestha and Jha (2010), studying *A. naviculare,* also reported higher vigor for the individuals examined in a lower elevation population. Plants may perform better simply because of favorable abiotic conditions (Billings, 1973; Körner, 2003), such as temperature and moisture (Körner, 2003). Plant performance is also enhanced in habitats with better abiotic conditions through facilitation (positive interactions), a common phenomenon at high elevation, which helps plants to cope with the harsh environment (Callaway *et al*., 2002). Thus, the higher vegetative vigor of *A. spicatum* observed at lower elevation is presumably related to the presence of appropriate abiotic conditions in which plants exhibit enhanced growth (Shrestha & Jha, 2010). The harsh environmental conditions at higher elevation generally impose constraints on plant growth (Körner, 2003). Similar trends of decreasing plant vigor with increasing elevation have been observed in other herbs, such as *Silene acaulis,* in which disturbance has been attributed to be beneficial for the performance of plants at lower elevation (Chardon *et al*., 2018).

The decreasing number of flowers and fruits set observed along the elevation gradient is likely related to plant size but could also be due to the time of flowering, which is influenced by the ambient temperature and the timing of snow melt (Kudo & Hirao, 2006). Flower production is often correlated with plant size, which generally increases with resource availability and decreases with increasing plant density (Weiner & Thomas, 1986). Moreover, flower and fruit production are shaped by the interactions between abiotic and biotic environment (Agren *et al*., 2008; Ma *et al*., 2010). For example, population density negatively affects fruit production of individual plants through its effect on flower number (Agren *et al*., 2008). Disturbances like trampling frequently break the inflorescence, and repeated disturbances year after year also reduce the reproductive potential of the individuals, thus limiting the production of flowers and fruits (Chardon *et al*., 2018).

Like many high-elevation plants (e.g., *Arenaria kansuensis, Rhodiola quadrifida, Tribulus terrestris* etc), *A. spicatum* also allocated a large amount of biomass to its underground structure and, as a result, aboveground reproductive organs are reduced and roots are enlarged (Ma *et al*., 2010). A high proportion of belowground biomass has often been interpreted as an adaptive response to severe environmental conditions (Webber & May, 1997). Particularly for high-elevation plants, subterranean organs are dedicated to produce annual aerial parts shortly after snow melt and, thus, high elevation plants are found to invest more in belowground parts as an adaptive strategy.

Despite the lower number of flowers and fruits set per individual in alpine populations, in terms of reproductive allocation, these plants performed far better than subalpine and lower alpine populations. The ratio of dry biomass of reproductive to aerial vegetative parts was higher in plants from the alpine population than in plants from subalpine and lower alpine populations, and this clearly indicates that plants from very high elevation invest more in reproduction. The subalpine population had larger fruits than the two other populations and, as a result, they produced higher number of seeds per fruit. Contrary to this, though producing the smallest number of fruits, the plants from the alpine population, compared to the two other populations from lower elevation, set higher number of seeds per unit volume of fruits, and these were seeds with a higher seed mass. Seed production by species from high elevations depends on environmental conditions (short, cool growing seasons) and is temporally and spatially variable (Chambers, 1995). The increasing seed mass with increasing elevation can be explained by more resources being allocated to the individual seed in alpine populations, thereby increasing their viability and fecundity to cope with a stressful environment. The increase in seed mass together with a reduced number of seeds per individual in populations from higher elevations may indicate that *A. spicatum* allocates more resources to the seeds and may reflect a strategy that helps increasing fitness and long-term population persistence in harsh alpine environments.

2.5 Conclusion

Human disturbance and topographical factors are related to plot-level microclimatic conditions, which contribute to the variation in density and the studied life history traits. Among the environmental variables tested, harvesting, animal droppings and fire appeared to be the most important (proxy) factors affecting the density of *A. spicatum*. The population at the lowest elevation was exposed to the highest anthropogenic pressure, the level of which decreased with increasing elevation. The prevailing harvesting pattern was destructive as it involved uprooting of the whole plant and was probably a main reason for the lower recruitment and reduced density in the subalpine population. The enhanced vegetative performance of adults and the higher number of flowers and fruits set per reproductive individual in the subalpine population cannot compensate for the lower recruitment and loss of younger plants due to high disturbance. The lower recruitment in the subalpine population was presumably also related to reduced seed mass, and decreased seed viability. In terms of reproductive effort (higher seed set per unit fruit volume, higher seed mass, and higher seed viability and fecundity), the alpine populations were performing better. This confirmed that alpine plants developed self-sustaining adaptive strategies by increasing their resource allocation in reproductive parts, especially in seeds, to cope with the harsh environment. As the subalpine population was suffering from reduced reproductive performance, the persistence and growth of *A. spicatum* at low elevations will depend on how effectively management will address the protection of younger life stages and control over harvesting and premature harvesting of tubers. Although the adult individuals were found tolerant to animal droppings and fire, the viability of *A. spicatum* populations not only depends on controlling over harvesting and premature harvesting but also on mitigating other common anthropogenic disturbances, like grazing and trampling, to ensure completion of flowering, fruiting and dispersal of viable seeds. It is therefore recommended that when formulating management guidelines, measures aiming to mitigate such anthropogenic disturbances should be considered. As science-based harvesting and conservation strategies have not yet been developed for *A. spicatum* and other Himalayan MAPs, and harvest and trade are conducted on ad hoc basis, this study will contribute to the conservation of MAPs by helping government authorities to formulate scientifically informed conservation management strategies for *A. spicatum* and other similar MAPs.

CHAPTER 3

SUSTAINABILITY OF HARVEST OF *Aconitum spicatum* ALONG AN ELEVATION GRADIENT IN NORTH-CENTRAL NEPAL: MATRIX MODELLING OF HARVESTING EFFECTS

Abstract

Demography of a commercially threatened medicinal plant, *Aconitum spicatum* was studied and the effect of different harvesting intensities on its population dynamics was explored. Different harvesting treatments (control (0%), 25%, 50% and 75%) based on local practices were applied where (0-25%) represents low harvest intensity and 50-75% represents high harvest intensity in three different populations along the elevation in north-central Nepal. Data on recruitment, growth and survival of individuals classified by size were collected for four years. Matrix modelling approach was used to identify the pattern of variation in the demographic vital rates.

The asymptotic population growth rate (λ) was >1 in low harvest intensity (0-25%) in all the populations in all the transitions (2015-2018) indicating that the harvesting of this plant at low harvest intensity is sustainable. In the high harvest intensities (50-75%), the lambda value was <1 in lower elevation populations indicating that these populations are sensitive to high harvest. The results showed that the extinction probability derived from the stochastic simulation (1000 runs) in 100 years has extremely low value for all the populations in both the intensities. Transient dynamics revealed that the low harvested alpine population shows gradual increase in its population reaching a size of approximately 4000 individuals during the projection period of 10 years. The deterministic projection revealed that the subalpine population is the most sensitive population. The populations of subalpine (both low harvest and high harvest) can reach the low harvest intensity of alpine in more than 20 years. The high harvest population of subalpine requires 22 years to reach its low harvest intensity in contrast to 7 years of lower alpine. The higher contribution of large size classes to λ indicates that the larger size classes are the critical stages in the life cycle of *A. spicatum* and conservation priority should focus on the protection of theses stages. This study further suggests that the populations of *A. spicatum* respond differently to harvest along the elevation and there are important differences in *A.*

spicatum's capacity to regenerate after harvest in alpine versus lower and sub alpine populations.

Keywords: Conservation, elasticity, extinction probability, population growth rate, sustainable harvest

3.1 Introduction

An accurate understanding of the driving mechanisms for the population dynamics of endangered plants and the relative contributions of anthropogenic disturbances and environmental factors is crucial for their sustainable management. Collection of long-term demographic data is considered essential for good management practices to help conserve endangered plant species (Lande, 1988; Schemske et al., 1994; Byers & Meagher, 1997). Schemske et al. (1994) noted that in order to implement species recovery, it is necessary to know the rate of population growth, the key life stages that influence it, and why differences in key life stage occur among populations and years. Population growth rate is considered a standard measure to predict the future fate of populations (Benton & Grant, 2000; Caswell, 2001). Accurate estimates of vital rates and a realistic population model are required to make reliable predictions of extinction and abundance probabilities (Coulson *et al.*, 2001, Ellner *et al.*, 2002). Population models are widely used to inform management decisions and to set conservation guidelines.

Although the introduction of the Integral Projection Model (IPM) has brought about a major change in the way many demographic models are conceptualized, traditional matrix models are still useful and represent the majority of previous demographic analyzes available for comparative and conservation work (Doak et al., 2021). Although several reviews have highlighted the superiority of IPM in recent years (Merow *et al.*, 2014; Rees *et al.*, 2014), these claims have not been critical or fully evaluated (Doak *et al.*, 2021).

Stage-based matrix models have been used extensively to assess the effects of harvest on plant population dynamics and population performance (Menges, 2000; Caswell, 2001). In such studies, stage-based matrix models are constructed from repeated measurements of vital rates (survival, growth, and reproduction) over several years.

The population growth rates (λ) are calculated from this data and assesses whether the population will grow, remain stable, or decline over the long term under current conditions. For harvested populations, $\lambda \geq 1$ is usually interpreted to reflect a sustainably harvested population, whereas $\lambda < 1$ is interpreted to reflect unsustainable harvest (Svenning & Macía, 2002; Endress *et al.*, 2006).

Elasticities derived from these models can be used to determine vital rates and stages that are most sensitive to environmental and other changes, and can inform recovery efforts for endangered species (de Kroon *et al.*, 1986; Caswell, 2001; Morris & Doak, 2002). In addition, elasticity analysis (Caswell, 2001) has been widely used to predict the impact of MAP harvesting and to suggest management strategies to mitigate the harvesting impacts (Ratsirarson *et al.*, 1996; Anderson & Putz, 2002; Freckleton *et al.*, 2003). Elasticity analysis predicts how λ would change in response to small changes in population vital rates. For example, a small change in a vital rate with high elasticity values will have a large effect on λ; similarly, changes in vital rates with low elasticity will result in very small changes in λ.

Changes in population size and habitat conditions such as nutrient enrichment and management practices were previously reported to have an impact on single vital rates. In particular, population size, nutrient availability and management practices may affect seed production (Oostermeijer *et al.*, 1998; Lennartsson & Oostermeijer 2001; Münzbergová, 2006), germination rate (Colling *et al.*, 2002; Lindborg & Ehrlén 2002) and seedling survival (Colling *et al.*, 2002). The same factors have been reported to influence the stasis and growth of individuals (Hegland *et al.*, 2001; Tomimatsu & Ohara, 2010; Dahlgren & Ehrlén, 2009).

A key for undertaking effective conservation actions is to understand the importance of stochasticity, demographic parameters and deterministic population dynamics on the performance of populations. So far, demographic parameters have been mainly used to gain insight into population viability (Reed *et al.*, 2002). Furthermore, important life history factors such as dormancy, seed banks and vegetative propagation are often neglected in research (Zeigler *et al.*, 2013). However, it is difficult to estimate the survival and re-emergence rates of dormancy-prone plant species (Alahuhta *et al.*, 2017). Therefore, the role of dormancy in life history is not well understood (Lesica & Crone, 2007. Dormancy is thought to be a means of buffering adult survival against environmental stochasticity in long-lived clonal plants (Shefferson *et al*., 2005). Many dormancy-prone plant species are rare and endangered (Reintal *et al*., 2010) and are important species for conservation (Alahuhta *et al*., 2016).

Fecundity and other vital rates are expected to vary with population size and resource availability (Horvitz & Schemske, 1995; Silvertown *et al*., 1996; Endels *et al*., 2004; Verheyen & Hermy, 2004; Valverde & Silvertown, 1998). Small populations had lower survival rates compared to large populations. Low recruitment resulted in significantly lower predicted population growth rates in small populations. Differences among populations in reproductive output are expected to influence the demographic parameters of interest. Available resources can vary spatially and temporally thus demographic processes need to be measured in multiple sites and years (Horvitz & Schemske, 1995; Menges, 2000; Lehtila *et al*., 2006).

Species with small populations are strongly influenced by demographic stochasticity (Dinnetz & Nilsson, 2002; Kery *et al*., 2003; Synder, 2003). The stochastic effect on survival varies among populations and stochastic simulations have shown that only large populations are viable, while small populations are expected to become extinct within decades (Dinnetz & Nilsson, 2002). Small populations may be at higher risk of extinction not only due to overall worse performance of individuals, but also due to higher potential effects of demographic and environmental stochasticity. Such potential hazards of small populations motivated recent studies that explored the effect of population size on the plant performance, population growth rate, extinction probability and variation in response to the population on different harvesting regimes.

Thus, the major aim of this research is to study the effect of harvesting on the population dynamics of *Aconitum spicatum* in three different populations along the elevation gradient.

Specifically, the following questions were addressed

1) How the population growth rates vary between the studied populations and years?
2) Which demographic processes are most important in producing the changes in growth rate caused by harvesting?

3) How do different harvesting regimes influence future population size?

4) What is the risk of extinction of the studied populations?

Answers to these questions provide a basis for recommendations aimed at the sustainable management of *A. spicatum* and may help to define other needed studies.

3.2 Materials and methods

3.2.1 Study species

Refer to study species in chapter 2

3.2.2 Study area

The field work was performed during 2015 to 2018 in three populations in Annapurna Base Camp region of Annapurna Conservation Area (N28°31.733' and E83°53.661' to N28°29.856' and N83°53.855') in Central Nepal (details in chapter 2). The three studied populations (subalpine-3200 m, lower alpine-3400 m and alpine-3700 m) were selected for permanent plot establishment. The three populations differed in elevation and other environmental variables (Table 5, chapter 2).

3.2.3 Sampling design and demographic data collection

Three permanent belt transects of 2 m × 14 m were laid down in each population. Each transect was divided into seven 2 m × 2 m subplots. Four alternate subplots were considered systematically for harvest simulation and detailed biological studies. Detailed demographic observations were conducted in three populations over four years from 2015 to 2018. The studied populations differed in size, the largest population consisted of c. 1500 individuals and the two smaller populations consisted of 262 and 185 individuals (including all the stages during 4 years period). Two smaller populations occurred along the graveled trekking trail prone to landslides, while the largest population occupies the alpine grassland meadows. The life cycle of *A. spicatum* was divided into four life stages based on morphological and reproductive criteria: (1) seedling (Sd), (2) juvenile (Jv) (3) adult vegetative (Adv) (4) adult reproductive (Adr) (see chapter 2) and dormant adult (Dr) (Figure 6) Additionally the dormant stage was added during further classification where the individuals remain alive by tubers.

Seed germination experiments were carried out. The seeds sown in 2016 germinated in 2017 and no new germinated seedlings were recorded in 2018. It is thus assumed that this species has no permanent seed bank, so seed bank category was not included in the matrices.

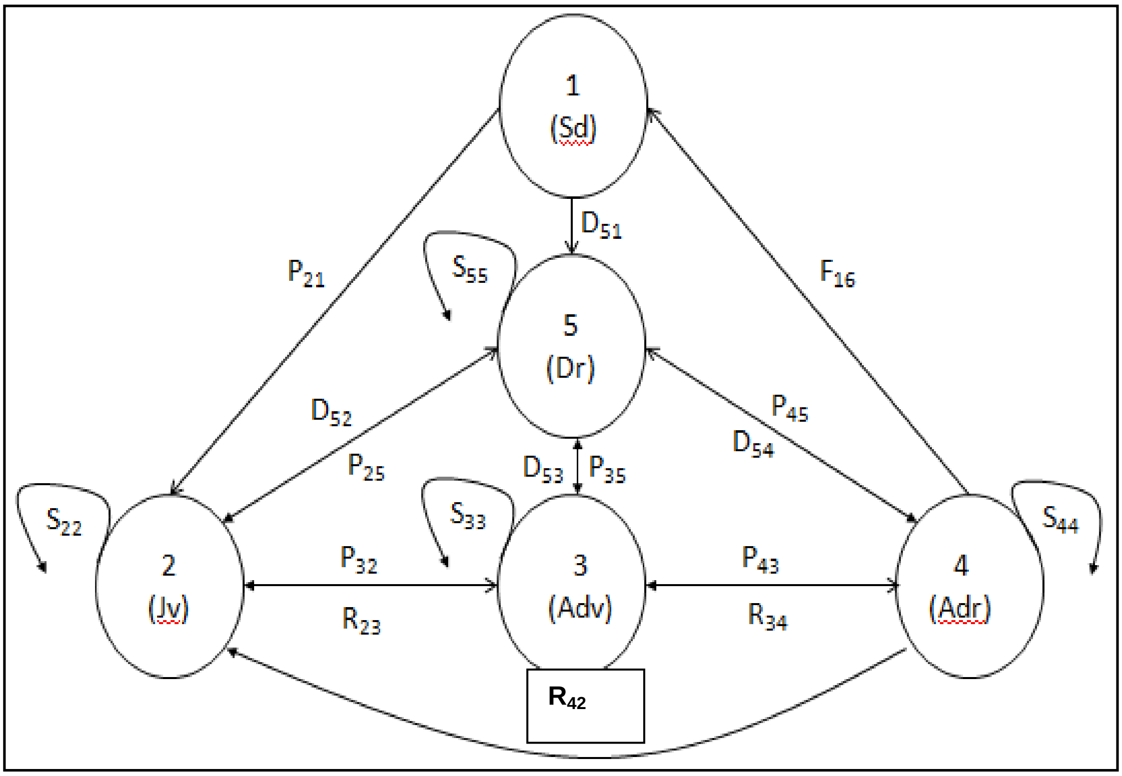


Figure 6: Life cycle graph of *A. spicatum*

The stage of 'seedling' was defined as newly established individual with cotyledon leaf that developed directly after germination of the seeds. The stage 'juvenile' was defined as an individual plant older than one year, with a maximum of four leaves and stems girth <3.0 mm. Larger vegetative individual with >3.0 mm stem girth are considered as vegetative adult. All reproductive (flowering and fruiting) individuals were placed in the adult reproductive stage. Vegetative and reproductive individuals could not be further divided into different size classes, as the main difference lies in plant size between population levels. The stage 'dormant adult' was defined as individuals in a dormant state, without any organs visible above ground but the plant has alive tuber. To identify individuals that died in the last year of the study, 20% of the dormant individuals were excavated and marked as dead or dormant, and the rest were assigned a calculated value and marked as dormant or dead. None of the monitored individuals dormant for two consecutive years appeared to be alive in the third year. Thus, the individuals which were dormant for two consecutive years were considered as dead. During the analysis the individuals whose stage was not determined in the last year was considered as dormant (except for seedlings, as the seedlings obtained only from the seeds were recorded during the data collection).

The extinction probability for each population was calculated using the population numbers in each population from the first year of demographic observations. Projections of the resulting population vectors were made over 10 years.

3.2.4 Harvest simulation and monitoring

The harvest treatment was applied following the local harvesting procedure. An agreement was made with the hotel owner association in the study area to take care of the permanent plots during the study period. Four different harvest simulations were applied systematically and randomly in each of the four plot which included 0% (control) no harvest, 25%, 50% and 75%. To create low harvest (0-25%) intensity individuals in 0% and 25% harvest plots were combined together. Similarly, to create high (50-75%) harvest intensity individuals in 50% and 75% harvest plots were combined together. Thus, each population received three replicates of high (50-75%) and low (0-25%) harvest intensity. The individuals in different stages (Sd, Jv, Adv, Adr and Dr) in each subplot were tagged using metallic plates (3×3 cm) with individual tag number. All the tagged individuals were monitored in early September in 2016, 2017 and 2018 during the peak growing season. All the newly found individuals were tagged during monitoring. The recorded parameters included plant height, number of leaves, size of leaf (length and breadth of two largest leaves), stem girth, length of floral axis and number of reproductive parts (bud, flower and fruits).

Small new vegetative plants found within the population were also tagged each year. The new adult individuals (developed from the dormant tubers) observed during the monitoring years were also tagged and all the demographic parameters were recorded.

3.3 Matrix construction and analysis

Population matrix model (Caswell, 2001) was used to estimate the demographic parameters. Due to the insufficient tagged individuals monitoired during all the transitions 0% and 25% harvest treatment were combined together and labelled it as low harvest intensity and 50 and 75% were combined together and labelled as high harvest intensity to run successful analysis. For each population and condition, annual transition matrices based on September/October censuses data were built. During this the demographic processes i.e., the proportion of individuals that transit into higher size class (P, progression), proportion of individuals that remain in the same stage class (stasis, S), proportions of individuals that regressed or shrank into a smaller size class (R, retrogression) and (Dr, dormant) remaining alive by tubers where the above ground part is not visible were recorded. A seed category was excluded from the life cycle model. It is assumed that germination of the seed takes place the year after the seed is produced and there is absence of the seed bank. The basic matrix model is given by: $n_{(t+1)} = A\ n_{(t)}$ where, n(t+1) and nt are vectors whose elements, aij are the number of individuals that belong to the ith category at time t and t + 1, respectively, and where **A** is a 5 × 5 stage-based matrix with the matrix elements representing transition probabilities among stages, n(*t*) is a vector of the number of individuals in each of the stage classes in year t, and n(t + 1) is the population vector in the next year t + 1.

A set of average matrices were constructed for each condition in each population. Each average matrix was a weighted mean of the three annual matrices, constructed by weighting each transition within a column by the sample size. Stochastic simulations of transition matrices were also performed to estimate extinction probabilities, transient dynamics, post harvest population recovery time. The fecundity data of *A. spicatum* obtained in chapter 2 were used for all the studied populations. Confidence intervals (95%) for lambda for each matrix were obtained by using the bootstrap method (Caswell, 2001). A bootstrap dataset is generated 1,000 times for each matrix by randomly resampling with permutations from the original dataset and compared those quantities between populations and among conditions. Elasticities were estimated to investigate the response of population growth rates to potential changes in vital rates caused by harvest (Silvertown *et al.*, 1993; Caswell, 2001).

3.4 Results

3.4.1 Stage structure and population growth rates

The more disturbed populations of lower elevation (with small population size), i.e., lower alpine and subalpine differ much in observed and stable stage distribution than in less disturbed alpine population as shown by higher value of Keyfitz's Δ (Appendix V).

In alpine population, all the matrix models predicted asymptotic population growth rates, $\lambda > 1$ even in the high harvest condition with the exception of low λ value (0.6203) in 2017-2018 transition in high harvest intensity. Asymptotic population growth rates (λ) were higher in low harvest than in high harvest populations in all the transition years in alpine populations. While making comparision between low harvest and high harvest populations there is statistically significant decrease in population growth rate in 2017-2018 transition as indicated by non overlapping CI (Table 7)

The alpine population has higher asymptotic population growth rates (λ) than the lower and subalpine populations in all the transitions in both types of harvest treatment except 2017-2018 transition in high harvest treatment (0.6203) (Table 7).

In lower alpine and sub alpine populations asymptotic population growth rates (λ) were higher in low harvest than in high harvest populations and has decreasing lambda value from first to third transition year. The asymptotic population growth rates (λ) during 2016/2017 and 2017/2018 transition showed significant differences as indicated by non overlapping CI (Table 7).

Table 7: Asymptotic population growth rates (λ) and 95% CI for low and high harvest and year in three different populations in Annapurna Conservation Area, North-Central Nepal

Populations	**Transition periods**	**Low harvest (0-25%)**		**High harvest (50-75%)**	
		Lambda	**95% CI**	**Lambda**	**95% CI**
Alpine	2015-2016	1.3513	1.3010- 1.3959	1.3330	1.2833- 1.3761
	2016-2017	1.0349	0.9853- 1.0810	1.0452	0.9901- 1.0972
	2017-2018	1.2372	1.1420- 1.3089	0.6203	0.5639- 0.6779
	Average (2015-2018)	**1.2438**		**1.0423**	
	Average (stochastic)	**1.2541**	0.9886-1.5976	**1.0448**	0.6023- 0.6779
Lower alpine	2015-2016	1.1767	1.0567- 1.2701	1.0966	0.9874- 1.1974
	2016-2017	1.1642	1.0590- 1.2459	0.9691	0.8401- 1.0407
	2017-2018	1.0865	0.9639- 1.1995	0.8716	0.2535- 0.8000
	Average (2015-2018)	**1.1449**		**0.9788**	
	Average (stochastic)	**1.1480**	1.0826 – 1.2398	**0.9856**	0.7718-1.2569
Subalpine	2015-2016	1.0761	1.0000- 1.2343	1.0504	0.7833- 1.1493
	2016-2017	1.0270	0.9347- 1.1266	0.9159	0.8257- 1.0052
	2017-2018	1.0390	0.9080- 1.2176	0.8671	0.2105 – 1.0000
	Average (2015-2018)	**1.0300**		**0.9763**	
	Average (stochastic)	**1.0373**	0.8700- 1.1663	**0.9823**	0.7911-1.2172

3.4.2 Elasticity analysis

The elasticity analysis revealed that in the alpine population the demographic process progression (P growing to a larger size class or a vegetative growth) has highest contribution to lambda followed by stasis (S, survival and remaining in the same stage class) in both the harvest treatments. In lower alpine population progression has higher contribution to lambda in low harvest and stasis in high harvest intensities. Likewise in sub alpine population progression and stasis has almost equal contribution to lamda in both the harvest intensities. The highest elasticity values were due to the contribution of individuals from large size classes, i.e., adult vegetative and adult reproductive (Figure 7 d). Fecundity has lowest contribution to lambda in subalpine population as this population is exposed to high disturbance pressure and thus even the low harvest treatment is highly sensitive.

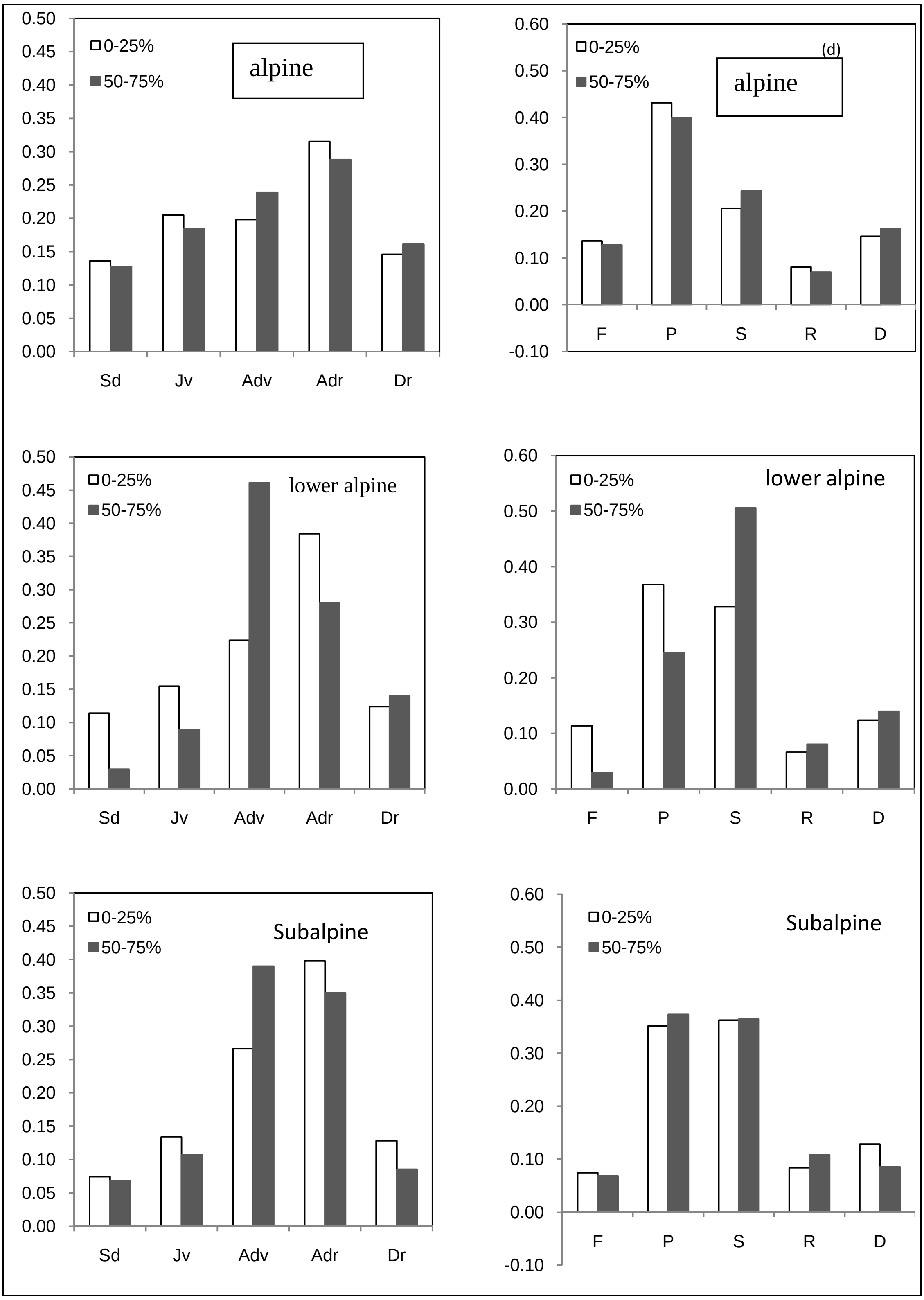


Figure 7: Sum of elasticity values for five stages (Sd = seedling, Jv = juvenile, Adv = adult vegetative, Adr = Adult reproductive and Dr = dormant) and demographic processes (F = Fecundity, P = Progression, S = Stasis, R = Retrogression, D = Dormancy). Elasticity values were calculated from weighted average matrices, in which elasticity values were for the year 2015–2018

3.4.3 Transient dynamics, stochastic simulation and population recovery

The analysis of transient dynamics obtained from the average matrices (Figure 8) indicated that the alpine populations if subjected to low harvest intensity there is gradual increase in population size (approximately 4000 individuals) during the projection period. In high harvest intensity population size is maintained almost constant during the projection period. In lower alpine population in low harvest there is slight increase in the population size while in high harvest there is slight decrease in the population size during the projection period. The subalpine population showed slight decrease in population size in both low and high harvest intensities during the projection period of ten years (Figure 8).

Population growth rates calculated from stochastic (bootstrapping) simulations were very similar to those obtained from time invariant deterministic projections (Table 7). Stochastic simulation showed that none of the studied populations go to extinction (1000 runs) in each 100 years.

Deterministic projection of transition matrices (where firstly 3 matrices selected for corresponding conditions and thereafter average matrix of low harvested alpine population selected) revealed that while comparing the lower elevation populations with low harvest intensity of alpine population, the low harvested population of lower alpine will reach that of low harvested intensity of alpine in 5 years and that of high harvest reach in twelve years (Figure 9a). Likewise, the low harvested population of subalpine will reach the size of low harvested intensity of alpine population in eight years and that of high harvest will reach at thirteen years.

Deterministic projection of transition matrices (firstly 3 matrices selected for corresponding conditions and thereafter average matrix of respective low harvest population selected) to compare whether lower alpine and subalpine population reach to that of alpine level revealed that the low harvest population of lower alpine will reach that of low harvest condition of alpine in six years and that of high harvest reach in sixteen years. Likewise, the low harvest and high harvest populations of subalpine will reach the size of low harvest condition of alpine population in more tha twenty years (Figure 9b).

Similarly, while making comparision between the high harvest populations with low harvest population of the respective population the high harvest population of lower alpine will reach the size of its low harvest population at seven years while that of subalpine will reach at twenty-two years (Figure 9c).

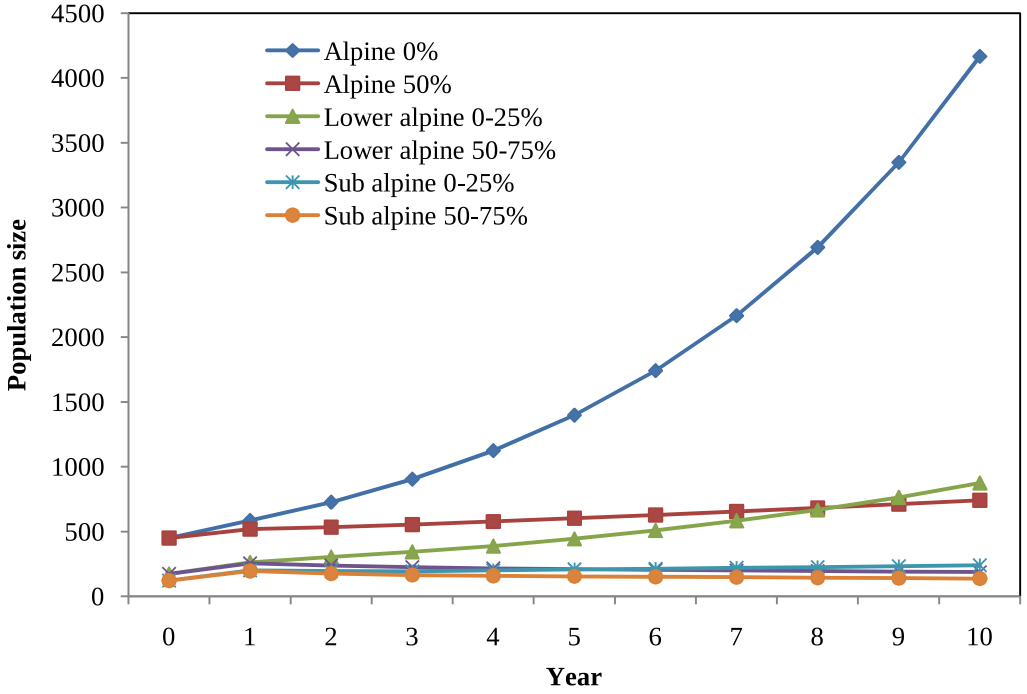
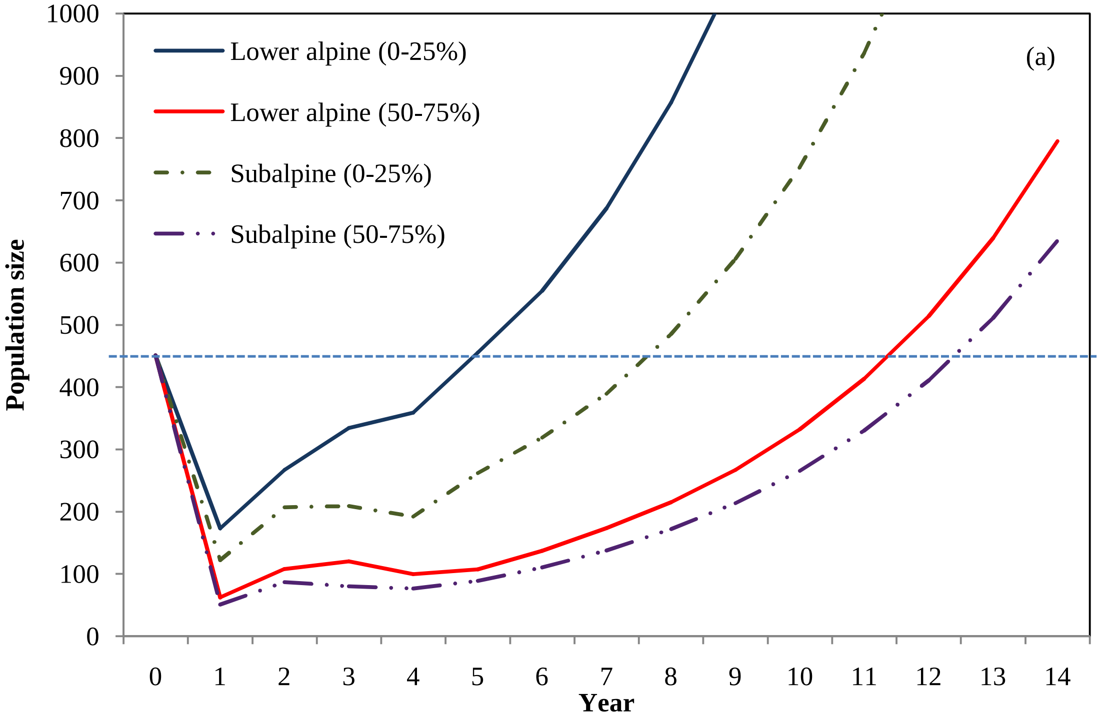
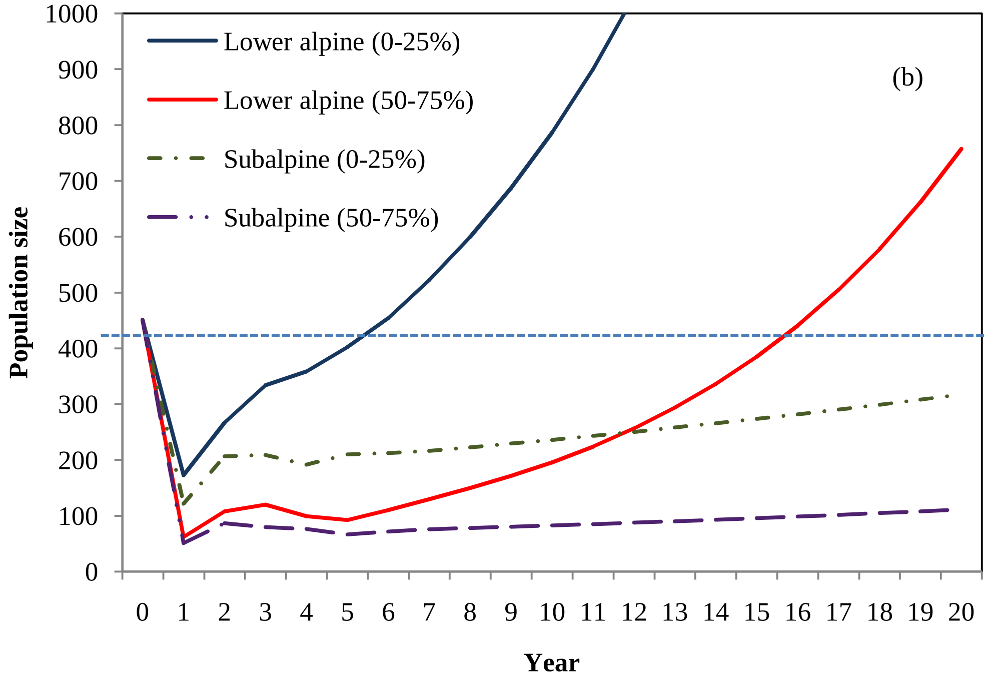


Figure 8: Transient dynamics of *Aconitum spicatum* populations based on 2015-2018 transition matrices. Projection of population size for 10 years from average matrix





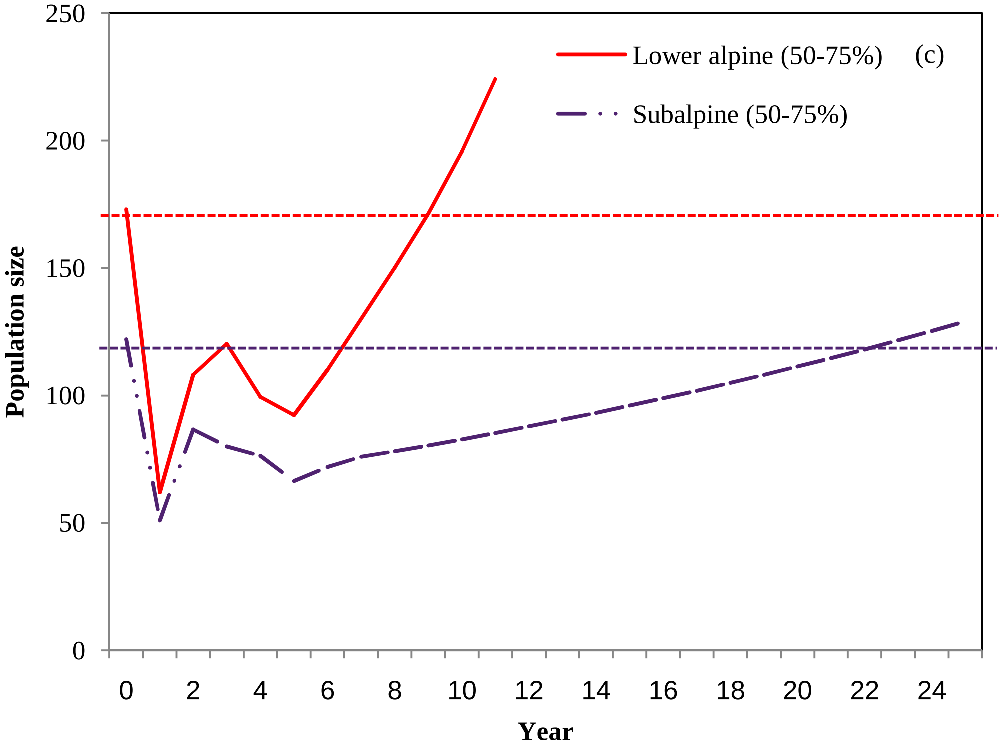


Figure 9: Deterministic projection (a) In relation to the low harvest intensity of alpine population (Deterministic projection: firstly 3 matrices selected for corresponding conditions and thereafter average matrix of unharvested alpine population selected) (b) In relation to the low harvest intensity of alpine population (Deterministic projection: firstly 3 matrix selected for corresponding intensities and thereafter average matrix of respective low harvest population selected to compare whether lower alpine and subalpine population reach to the alpine level) (c) In relation to the low harvest intensity of respective population

3.5 Discussion

For species harvested commercially by humans, differences in population growth rates in populations along the altitudinal gradient has important conservation and economic implications.

3.5.1 *Aconitum spicatum* population dynamics in different elevation populations

Although variation in the population growth rates was recorded, it may be confounded to a certain degree by other factors (for instance, temperature, snow cover, time of snow melt, time of first snow, snow melt period of the sites, age of the differing populations etc).

The present results are consistent with other studies. Ghimire *et al*. (2008) recorded higher population growth rate of *Nardostachys grandiflora* in meadow population than in outcrop populations. Ticktin and Nantel (2004) found higher population growth rate of *Aechmea magdalene* in secondary forests than in old growth forest. Villegas (2001) found that populations of *A. magdalene* growing in off- season forests had lower rates of growth than those growing in forests with a pronounced dry season.

Population growth rates of *A. spicatum* in all the studied populations along the elevation gradient in low harvest condition is slightly more than 1, which corresponds to the stable population growth rate of many perennial plant species (García *et al*., 2008; Rokaya *et al*., 2017). The perennial plant species showed more stable population dynamics than the short-lived species. The underground parts present in the perennials help the plant to adapt better in the harsh environmental condition (chapter 2) but also makes more sensitive to harvesting (Ghimire *et al*., 2008). In this study the alpine population with high harvest intensity, average population growth rate is more than 1. Astonishing range of life history traits with unique adaptive strategy of *A. spicatum* individuals (presence of under ground tubers with multiple numbers of sprouting buds) might be the probable reasons for this increasing population population growth rate (chapter 2). Poisonous nature of the plant reduces the chances of consuming their fruits and seeds by insects and animals. *A. spicatum* of alpine population are with better reproductive performances (higher seed mass, higher number of seeds per unit volume of fruit, higher seed viability and higher fecundity) as compared to the lower and subalpine populations (chapter 2). Further, long lived plants in high elevation grow in safe sites like rocky places or the edges of the large rocks which provide protection from the environmental harshness to certain extent.

In the final year of this study (2018) plant individuals infested with pests were recorded in some of the studied alpine plots which could be the reason of decrease in the population growth rate in 2017-2018 transition. The variation in the population growth rate of *A. spicatunm* in high harvest intensities in the studied populations might be due to the site-specific response of high-elevation plants as also recorded by Rokaya *et al*., 2017 in *Rheum* species.

The declining population growth rates of subalpine population might be due to low density, higher disturbance pressure (chapter 2) and low recruitment. In such cases, the long lifespan of dormant individuals could be an excellent strategy for avoiding local extinctions caused by high levels of disturbance, rare and unpredictable recruitments, and large population fluctuations (Garcia *et al*., 2008). Further the ability of the individuals to enter dormancy may prevent it from being harvested by the harvesters as they become invisible.

In all the three populations, population growth rates did not differ much among years. The extremely low extinction probability of *A. spicatum* individuals in all the populations indicates that the plant might have some adaptive strategy for instance multiple sprouting buds in daughter tuber which can undermine the harvest pressure.

High survival of larger stage class individuals and tendency of adults to remain at the same size (stasis) was found in *A. spicatum* which also characterize other perennial species (Silvertown *et al*., 1993; Silvertown *et al*., 1996; Ghimire *et al*., 2008).

3.5.2 Effect of harvesting

The alpine population does not suffer much from harvesting and has shown better reproductive performances. The subalpine (lowest elevation) population was found highly sensitive to harvesting as revealed by deterministic projection and are suffering with respect to reproductive fittness (chapter 2). The impact of harvesting on *A. spicatum* which possess dormant underground tubers and multiple buds was found varing along the elevation indicating the proability of the presence of such trends in other similar MAPs of the Himalayas.

3.5.3 Conservation implications of *Aconitum spicatum*

Like many MAPs, the commercial harvest of *A. spicatum* has flourished since the past few decades (Olsen, 1998). Only recently the Government of Nepal has included it in the list of priority species for conservation and economic development (DPR, 2012). The plant is found responding differently to different harvest intensities along the elevation. Though the plant did not show high probability of extinction, the lower elevation populations are declining. The lower elevation populations were found highly sensitive to prevailing disturbance factors. Thus, the existing alpine population should be conserved with regular monitoring and the lower elevation populations are to be conserved with great priority as these populations are found extremely sensitive to harvest and with slow recovery rate.

3.6 Conclusion

Populations of *Aconitum spicatum* at different elevations respond differently to harvest with different recovery rate. The alpine population allows the harvest of 10-20 percent of the individuals for the local use maintaining the equilibirium. At low harvest intensity all the populations show asymptotic growth rate more than 1, thus the probability of extinctly is extremely low. Subalpine (lowest elevation) population is the most sensitive population to harvest and should be conserved with greater priority. Higher contribution of larger size classes (adult vegetative and adult reproductive) to population growth rate indicated that these stages are the critical stages in the life history of *A. spicatum* and thus the conservsaion priority should focus on protection of these stages. This study demonstrates that there are important differences in the ability of *A. spicatum* to regenerate after harvest along the Himalayan elevation gradient in Nepal. This study recommends that the present populations of the *A. spicatum* should be protected with regular monitoring by the biologist, local community and the management authority

CHAPTER 4[*]

ILLEGAL HARVESTING AND LIVESTOCK GRAZING THREATEN THE ENDANGERED ORCHID *DACTYLORHIZA HATAGIREA* (D. DON) SOO IN NEPALESE HIMALAYA

Abstract

Harvesting of orchids for medicine and salep production is a traditional practice and its increasing market demand is spurring illegal harvest. Ethno-ecological studies in combination with the effect of anthropogenic disturbance is lacking for orchids. Population density and structure, and tuber biomass of *Dactylorhiza hatagirea* (D. Don) Soó were compared for three years in two sites: Manang, where harvesting of medicinal plants was locally regulated (protected) and Darchula, where harvesting was locally unregulated (unprotected). Six populations were studied along an elevation gradient by establishing 144 temporary plots (3×3 m^2) from 3400 to 4600 m elevations. Semi-structured interviews were conducted with informants (n = 186) in the villages and at the ecological survey sites. Mean density of *D. hatagirea* was significantly higher in the locally protected (1.31 ± 0.17 plants/m^2) than in the unprotected (0.72 ± 0.06 plants/m^2) site. The protected site showed stable population density with high reproductive fitness and tuber biomass over the three-year period. A significant negative effect ($P<0.1$) of relative radiation index (RRI) on the density of the adult vegetative stage and a positive effect of herb cover on juvenile and adult vegetative stages were found using mixed Zero-inflated Poisson (mixed ZIP) models. The densities of different life-stages were highly sensitive to harvesting and livestock grazing. Significant interactions between site and harvesting and grazing indicated particularly strong negative effects of these disturbances on densities of juvenile and adult reproductive stages in the unprotected site. Interview results showed that at the protected site people are aware of the conservation status and maintain sustainable populations, whereas the opposite was the case at the unprotected site where the populations are threatened. Sustainability of *D. hatagirea* populations, therefore, largely depends on controlling illegal and premature harvesting and unregulated livestock grazing, thus indicating the need for regular monitoring of the species.

---

[*] **Chapagain, D.J.**, Meilby, H., Baniya, C.B., Magar, S.B. & Ghimire, S.K. (2021). Illegal harvesting and livestock grazing threaten the endangered orchid *Dactylorhiza hatagirea* (D. Don) Soo in Nepalese Himalaya. *Ecol Evol.* 2021; 00:1-16. https://doi.org/10.1002/ece3.7520

Keywords: Anthropogenic disturbances, interview survey, orchid, population density, population monitoring, salep, sustainability

4.1 Introduction

Globally, 100 to 1,000 species per million become extinct every year, mostly due to anthropogenic habitat deterioration and fragmentation, invasion alien species, land use change, urbanization, atmospheric nitrogen deposition and climate change. Consequently, a large number of species, including many plants, are categorized as vulnerable or threatened (Jacquemyn *et al*., 2005a; Kull & Hutchings, 2006; Pimm *et al*., 2014; Di Marco *et al*., 2018). The Orchids typify the problem faced by the medicinal and aromatic plant species (MAPs). Orchids, with their complex biology (Rasmussen, 1995; Van der Cingel, 1995), tend to have small and isolated populations and exhibit high sensitivity to environmental changes (Vakhrameeva *et al*., 2008), and are at greater risk of extinction than most other plant groups (Kull & Hutchings, 2006; Warghat *et al*., 2013; IUCN, 2020). Human-mediated disturbances, mainly harvesting and habitat destruction, fragmentation or loss of habitats are the most significant threats to the survival of orchid populations (Saunder *et al*., 1991; Laurance & Bierregaard, 1997; Ehrlich, 1998; Zhang *et al*., 2019). Also, increasing human activities may create novel environments (Zhang *et al*., 2019) which limit the persistence of orchid populations. Human disturbances may result in the breakdown of ecological connections between orchids and their pollinators and mycorrhiza, changes in edaphic and micro-climatic conditions and introduction of pests and diseases (Light *et al*., 2003; Fay *et al*., 2015). Disturbances may interrupt interspecific interactions leading to reduced reproductive output and eventually altering the plant demographic dynamics (Steffan-Dewenter *et al*., 2006). Studies have shown that, generally, the magnitude of disturbance impacts on plant populations depend on plant life stage and features of their reproductive system (Calvo, 1990). In the case of orchid populations, the severity of disturbance impacts also depends on the level of specificity of plant-animal interactions (e.g., interactions with pollinators) and the availability of sites suitable for seedling recruitment (Schulze *et al*., 2019).

The distribution and abundance of orchid populations depend on a suite of biological and ecological factors including seed production and dispersal, recruitment, availability of mycorrhizal fungi and appropriate environmental conditions (McCormick & Jacquemyn, 2014). However, in case of the small orchid populations the seed output may be insufficient to ensure their long-term persistence (Faast *et al*., 2011). The life stage dynamics of orchid populations further depend on the elevation of their habitat. Environmental conditions and interactions associated with elevation play a significant role in the composition and distribution of orchid populations (Jacquemyn *et al*., 2005; Djordjevic *et al*., 2016; Djordjevic *et al*., 2020; Djordjevic & Tsiftsis, 2020). Alpine and subalpine grassland suffer from reduced nutrient availability and harsh environmental conditions, and plants growing in these habitats presumably develop adaptive coping strategies (chapter 2). Disturbance regimes, like harvesting, grazing, trampling, and fire also play influential positive (Chen *et al*., 2014; Dai *et al*., 2019) or negative (Kreziou *et al*., 2015) roles in determining the growth and persistence of plants and could be important elements of an optimal grassland management strategy for alpine meadows. Harvesting of whole plants or plant parts affect reproduction, survival and growth, and thereby also affect plant population dynamics (Ticktin, 2004; Ghimire *et al*., 2005; Ghimire *et al*., 2008; Gaoue *et al*., 2013; Huai *et al*., 2013). The extent of harvest impacts on plant populations, however, varies depending on habitat conditions, plant growth strategies, regeneration patterns, and microbial interaction, such as mycorrhizal association (Gaoue *et al*., 2013; Ticktin, 2015).

Inherently slow growth, high habitat specificity, dependency on pollinators, need of mychorrhiza for reproduction and germination, narrow range of ecological substitution options, unsustainable exploitation and climate change are major challenges for the growth and development of orchids, such as *Dactylorhiza hatagirea* (Hinsley *et al*., 2017; Reiter *et al*., 2017; Yeung, 2017; Hutchings *et al*., 2018; Rasmussen & Rasmussen 2018; Dhiman *et al*., 2019; Shrestha *et al*., 2021). Due to a marked decline in its natural populations, *D. hatagirea* has been listed as an endangered species in Nepal by Conservation Assessment and Management Plan (CAMP, 2001). According to the Forest Act of Nepal (2019), collection, use, sale, trade and export of *D. hatagirea* is prohibited and is strictly protected species in list I of Government of Nepal (GoN, 2011). It is also listed under appendix II in Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2020). Nevertheless, due to its high medicinal potency, *D. hatagirea* is still collected illegally at all life stages and traded to, especially, India and China (Olsen & Helles, 1997; Subedi *et al*., 2013) which has pushed local populations towards extinction (Manandhar, 2002).

Given that many orchids like *D. hatagirea* are currently threatened or endangered, a better understanding of the factors that influence orchid population ecology and dynamics may be critical to their long-term conservation (Shefferson *et al.*, 2020). Formulation of strategies for conservation of a species requires a sound knowledge of environmental factors, population ecology and demographic parameters (Margules & Pressey, 2000). Disentangling factors determining successful orchid establishment and its persistence under changing conditions is a major challenge. For the long-term conservation of endangered orchids, the development of concrete conservation plans based on indigenous knowledge, long-term monitoring, genetic analysis, and scientific inputs is crucial (Dobriyal *et al.*, 2002; Jacquemyn *et al.*, 2007). Endangered orchid species are in need of specific conservation actions (Tsiftsis *et al.*, 2011; Mincheva *et al.*, 2018; Charitonidou *et al.*, 2019), and more attention should be paid to the management of existing sites of orchids (Stipkova & Kindlmann, 2015).

Thus, the objectives of this chapter are to (a) analyze the variation in population density, structure, and tuber production in *D. hatagirea* over three years in two sites subjected to different levels of anthropogenic disturbances, (b) study the impact of elevation and anthropogenic disturbances on the population density of *D. hatagirea*, (c) examine the interaction between site and environmental factors (harvesting, grazing and herb cover) and its effect on population density of *D. hatagirea*, and (d) study the socio-cultural role of *D. hatagirea* and assess peoples' perception of its status.

To meet these objectives, locally unregulated site was identified in the western part of Nepal and a well-managed, locally regulated site in the central part of Nepal. At each site, a survey among local people was carried out and permanent plots were established at different elevations.

4.2 Materials and methods

4.2.1 Study area

The study of *D. hatagirea* was carried out in two sites: (i) Lolu-Pilkanda (N29˚60.095 and E080˚56.754 to N29˚57.719 and E080˚57.672) within Api-Nampa Conservation Area (ANCA) in Darchula District, north-west Nepal, and (ii) Bhimthang (N28˚37.607 and E084˚28.343 to N28˚40.284 and E084˚29.166), located within the strip of land separating Annapurna Conservation Area (ACA) and Manaslu Conservation Area (MCA) in Manang District, north-central Nepal (Figure 10).

The Lolu site, which lies in the upper Chamelia valley within ANCA, experiences high human pressure related mainly to livestock grazing and commercial harvesting of medicinal and aromatic plants (MAPs) (DNPWC, 2015). The site is managed by ANCA under the Department of National Parks and Wildlife Conservation (DNPWC) of the Government of Nepal, and due to poor implementation of regulatory mechanisms the human exploitation of natural resources at this site is very heavy (Pyakurel *et al*., 2018). Further, the local community has not taken any particular initiatives to conserve the area. Commercial and illegal trade of MAPs from the area has increased drastically in the last few decades (Pyakurel *et al*., 2018). Hence, Lolu site is hereafter referred as the locally “unprotected site”.

ANCA is the youngest and the most remote conservation area of Nepal. The climate is temperate to nival with an annual average precipitation of 2100 mm and annual mean minimum and maximum temperatures of 4.7°C and 27°C, respectively (DNPWC, 2015). Important livelihood activities are collection and trade of high-value MAPs, notably *Ophiocordyceps sinensis, D. hatagirea, Fritillaria cirrosha, Neopicrorhiza scrophulariiflora* (Pouliot *et al*., 2018; Pyakurel *et al*., 2018), and traditional mountain farming systems tightly integrated with transhumance and other livestock systems (DNPWC, 2015). The area has legal permission for commercial harvest of MAPs, and over 25,000 collectors from different parts of western Nepal harvest valuable MAPs from rangelands within ANCA (DNPWC, 2015).

The Bhimthang site, which lies in Gyasumdo valley (lower Manang), experiences low human pressure in terms of livestock grazing and commercial and illegal harvesting of MAPs. This site is rich in biodiversity and natural beauty and is located in one of the major tourist areas of Nepal. Thus, tourism is the major source of income for the local community. The area is managed by the community in collaboration with Annapurna Conservation Area Project (ACAP) and the exploitation of valuable MAPs is very limited due to strict regulation by the community (NTNC, 2016). The locals are involved in patrolling to prevent illegal harvesting of MAPs during the maturation period. Further, the local people practice rotational harvest (one or two years depending on the availability of MAPs) of highly valued MAPs (NTNC, 2016). Therefore, the Bhimthang site is hereafter referred to as the locally “protected site”.

At the protected site in Manang the climate varies from temperate to nival and is influenced by the summer monsoon. The annual mean minimum and maximum temperatures are 4.7°C and 16.8°C, respectively, and the average annual precipitation is 972 mm (DNPWC, 2015). The basic livelihood activities include and combine traditional mountain farming systems, transhumance and animal husbandry, small-scale trade at lower altitude during winter, tourism and the collection and trade of highly valued MAPs (Subedi & Chapagain, 2011; Chhetri, 2014).

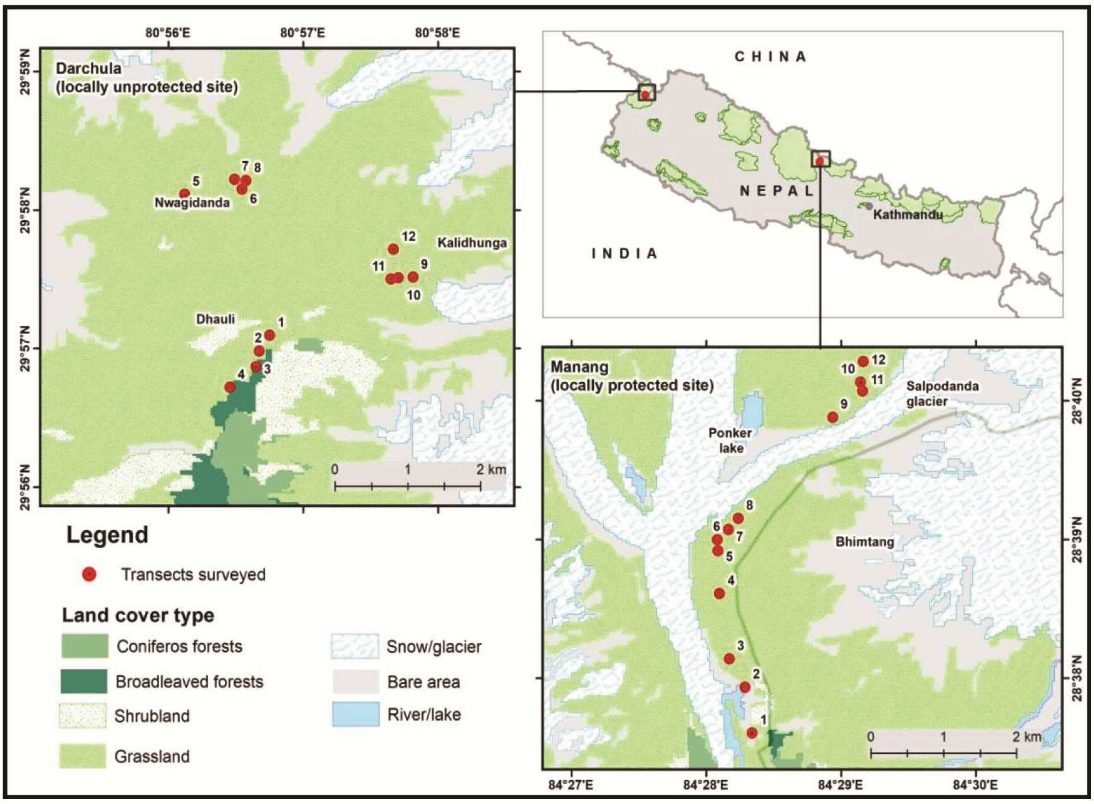


Figure 10: Map of the study area and the locations of the study plots of *Dactylorhiza hatagirea.* Source of land cover map: Department of Survey, Government of Nepal

These study sites differ slightly with respect to edaphic, topographic and substrate conditions. Both the sites have loamy soil rich in herbs, grasses, mosses, lichen and litter. *D. hatagirea* is found in slopes ranging between 1-66°. The sites do not vary much in terms of relative radiation index (RRI) (Appendix II).

The sites vary greatly in terms of anthropogenic disturbances. The unprotected site is subjected to higher anthropogenic pressure as revealed by higher disturbance scores (harvesting, grazing, trampling and animal droppings) than observed at the protected site.

4.2.2 Study species

Dactylorhiza hatagirea (D. Don) Soo

Dactylorhiza belongs to the family orchidaceae and the genus has approximately 75 sepcies in the world. Two of the species are recorded from Nepal. *Dactylorhiza hatagirea* hereafter *D. hatagirea* is locally known as Panchaunle ('five fingered hand'). It is distributed in Nepal, India, Bhutan, Pakistan, China, Afganistan and Mangolia (Ghimire *et al*., 1999). It prefers growing in wet places with high soil Ca content (Thakur *et al*., 2021) along forest margins, shrubby slopes, wet meadows and marshy places at an elevation ranging between the elevation of 2800- 4600 m (Ghimire *et al*., 2021).

It is an erect perennial herb (30-90 cm tall) with palmate tubers, 5-7 lanceolate or oblong leaves, which are progressively smaller towards the top and has a robust stem (Figure 11 left). Inflorescence ranges up to 15 cm long, with densely packed large number of flowers. Flowers are resupinate purple to light pink, arranged around the rachis resembling a hyacinth (Ghimire et. al., 2021). Capsules bear thousands of dusts like seeds. Seeds are minute and lack the storage reserve, i.e., endosperm impelling the orchid to depend partly on the mycorrhizal fungi *Rhizoctonia* for nutrition (Giri & Tamata, 2012; Warghat *et al*., 2014). The plant posses very slow rate of vegetative propagation and very poor natural seed germination, i.e., 0.2%-0.3% (Vij, 2001).

The tubers of *D. hatagirea* (Figure 11, right) yield a high quality salep (a beverage made from the powder of orchid tuber) which is used as an aphrodisiac or a nutritive and restorative tonic and is also eaten raw as farinaceous food (Vij, 1995; Sood *et al*., 2005; Watanabe *et al*., 2005; Baral & Kurmi, 2006; Thakur & Dixit, 2007). It is also used in the treatment of diabetes, chronic diarrhea, dysentery, cough, hoarseness of voice, paralysis, fractures, during convalescence and to correct malnutrition (Das, 2004; Singh & Duggal, 2009). The whole plant posses anti bacterial properties and is used in curing various bacterial diseases (Ranpal, 2009).

The secondary metabolites such as dactylorhins A–E, dactyloses A–B, glucosides, starch and albumin present in the plant are responsible for the wide spectrum of pharmacological activities (antioxidant, antimicrobial, antiseptic, anticancer, and immune enhancing activities) (Kizu *et al*., 1999; Lama *et al*., 2001; Wani *et al*., 2020). Recent findings indicate that *D. hatagirea* also has anti-cancerous properties

(Popli & Sood, 2017). The plant also acts as a good indicator of environmental quality (Kindlmann *et al*., 2006).

The salep produced from its tuber has spurred its market value which has promoted its illegal harvest (Subedi *et al*., 2013). Incessant overexploitation resulted in the dwindling of its populations in the wild. Overexploitation, illegal trade, habitat destruction, and climate change are the major threats to its survival (Shrestha *et al*., 2021).

Figure 11: a. Left: Blooming *Dactylorhiza hatagirea* (D.Don)Soo (PC: Suresh Kumar Ghimire). Right: Tubers of *Dactylorhiza hatagirea* beaded for drying by the locals (PC: Chandra Kanta Subedi)

Figure 11: b. Capsules of *D. hatagirea* **c.** Seeds of *D. hatagirea* (enlarged under microscope)

4.2.3 Sampling design used in the vegetation survey

This study was carried out between 2015 and 2017 to understand the population dynamics of *D. hatagirea* in unprotected site (Darchula) and protected site (Manang). Here, vegetation was sampled in two ways: (i) to meet the first objective regarding variation in the populations over time three permanent plots (20 × 20 m) were established in each population at the two study sites. (ii) Similarly, to meet the second and third objectives regarding impacts of environmental variables on population density a total of 144 temporary plots (3 × 3 m), 72 at each site, from the lowest to the highest elevation to cover the whole distributional range of *D. hatagirea* at the study sites was established.

In each site three fairly dense populations (with all four stages) were subjectively identified. In each population, three randomly located permanent 20 m × 20 m plots were established and each plot was divided into four 10 m x 10 m subplots. In each subplot all individual plants were tagged in 2015, categorized with regard to life stage and monitored in 2016 and 2017.

Temporary plots were established using the methods described in chapter 2. At each site three populations of *D. hatagirea* were identified along an elevation gradient ranging from 3400–3900 m along the Upper Chamelia valley of ANCA and 3400-4600 m along the Gyasumdo valley in Manang (Figure 10, Appendix II). In each population four transects were established at a minimum vertical distance of approximately 100 m. In each transect six (3 m × 3 m) plots at a minimum horizontal plot to plot distance of 10 m were established. Each plot was divided into nine 1 m × 1 m subplots and the four corner subplots were systematically sampled and measured.

For each plot the geographical location (latitude and longitude) and topographical characteristics (elevation, slope and aspect) were recorded and used to calculate the relative radiation index (RRI) (Oke, 1987; Vetaas, 1992a; 1992b). In each subplot the ground cover (%) for vascular plants (grasses, herbs and shrubs), non-vascular plants (lichens and bryophytes), litter, bare ground, rock and scree cover were estimated using standard methods (Pauli *et al*., 2015). Disturbance (harvesting, trampling, grazing, and animal dropping) scores ranging from 0 (none) to 4 (very high) were recorded for each subplot after careful observation of the evidence, e.g., large holes caused by excavation, wilted or fresh uprooted aerial parts, tuber fragments, browsed plant parts, defoliated aerial parts and animal droppings.

The individual plants were classified into four life stage classes based on the number and size of leaves and presence of reproductive structures. The four stages are: seedlings (Sd; leaf breadth ≤ 1 cm, leaf number = 1-2), juveniles (Jv; leaf breadth ≥ 1 cm, ≤ 2cm, leaf number = 2-3), vegetative adults (Adv; leaf breadth ≥ 2 cm, leaf number > 2, non-flowering) and reproductive adults (Adr; flowering or fruiting individuals). Individuals at different stages were counted in each subplot to calculate the density. The population structure was described as the proportion of each life stage within the studied population.

To estimate reproductive traits, fifteen mature individuals from each population at the two sites were selected and recorded the reproductive traits (number of flowers and fruits). For the estimation of dry biomass of tubers, the weight of fifteen dried tubers of *D. hatagirea* from the local MAPs collectors were recorded.

4.2.4 Interview survey

Semi-structured interviews were conducted during 2015-2017 among 117 persons in the unprotected site (Darchula) and 69 persons in the protected site (Manang). (Details in chapter 2)

4.2.5 Data analysis

A relative radiation index (RRI), which is a relative measure of the exposure to solar radiation at noon at a specific location (Oke, 1987; Vetaas, 1992a, 1992b) was calculated for each plot as a function of aspect, latitude, and slope:

$$\mathrm{RRI} = \cos(180° - \Omega) \times \sin(\beta) \times \sin(\Phi) + \cos(\beta) \times \cos(\Phi),$$

where Ω is aspect (slope azimuth in degrees), Φ is latitude (degrees) and β is slope inclination (degrees).

The densities of different stages (seedling, juvenile, vegetative and reproductive adults) were compared by Kruskal-Wallis tests, and the reproductive traits were compared among the three populations in each of the two sites using one-way ANOVA.

Direct field observations confirmed the plant as rare in the study site and it is therefore expected that the data collected would exhibit a large number of zeros. Different model alternatives were tried but based on the Akaike Information Criterion

(AIC) the best fit was obtained using mixed Zero-inflated Poisson (ZIP) models. The mixed ZIP model allowed to analyze relationships between density of *D. hatagirea* plants at different stages and a set of independent variables including population, cover of shrubs or herbs, relative radiation index (RRI), and anthropogenic disturbance indicators such as harvesting, trampling, grazing and animal droppings.

Ten sets of candidate models were prepared using the glmmTMB package (Appendix VI) and finally prepared an average model based on the set of five best candidate models (selected on the basis of delta AIC) using the MuMIn package (Barton, 2018). In practice, the final average models were prepared using five models with delta AIC values ≤ 402 for the seedling stage, ≤ 748 for the juvenile stage, ≤ 645 for the adult vegetative stage and ≤ 763 for the adult reproductive stages. The full models were in all cases expressed as:

Density (of a particular stage of *D. hatagirea*) = a + b (population) + $c_1 \mathrm{RRI}_{ij}$ + c_2 Herb cover$_{ij}$ + c_3 Harvesting$_{ij}$ + c_4 Trampling$_{ij}$ + c_5 Grazing$_{ij}$ + c_6 Animal dropping$_{ij}$

Where a (intercept), b (population) and $c_1 \ldots c_{6}$ $_{\text{are}}$ fixed model parameters. i = 1…144 is the plot (included as a random effect), j = 1…4 is the subplot. The population variable had six categories, three at each site (see Study area in chapter 1). Confounded variables were excluded from the analysis.

4.3 Results

4.3.1 Variation in population density and structure

The population density of *D. hatagirea* at the unprotected site ranged from 0.60 to 0.79 individuals/m^2 while the density ranged from 0.70 to 2.16 individuals/m^2 at the protected site. Population densities were highest in mid-elevation populations at both sites (Table 8). At the unprotected site, all populations showed highest densities for the juvenile and adult vegetative stages, whereas at the protected site the density of the adult reproductive stage was mostly higher than for the juvenile and adult vegetative stages (Table 8). At the protected site, the variation of density among the populations was significant (Kruskal Wallis test, $P<0.05$), overall and for all stages except the adult vegetative stage, but at the unprotected site there was no significant variation among populations ($P > 0.05$).

The population structure varied between the sites (Figure 12). For all populations, the proportion of seedlings was lower at the unprotected site than at the protected site. The proportion of juvenile and adult vegetative plants was highest at the unprotected site while the proportion of the adult reproductive stage was highest at the protected site. Comparing the mean densities across three consecutive years (2015-2017), a drastic decrease was observed at the unprotected site (approximately by one third), while almost no change was seen at the protected site (Figure 13).

Table 8: Population density (m^{-2}) for different life stages of *Dactylorhiza hatagirea* in three populations in each locally unprotected (Darchula) and locally protected (Manang) sites. Densities are stated as mean ± SE. χ^2 and *p*-values were based on Kruskal Wallis test, df = 5, n = 144. up = locally unprotected site, p = locally protected site † Life stage classes: Sd: seedling; Jv: juvenile; Adv: adult vegetative; Adr: adult reproductive

Population	**Elevation (m asl)**	**Life stage class**				
		Sd	**Jv**	**Adv**	**Adr**	**Total**
Dhauli^{up}	3605	0.05 ± 0.03	0.20 ± 0.03	0.20 ± 0.04	0.13 ± 0.02	0.60 ± 0.06
Nwagidanda^{up}	3799	0.06 ± 0.02	0.39 ± 0.09	0.21 ± 0.04	0.12 ± 0.03	0.79 ± 0.10
Kalidhunga^{up}	3976	0.08 ± 0.03	0.25 ± 0.04	0.28 ± 0.07	0.17 ± 0.03	0.78 ± 0.12
Mean^{up}		0.07 ± 0.01	0.29 ± 0.03	0.23± 0.03	0.14 ± 0.01	0.72 ± 0.06
χ^2 value		0.70	2.56	0.98	1.39	2.57
p-value		0.70	0.28	0.61	0.50	0.28
Bhimthang^{p}	3713	0.17 ± 0.05	0.46 ± 0.10	0.18 ± 0.07	0.27 ± 0.06	1.07 ± 0.17
Ponker Hill^{p}	4046	0.44 ± 0.17	0.49 ± 0.14	0.40 ± 0.10	0.82 ± 0.17	2.16 ± 0.40
Salpodanda^{p}	4437	0.03 ± 0.02	0.09 ± 0.03	0.19 ± 0.05	0.40 ± 0.04	0.70 ± 0.07
Mean^{p}		0.21 ± 0.07	0.34 ± 0.07	0.26 ± 0.04	0.50 ± 0.07	1.13 ± 0.17
χ^2 value		13.62	8.86	3.09	10.63	14.24
p-value		<0.01	<0.05	0.21	<0.01	<0.01
Combined						
χ^2 value		18.64	14.82	3.92	42.99	21.77
p-value		<0.001	<0.01	0.41	<0.0001	<0.001

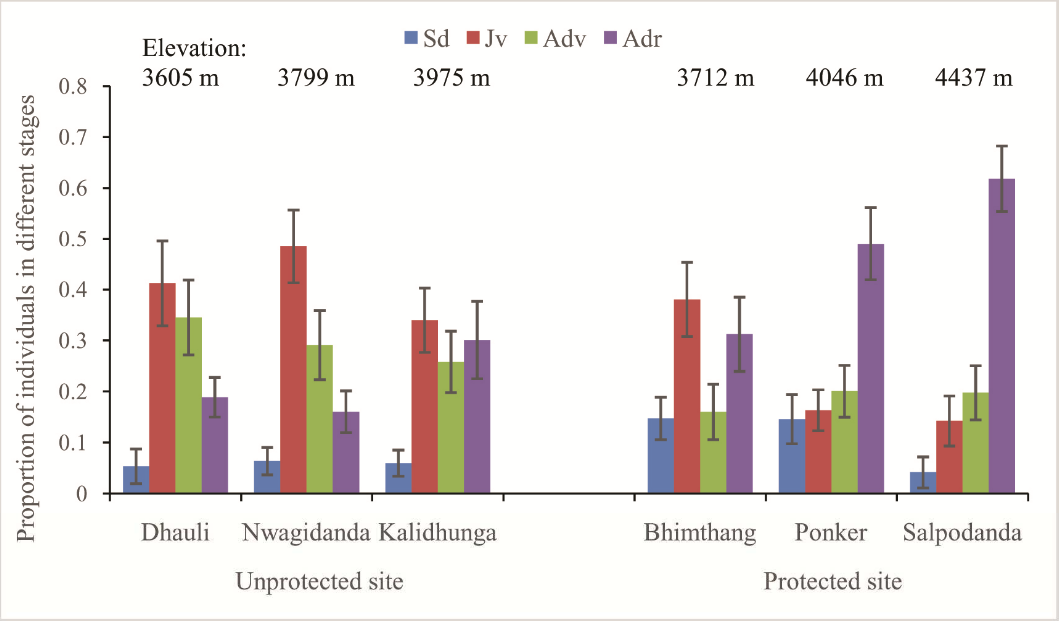


Figure 12: Population structure of *Dactylorhiza hatagirea* in (left) locally unprotected and (right) locally protected sites. Life stage classes: Sd = seedling; Jv = juvenile; Adv = adult vegetative and Adr = adult reproductive

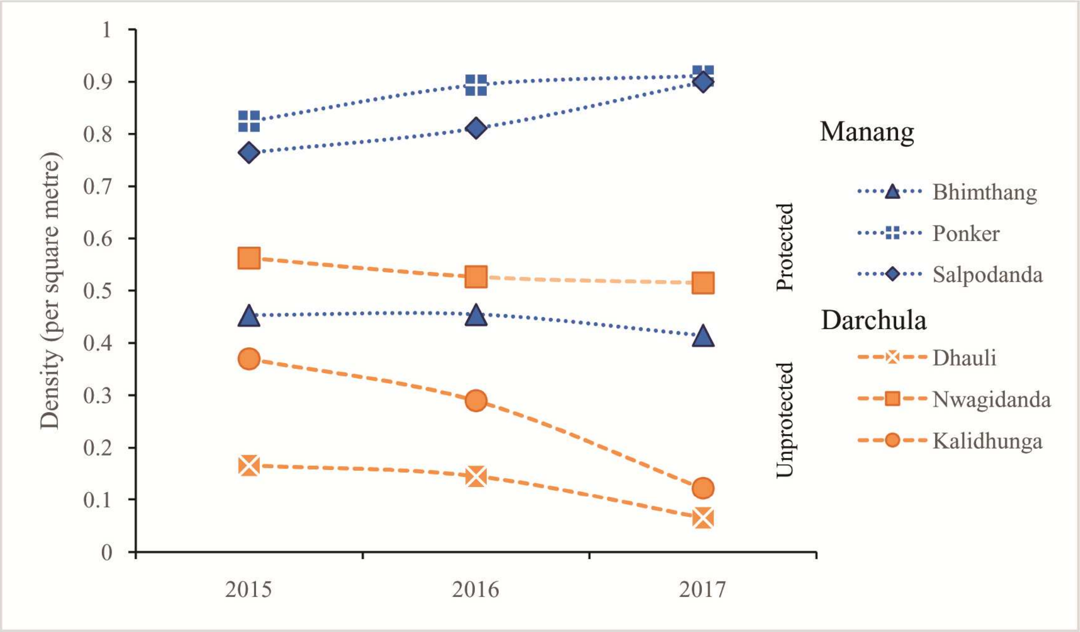


Figure 13: Density of *Dactylorhiza hatagirea* at the protected (Manang) and unprotected (Darchula) sites in 2015, 2016 and 2017

4.3.2 Variation in reproductive traits and tuber production

At the protected site the reproductive output and tuber biomass were about three times higher than at the unprotected site. In comparisons between populations the reproductive traits (number of flowers, number of fruits, and total reproductive output per individual) were found to decrease from the lowest to the highest elevation at both sites (Table 9). The dry biomass of daughter tubers showed similar trends at both study sites. A comparison of tuber production across three consecutive years (2015, 2016 and 2017) showed reduced tuber production in 2017 at the unprotected site and increasing tuber production at the protected site (Figure 14).

Table 9: Variation in reproductive output of *Dactylorhiza hatagirea* in three populations in each locally unprotected (Darchula) and locally protected (Manang) sites. Values are stated as mean ± SE. *F* and *p*-values were based on one way ANOVA, df = 5, n = 144. up = locally unprotected site, p = locally protected site

Population	Number of flowers per individual	Number of fruits per individual	Total reproductive output per individual	Dry weight of daughter tuber (g)
Dhauli^{up}	15.27 ±3.06	27.87 ±1.94	39.07 ±4.30	0.73 ±0.08
Nwagidanda^{up}	14.89 ±1.20	26.33 ±1.92	34.93 ±2.67	0.63±0.13
Kalidhunga^{up}	13.11±3.65	24.5±2.07	27.40±2.96	0.61±0.09
Total				
F-value	0.49	2.22	9.47	3.06
p-value	0.49	0.14	0.15	0.09
Bhimthang^{p}	29.5±2.39	40.7±3.05	71.2±2.93	1.74±0.13
Ponker Hill^{p}	27.9±3.27	36.9±2.18	64.8±4.40	1.42±0.16
Salpodanda^{p}	21.9±1.61	31.4±1.84	53.3±2.32	1.17±0.14
Total				
F-value	9.78	5.11	12.89	8.82
p-value	0.01	0.02	0.00	0.00
Combined				
F-value	0.26	29.8	29.8	92.3
	0.61	0.00	0.00	0.00

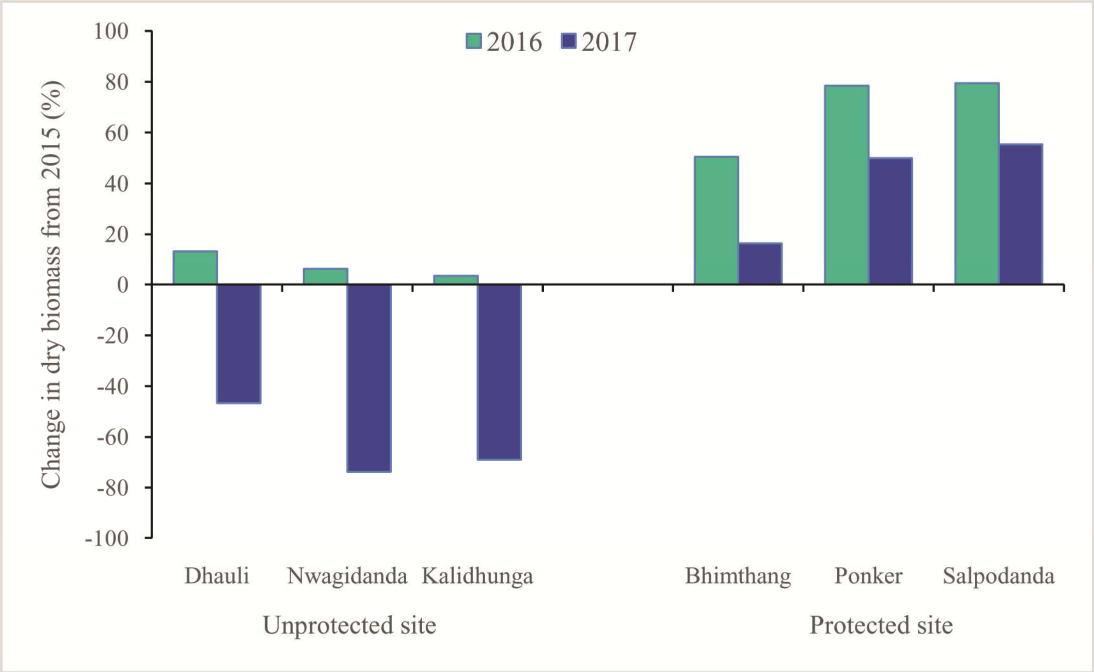


Figure 14: Variation in tuber production of *Dactylorhiza hatagirea* during 2015 to 2017 in three populations each of locally unprotected (first three in the graph) and locally protected site (last three in the graph)

4.3.3 Effect of environmental variables on densities of different life stages and their interaction among the sites

Mixed zero–inflated Poisson (mixed-ZIP) models showed significant negative effects of harvesting on the densities of juvenile and adult stages (both vegetative and reproductive). Grazing had a significant negative effect on juvenile ($P<0.001$) and adult vegetative ($P<0.05$) stages. Herb cover showed significant positive effects ($P<0.05$) on the juvenile and adult vegetative stages. The relative radiation index had a weakly significant negative effect ($P<0.1$) on the density of the adult vegetative stage. The zero-inflation model component was significant only for the adult reproductive stage (Table 10).

The interaction effects of harvesting and grazing within population among the sites indicated that unprotected populations show significant negative effects of harvesting and grazing, while the effects were not distinct for the protected populations. The herb cover did not show any significant effects within populations among the sites (Table 11).

4.3.4 Interview survey

About forty-three per cent of the MAPs users interviewed at the unprotected site (n = 117) were aware that the harvesting of *D. hatagirea* was strictly protected, seventy one percent were aware of its use value, five per cent were aware of its population ecology, forty two percent harvested *D. hatagirea* for local uses to treat cuts and wounds, boils, fractures, and to use it as a tonic. Ninety-two per cent of the informants claimed that illegal harvesting of *D. hatagirea* was a common practice and that the population had decreased drastically over the last few decades in the unprotected site. They further disclosed that there are illegal traders in the district headquarters of Darchula who motivate MAPs users and cattle herders to harvest *D. hatagirea* by promising to buy the dried tubers. Further, perceived difficulties to collect *Ophicordyceps sinensis* and *Fritillaria cirrhosa* increased the temptation to carry out illegal and premature harvest of *D. hatagirea.* The informants mentioned that for every 1 kg of dried tubers sold; approximately 500-1000 mature plants are harvested. Eighty-one per cent of the informants were of the view that the major source of their livelihood is MAPs collection.

At the protected site, about ninety three percent of the MAPs users interviewed (n = 69) were aware of the harvesting ban, ninety-eight percent were aware of the species' use value, nineteen precent were aware of its population ecology and twenty-one percent occasionally harvested a few (2-5) individuals of *D. hatagirea* for home use to treat cuts and wounds, burns and boils and for religious purposes by Buddhist Lamas. About twenty four percent were of the opinion that the population is decreasing, while seventy three percent thought that the population has been almost constant in the last few decades as the local community is involved in patrolling the area to control illegal collection during the maturation period. Interview results also revealed that none of the families totally rely on MAPs collection for their livelihood as they had access to income from the flourishing tourism. It is also observed that awareness programs were run at community level about the sustainable use of available MAPs. Further, they also used a specific grazing and trampling route to protect sensitive plants from damage.

Table 10: Mixed Zero-inflated Poisson regression models for the density (m^{-2}) of seedling, juvenile, adult vegetative and adult reproductive stages of *Dactylorhiza hatagirea*. Density of different stages were modelled as a function of herb cover (%), relative radiation index (RRI) and disturbance (harvesting, grazing and animal droppings), which were assessed using an ordinal 0-4 integer scale. Zi is the intercept in the Zero-inflated component: all other parameters refer to the count component of the model. Parameter estimates with standard errors in brackets for Full average and Conditional average models. Significance levels are stated as: *: P < 0.1, **: P < 0.05, ***: P < 0.01, ****: P < 0.001

Life stages			**Locally protected site (Manang)**			**Locally unprotected site (ANCA)**							
	Average model	**ZI component**	**Count component**										
		Zi	**Bhimthang: intercept**	**Ponker hill**	**Salpodanda**	**Dhauli**	**Nwagidanda**	**Kalidhunga**	**Herb cover**	**RRI**	**Harvesting**	**Grazing**	**Animal droppings**
Seedling	Full	-0.702	-1.982***	0.537	-2.037**	-0.677	-0.740	-0.514	0.009		-0.606	-0.424	0.019
		(0.691)	(0.619)	(0.553)	(0.818)	(0.876)	(0.720)	(0.750)	(0.010)		(0.369)	(0.394)	(0.116)
	Conditional	-0.702	-1.982***	0.537	-2.037**	-0.677	-0.740	-0.514	0.013		-0.606	-0.606	0.147
		(0.691)	(0.691)	(0.553)	(0.818)	(0.876)	(0.720)	(0.750)	(0.009)		(0.369)	(0.336)	(0.292)
Juvenile	Full	-2.150	-0.919**	-0.133	-1.854****	-0.070	0.002	-0.213	0.010		-0.367**	-0.644****	-0.080
		(1.401)	(0.363)	(0.346)	(0.470)	(0.488)	(0.381)	(0.424)	(0.007)		(0.177)	(0.188)	(0.149)
	Conditional	-2.150	-0.919**	-0.133	-1.854****	-0.070	0.002	-0.213	0.011**		-0.376**	-0.644****	-0.197
		(1.401)	(0.363)	(0.346)	(0.470)	(0.488)	(0.381)	(0.424)	(0.004)		(0.169)	(0.188)	(0.179)
Adult Vegetative	Full	0.122	-1.080 *	0.953**	-0.201	1.026*	0.437	0.720	0.009	-0.106	-0.657***	-0.530*	-0.014
		(0.307)	(0.560)	(0.421)	(0.461)	(0.590)	(0.456)	(0.490)	(0.007)	(0.459)	(0.230)	(0.290)	(0.010)
	Conditional	0.122	-1.080*	0.953**	-0.201	1.026*	0.437	0.720	0.010**	-.594*	-0.657***	-0.595**	-0.071
		(0.307)	(0.560)	(0.421)	(0.461)	(0.590)	(0.456)	(0.490)	(0.006)	(0.903)	(0.230)	(0.238)	(0.217)
Adult reproductive	Full	-0.781**	0.894**	1.138****	0.394	0.353	-0.106	0.224	0.000	-0.098	-0.801****	-0.057	-0.010
		(0.370)	(0.426)	(0.287)	(0.307)	(0.413)	(0.389)	(0.373)	(0.001)	(0.411)	(0.202)	(0.134)	(0.061)
	Conditional	-0.781**	-0.894**	1.138****	0.394	0.353	-0.106	0.224	0.000	-0.583	-0.801****	-0.177	-0.103
		(0.370)	(0.426)	(0.287)	(0.307)	(0.413)	(0.389)	(0.373)	(0.004)	(0.853)	(0.202)	(0.188)	(0.173)

Table 11: Mixed Zero-inflated Poisson regression models expressing the interaction effect between site and environmental factors (harvesting, grazing and herb cover) on density (m^{-2}) of seedlings, juvenile, adult vegetative and adult reproductive stages of *Dactylorhiza hatagirea*. Parameter estimates with standard errors are in brackets. Significance levels are stated as: *: P < 0.1, **: P < 0.05, ***: P < 0.01, ****: P < 0.001

	Independent variables	**Harvesting**				**Grazing**				**Herb cover**			
Life stages	**Model**	**Intercept: locally unprotected site**	**Locally protected site**	**Harvest**	**Interaction locally protected* harvest**	**Intercept: locally unprotected**	**Locally protected**	**Grazing**	**Interaction locally protected* grazing**	**Intercept: Locally unprotected**	**Locally protected**	**Herb cover**	**Interaction locally protected* herb cover**
Seedling	Conditional	-2.561****	0.180	-0.860***	1.012	-2.338****	0.178	-1.011***	0.317	-3.569***	1.209	0.002	-0.004
		(0.540)	(0.461)	(0.307)	(1.207)	(0.553)	(0.488)	(0.348)	(0.558)	(0.899)	(0.916)	(0.011)	(0.014)
	Zero Inflation	0.774				-0.789				-0.667			
		(0.549)				(0.557)				(0.719)			
Juvenile	Conditional	-0.546***	-0.708***	-0.833****	2.250**	-0.354*	-0.833***	-1.081****	0.757**	-1.230***	0.132	0.000	0.000
		(0.201)	(0.244)	(0.138)	(0.922)	(0.212)	(0.262)	(0.168)	(0.329)	(0.417)	(0.472)	(0.006)	(0.009)
	Zero Inflation	-1.327****				-1.614****				-0.668			
		(0.466)				(0.466)				(0.377)			
Adult	Conditional	-0.200	-0.654**	-0.963****	0.903	0.019	-0.787**	-1.126****	0.650	-0.667	0.034	0.001	0.006
Vegetative		(0.251)	(0.284)	(0.180)	(0.842)	(0.263)	(0.321)	(0.207)	(0.408)	(0.476)	(0.541)	(0.007)	(0.010)
	Zero Inflation	0.093				0.047				0.732***			
		(0.308)				(0.308)				(0.267)			
Adult	Conditional	-0.851****	0.378*	-0.949****	1.039**	-0.660***	0.299	-0.956****	0.732**	-1.551****	1.279***	-0.000	-0.002
reproductive		(0.234)	(0.230)	(0.204)	(0.501)	(0.256)	(0.256)	(0.200)	(0.294)	(0.464)	(0.478)	(0.007)	(0.008)
	Zero Inflation	-0.760*				-0.666*				-0.474			
		(0.389)				(0.368)				(0.337)			

4.4 Discussion

The study is the first of its kind in this region, but similar research on *Dactylorhiza sambucina* has been done in Epirus, Greece (Charitonidou *et al.*, 2019). The present work provides results based on a mediun-term (three years) study from the Nepal Himalaya and should therefore be of significance to the conservation management of the endangered orchid *D. hatagirea*. Environmental variables and human-mediated disturbances such as harvesting and livestock grazing had significant effects on the population structure, density, reproductive traits and tuber production of *D. hatagirea*.

4.4.1 Variation in population density and structure

A maximum population-level mean density of 1.31 plants/m^2 was recorded at the protected and 0.72 plants/m^2 at the unprotected site. The population density at the protected site was thus roughly twice as large as for the unprotected site. Particularly low proportions of seedlings and adult reproductive plants observed at the unprotected site could be due to over-harvesting and grazing, since harvesters presumably tend to target the adult reproductive stage and recently established seedlings are sensitive to grazing. Moreover, seedlings browsed by livestock may be hard to find and could therefore be overlooked more frequently than the larger plants characterizing later life stages.

Low proportion of seedlings at both the sites could partly be explained as a consequence of orchids being habitat specific and seedling establishment depending on a suite of environmental factors, which are rarely present at the same time and place (Sherfferson *et al.*, 2020). The results compare well with previous researches as small populations have substantially lower viability compared to larger populations, and seedling recruitment rates can be considerably lower in small populations, which results in significantly lower population growth rate and density (Pellegrino & Bellusci, 2014; Hens *et al.*, 2017). The low proportion of reproductive plants observed at the unprotected site compares well with observations made by Pellergrino & Bellusci (2014) for the orchid species *Serapias cordigera*, where human disturbances were noted to have a negative effect on the population size. The traditional practices of transhumance, harvesting, and habitat fragmentation are likely the major anthropogenic factors responsible for reducing the flowering density as well as the population size of *D. hatagirea* in the unprotected site. Human induced disturbances like harvesting, grazing and fire have negative effects on population density and performances (McKinney, 2002; Aguilar *et al*., 2006) however, there are also examples of the opposite cases, and Chen *et al*. (2014) actually reported a positive effect of human disturbance on some orchid species. The results further suggest that at both sites the density of *D. hatagirea* reached a peak in mid-elevation populations (3799 m asl in the unprotected and 4046 m asl in the protected site). This might indicate that present sampling actually managed to cover the elevation range and that the best habitats are thus found approximately in the middle of the range. It is also consistent with the idea that when examining small populations across environmental gradients, the peak density usually occurs at intermediate levels, as also noted by Chen *et al*. (2014).

4.4.2 Variation in the reproductive traits

Reduced reproductive fitness in *D. hatagirea* was found at the unprotected site. Alterations due to anthropogenic disturbances in natural habitats often reduce the size and density of populations (McKinney, 2002; Aguilar *et al*., 2006). Anthropogenic disturbances increase the spatial distance between the plant populations as well as between individuals within a population, thereby disrupting insect movement between plants (Öckinger *et al*., 2009), decreasing pollinator abundance (Liu & Koptur, 2003) and altering their behavior and the frequency of flower visits (Aguilar *et al*., 2006). This process ultimately decreases the reproductive fitness of the plants (Peterson *et al*., 2008). The level of inbreeding may be higher in small, isolated populations (Miao *et al*., 2014) because of the higher rate of selfing and more frequent mating between close relatives. The resulting inbreeding depression can reduce the fitness of the plants compared with those in larger populations. Consequently, decreased out-crossing in small, sparse populations may reduce population fitness, potentially increasing the probability of extinction (Gargano *et al*., 2009; Stachurska-Swakon *et al*., 2011). The weaker performance in terms of reproductive output at the unprotected site could further be attributed to the interruption of plant development caused by breakage of inflorescences during heavy grazing and trampling. A long history of grazing and trampling at the unprotected site (DNPWC, 2015) might have resulted in the production of reduced or defective reproductive parts. Grazing is likely to affect *D. hatagirea* in numerous ways, both directly by damaging above-ground parts and indirectly by changing habitat characteristics (light intensity, litter accumulation, temperature, etc.) and interaction with other individuals or species (intra- and inter-specific competition, pollination/herbivory).

The unprotected populations had a lower population size and a lower fruit set than did protected populations, suggesting that the latter populations are better buffered. This could be a consequence of inadequate pollinator visitation in small populations, resulting in insufficient pollen transfer, poor pollination and lower seed set (Tremblay *et al*., 2005; Smithson, 2006; Xia *et al*., 2013). By contrast, larger populations of plants are likely to be more attractive to pollinators, resulting in higher visitation rates and therefore higher pollination success (Mustajärvi *et al*., 2001). Habitat fragmentation and disturbance lead to the interruption of interspecific interactions, indirectly causing changes in plant demographic dynamics via reduced reproductive output (Steffan-Dewenter *et al*., 2006; Sherfferson *et al*., 2020). Some studies have shown that plants in disturbed habitats undergo a reduction in pollination efficiency and reproductive success as well as recruitment and survival rates, all of which negatively affect plant demographic dynamics (Aguilar *et al*., 2006; Bruna *et al*., 2009).

Transhumance and MAPs collection are common practices in the alpine and subalpine pastures of the unprotected site and have existed for a very long time (DNPWC, 2015). The site is legally open for commercial harvest of highly valued MAPs, such as *Ophiocordyceps sinensis* and *Fritillaria cirrhosa* and the locals are highly dependent on the collection and trade of MAPs for their livelihood. A huge influx of collectors (locals as well as from other parts of the country) enters the site to collect *O. sinensis* and *F. cirrhosa* during the late spring when *D. hatagirea* also emerges. The resulting trampling presumably has a huge negative impact on its growth and development by breaking the aerial parts before fruit maturation and seed dispersal. Besides, collectors who are unable to collect sufficient amounts of *O. sinensis* and *F. cirrhossa* are tempted to illegally harvest the tubers of *D. hatagirea* irrespective of its degree of maturity. Such practices are also common in other parts of the world (Ghorbani *et al*., 2014; Kreouzi *et al*., 2015). Further, the harvesting of the orchids involves destructive uprooting of the daughter tubers, which kills the plants.

When ascending from lowland to alpine environments in the Himalayas, plant species experience a large variation in abiotic conditions over an extremely short distance

(Korner, 2003). With increasing elevation, changes in pressure, temperature, wind speed, UV exposure, and soil properties have been shown to affect different phenological and morphological properties of plants (Hodkinson, 2005; Djordjevic & Tsiftsis, 2020), thus also influencing growth and reproductive performance. The decreasing number of reproductive parts observed along the elevation gradient could be further attributed to the time of flowering, which is influenced by the ambient temperature and the timing of the snow melt (Kudo & Hirao, 2006).

4.4.3 Effects of different environmental variables on the density of different stages

The occurrence and distribution of orchid species are influenced by environmental and topographical factors such as latitude, altitude, slope and aspect (Bulafu *et al*., 2007; Djordjevic *et al*., 2016; Djordjevic *et al*., 2020). This study recorded a very weak negative effect of the Relative Radiation Index (RRI) on the adult vegetative density ($P<0.1$) of *D. hatagirea*. Although the effect is weak, it may indicate that *D. hatagirea* prefers growing in humid places. Mixed ZIP models revealed a significant positive effect of herb cover on the juvenile and adult vegetative density. It may be possible to explain this as a consequence of the herbs providing shade and shelter for the growth and development of *D. hatagirea* individuals at different life stages. The harvesting showed significant negative effects on the density of all stages except seedlings, as also observed in other parts of the world (Ghorbani *et al*., 2014; Kreziou *et al*., 2015), whereas grazing showed significant negative effects on juvenile and adult vegetative stages only. Contrasting results from other parts of the world also exist. For example, Charitonidou *et al*. (2019) showed that the current level of collection of *Dactylorhiza sambucina* in Greece is not significantly affecting the abundance of this orchid, and Mincheva and Kozuharova (2018) reported that wild orchids are not threatened by harvesting in Bulgaria.

Grazing and trampling adversely affect aboveground parts and disturb the life cycle. In some cases, the underground parts are also exposed and eventually destroyed. The weak positive effect of harvest and grazing on plant density at the protected site for all the different stages could be linked to the narrow range of harvest and grazing intensities observed. By contrast, at the unprotected site, the negative impact of harvesting and grazing on density of different stages was highly pronounced. The prevailing disturbance practices (grazing, trampling and over-exploitation) and lack of awareness of the population ecology and the conservation status of the plant are the major challenges for sustainable management at the unprotected site (Pouliot *et al*., 2018). This is also in agreement with Poudeyal *et al*. (2019) who observed that intense human disturbances, especially harvest, played a crucial role as determinants of the density and structure of *Neopicrorhiza scrophulariiflora* populations.

4.4.4 Local communities' perception

I observed a higher proportion of people at the protected site who knew about the harvest ban and the population ecology and use value of the species than at the unprotected site. The strict enforcement of MAPs harvesting rules by the community at the protected site is the key factor for the maintenance of sustainable populations of *D. hatagirea*. People at the protected site do not allow people from other districts to collect any types of MAPs in their territory (NTNC, 2016) and this helps to maintain a low harvesting pressure in populations of MAPs including *D. hatagirea.* Moreover, the Annapurna Conservation Area Project (ACAP), which has worked in this area for three decades, has also contributed to increase conservation awareness and promote sustainable use of natural resources (Baral & Heinen, 2007).

In contrast, in the unprotected site there is no strict local protection system and people from outside ANCA are also allowed to harvest MAPs. The local MAPs users were also found to have a low level of conservation awareness (in relation to *D. hatagirea*) as also reported for northwestern Greece by Kreziou *et al*. (2015). Moreover, due to the lack of protection measures and awareness, the MAPs users were found to engage in over- harvesting of *D. hatagirea*, and selling it to local traders (Pyakurel *et al*., 2018) despite the local legal acts protectio of *D. hatagirea.*

Additionally, a group of locals at the unprotected site has the view that the uncontrolled influx of unaware collectors from different parts of the country exacerbates the exploitation of the alpine vegetation. The prevailing unhealthy competition of MAPs collection among the collectors in the unprotected site sometimes terminate in social conflicts which promotes the illegal harvest of *D. hatagirea* challenging its persistence. Thus, unregulated harvesting of *D. hatagirea* could be one of the major reasons for decline in the populations of the species.

4.5 Conclusion and conservation implications for *D. hatagirea*

Harvesting and grazing and a low level of awareness about the population ecology and conservation among local people are the major challenges for conservationt of *D. hatagirea* populations at the unprotected site. Harvesting and grazing showed significant negative effects on the density of different life stages of *D. hatagirea* and played a crucial role in deteriorating plant populations through reduction in reproductive outputs. Hence, this study indicated significantly reduced fruit production and lower productivity in terms of tuber biomass for plants at the unprotected site. Moreover, disturbances have the potential to cause a reduction in number of recruits and adult individuals (both vegetative and reproductive) as intensive destructive harvesting techniques are used at the unprotected site, irrespective of life stage and maturity. Coupled with other prevalent disturbances, the intensive and destructive harvesting may lead to local extinction of the species. Therefore, a good strategy for long-term conservation of the species would involve strengthening people’s knowledge about the population ecology of *D. hatagirea*, provide regular awareness program, increasing the enforcement of current regulations and introducing permanent monitoring of the populations. This study also recommends that governmental and non-governmental organizations working in the field of conservation help identifying alternative sources of income for the locals at the unprotected site, so that their dependency on MAPs harvesting can be reduced, thus also reducing the pressure on populations of *D. hatagirea* and preventing local extinction of the species.

CHAPTER 5

POPULATION DYNAMICS OF AN ENDANGERED ORCHID *Dactylorhiza hatagirea* (D. DON) SOÓ IN NEPALESE HIMALAYA: EFFECTS OF HABITATS AND HARVESTING

Abstract

Explaining the variation in population growth rate and identifying the causes of that variation is important for effective conservation of endangered species. In this study, population dynamics of an endangered medicinal orchid, *Dactylorhiza hatagirea* was studied and its current status was evaluated in two sites, one protected from commercial harvesting by the locals (Manang) and the other unprotected (Darchula) and thus receiving high harvesting pressure from commercial collectors. In the unprotected site, further comparison of population demography was made in wetland and grassland habitats. Matrix projection models, based on three years of data of seven populations (three in each site in grassland plus one wetland habitat in unprotected site), were used to identify the patterns of variation in the demographic vital rates of this species. Demographic data included 3-year observations on growth, fecundity and survival of individuals classified by size. Transition probabilities for stage-based population projection matrices varied between populations and among years. The population growth rate (λ) was 0.826 and 0.553 in the unprotected site, and 1.166 and 1.003 in the protected site for the transition year 2015/2016 and 2016/2017, respectively. The wetland and grassland habitats in the unprotected site had λ of 0.847 and 0.826 respectively, in 2015/2016 and 0.780 and 0.553 respectively in 2016/2017. The elasticity analysis showed that the stasis in the juvenile stage in unprotected site, and reproductive adult stage in protected site contributed most to λ. Numerical simulations were performed by changing the value of specific matrix entries and directly evaluating the effect on λ. Population growth rate reached values above unity only when fecundity was increased 10-fold in unprotected grassland populations. Further, the wetland population in unprotected site required 25-fold increase in fecundity to reach the population growth rate of unity.

Management should focus on the maintenance of existing populations by maintaining the seeding recruitment rate in the protected site and increasing it in the unprotected site. The wetland populations should be conserved with great focus on increasing the existing fecundity. Given the limited range and low population density of the species, this study supports management practices aimed at increasing seedling germination and establishment success. The results recommend that in the protected site very limited harvesting (10%) can be allowed for local use. In unprotected site commercial harvesting should be strictly banned for certain period of time.

Keywords: Demography, fecundity, population growth rate, rotational harvest

5.1 Introduction

Explaining variations in population growth rate and identifying the underlying causes is critical for effective conservation of an endangered species like *Dactylorhiza hatagirea* (Thakur *et al.*, 2020). Population dynamics studies are an important foundation for understanding the future of plant populations and allowing predictions about the impact of different disturbance regimes and habitats on plant population performance. Disturbance regimes including over exploitation, habitat fragmentation, climate change and herbivory play crucial roles in altering the life history stages and population dynamics of plant species (Shrestha *et al*., 2021). Harvesting of MAPs affects survival, growth and fecundity and thereby also affects population growth rate and dynamics (Ghimire *et al*., 2008).

The transition matrix model is a commonly used technique to describe the population dynamics of endangered plants, predict future viability, and apply the results to sustainable management (Crone *et al*., 2013; Caswell, 2001). Matrix models have also been used to determine the population growth rates, life expectancy and population extinction risk, and to identify the specific vital rates that are most likely to influence the population growth rate. This approach integrates the effects of management on the life history components (survival, growth, reproduction, mortality) and population dynamics (Kaye *et al*., 2001).

Further, these techniques can be applied to assess impacts of harvesting on life-history processes, identify optimal harvest regimes, disentangle the role of habitats and other factors and devise sustainable and profitable management plans for various plant species (Ghimire *et al*., 2008; Schmidt *et al*., 2011; Mondragón Chaparro & Ticktin, 2011; Klimas *et al*., 2012). However, the fact that collecting detailed demographic data is very laborious and time-consuming hinders the wider application of transition matrix models. It is therefore appealing to use knowledge on population dynamics and resulting conclusions on optimal management obtained from one region (and/or species) to other regions and/or to closely related species. Different management regimes may serve as potential drivers of local adaptations and variations in grassland plant life histories. Much of the previously generated demographic information is now being used for all kinds of purposes, from management plans (Olmstead & Alvarez-Buylla, 1995) to life history analysis and conservation guidelines (Silvertown *et al*., 1993, 1996). Demographic studies also play an important role for evaluating the response of orchid populations to external factors, particularly management (Shefferson *et al*., 2001; Ke´ry & Gregg, 2003, 2004). Orchids can exhibit large inter-annual variations in mortality, fecundity, and other vital rates (Hutchings, 2010). Terrestrial orchids typically remain dormant for one or many years, emerging from a dormant state in a vegetative or flowering state, with only a limited proportion of plants growing above ground each year (Reintal *et al*., 2010).

Anthropogenic disturbances like harvesting, grazing and trampling are threatening the populations of many Himalayan orchids including *D. hatagirea* and achieving a sustainable harvest is a great challenge (Thakur *et al*., 2020). Even at low harvest intensities the exploitation of the species is likely unsustainable as the prevailing harvesting is destructive irrespective of life stage (chapter 4). Given that many orchids are currently endangered or on the verge of extinction, a better understanding of the factors driving orchid population dynamics will be important to their long-term conservation.

D. hatagirea (D. Don) Soó is an endangered medicinal orchid with high medicinal value. It has a restricted distribution range from 3400 m to 4600 m above sea level (m asl) in the Himalayan region. The species is found in loamy soil (chapter 4) at an elevation of 3400 to 4600 m asl in Manang district, north-central Nepal, 3600–4200 m asl in Darchula district, north-western Nepal, and in similar ranges in other parts of the Himalaya. *D. hatagirea* is generally found in populations with a relatively low density, e.g., 1.31 plants/m^2 in protected site (Manang) and 0.72 plants/m^2 in unprotected site (Darchula) (chapter 4). The species is endangered across its entire distribution range, and is of interest for nature conservation efforts in all countries within its range. It's populations are highly threatened due to anthropogenic disturbances. However, local participation in conservation activities emphasizing MAPs offers some protection of populations in some parts of Nepal, e.g., Manang (chapter 4).

Long-term studies pertaining to population dynamics of endangered plant species, especially orchids are very scarce in Nepal and this has hampered the process towards devising conservation strategies and actions. Government of Nepal has listed *D. hatagirea* as a protected species under the Forest Act (2019), but due to poor implementation and enforcement of the law and the existence of lucrative markets for tubers of the species, local harvesters are heavily involved in illegal harvesting and trade for their livelihoods. The rugged topography, difficult terrain and lack of transportation facilities in the high mountainous region of the country are the hindrances for the government authorities to reach to the growing site where *D. hatagirea* is actually harvested and traded. Due to this the implication of government laws and policies in the ground level is very weak. This is one of the major factors that hinder the prevention of illegal harvest of *D. hatagirea* tubers.

Habitat modification, climate change, and over-harvesting for ornamental or medicinal values are the factors thought to increase the vulnerability of orchid species (Swarts & Dixon, 2009; Wraith *et al.*, 2020). How orchids became endangered and how their fate is linked to demographic characteristics is still poorly understood. Better predictive and explanatory models are needed to study the vulnerability and extinction risk of orchids. The forecasting of extinction has been an important aspect of conservation biology, since habitat loss, introduced organisms, and over-exploitation of natural populations have long been a concern (Pimm *et al.*, 2014). In recent decades, the issue of climate change has placed new emphasis on extinction threats (Thomas *et al.*, 2005). However, studies of disturbance and habitat impacts on the population dynamics of endangered plant species are scarce in the Himalayas. Some studies, like Ghimire *et al.* (2008) and Rokaya *et al.* (2017) have explored the effects of harvesting on population dynamics of threatened Himalayan species in multiple habitat types and have stressed the importance of such long-term research for other endangered species.

Orchid species conservation priorities seem to be confused. For orchids, the current state of the art is to assess population health based on adult population and their geographic range. However, despite the growing awareness and surveillance of endangered orchids, little research has been done from a demographic perspective.

Demographic variation of an endangered orchid *D. hatagirea* were compared in six populations, three exposed to high anthropogenic pressure (Darchula district) and three experiencing very little anthropogenic pressure (Manang district). The demography of *D. hatagirea* was also compared in two contrasting habitats, grassland and wetland, in unprotected site and sustainable management strategies suggested by the present results were proposed. This research is based on multiple-season data from the higher Himalayan ranges of Nepal**.**

The main objective of this study is to describe the life cycle characteristics and the population dynamics of *D. hatagirea*. Specifically, (i) to explore its population dynamics, (ii) to contribute knowledge and evaluate the most important events in the life history, (iii) to disentangle the role of habitats (grassland and wetland) in the population demographics, and (iv) to predict the present sustainable harvest level of the species in the study sites.

5.2 Materials and methods

5.2.1 Study area

The demographic study of *D. hatagirea* was carried out in three different populations each in north-central (Manang district) and four different populations in north-western (Darchula district) Nepal (Figure 10). Manang populations were protected from commercial harvesting, but the Darchula populations received high harvesting pressure from commercial collectors. Due to contrasting levels of anthropogenic disturbances, these two sites were regarded as locally protected (Manang) and locally unprotected (Darchula) on the basis of degree of local participation on the conservation of MAP resources (chapter 4). The study sites are almost similar in topographic and edaphic factors (Appendix II). In protected site, all the three populations of *D. hatagirea* occur in grassland habitat. In unprotected site (Darchula), three populations from grassland and one from wetland habitat were selected (details in chapter 4).

5.2.2 Demographic data

Demographic data in all the seven populations (ranging in elevation from 3400 to 4600 m) of two sites were collected during 2015-2017. All the plants found within 21 permanent 20×20 m plots which were divided into eighty-four 10×10 subplots (thirty-six in each site, plus twelve in wetland habitat in Darchula) were located, numbered and tagged in each population on both the sites during June-July 2015 (n = 1316 in unprotected site, and 2488 in protected site). A light aluminum plate (3×3 cm) having individual identity number was used to tag each individual. Each tag was tied with the aid of a short twisting wire in a bamboo stick; the latter was inserted deep into the soil at the close vicinity of the plant taking enough care so that the stick will not damage the plant and its tuber. The purpose of tagging was to identify each individual to be re-measured a year after. In 2015, 2016, and 2017, all the available demographic parameters for each individual, *viz*., plant height (for adult reproductive), number of leaf, length and breadth of the largest leaf (in all the individuals), stem girth (5 cm) above the ground, only for adult reproductive), were recorded. During the fruiting season of each study year, inflorescence length and the number of reproductive parts per plant (intact and aborted, buds, flowers and fruits) were recorded. Seed germination experiments were performed in 2015 by establishing three 1mx1m plots in close vicinity of each population, removed all the plants present there and dusted the seeds from 50 capsules of 5 matured plant individuals in each plot. As the result was not satisfactory it was not included in the analysis here. So, to calculate the fecundity measure the counts of seedlings in 2016 and 2017 were used and estimated the fecundity as the number of seedlings per adult reproductive plant.

In a number of cases, where inflorescences were grazed prior to the data recordings, stem diameter of adults were measured and directly labeled those as adult reproductive as plants in other stages are smaller and do not produce thick stem. In 2016 and 2017, all the tagged individuals were revisited (during June-July) to determine the number of intact, wilted and grazed (when entire inflorescence is removed), and partially damaged inflorescences. I also measured and recorded all the biological details in 2016 and 2017. A proportion (0.38% in unprotectes and 0.36% in protected site) of individuals in permanent plots were dormant at 2016 recordings, i.e., they did not produce aboveground parts. A number of individuals were found harvested in 2016 (n = 324) and 2017 (n = 591) in unprotected site and labeled them as dead. To assess dormancy in 2016, I therefore revisited all populations in 2017 to record the survival of plants present in 2015 and absent in 2016. Of the 102 individuals that were present in 2015 but absent in 2016, only 39 reappeared in 2017 (from the unprotected populations). Similarly, out of 338 individuals present in 2015 but absent in 2016 only 121 reappeared in 2017 (from the protected populations). Higher proportions of plants in juvenile stage showed a tendency of entering into dormancy at both the sites (24 and 76 individuals in unprotected and protected site, respectively).

5.2.3 Matrix construction

Stage-based population projection matrix models (Lefkovitch, 1965) were constructed for all individual populations using a life cycle graph (Figure 15) to estimate demographic parameters. I used plant size measures and presence/absence of a flower stem to classify individuals of *D. hatagirea* into four stage classes (seedling, juvenile, adult vegetative, adult reproductive (details in chapter 4) In addition, dormancy stage was added as an additional classification (when the plant remains dormant in 2016) and a state known as "dead" when plants were uprooted or remained dormant for the two consecutive years (Table 12).

Table 12: Criteria of Stage categories used to describe the demography of *Dactylorhiza hatagirea*

Life cycle stage	**Abbreviation**	**No. of leaves**	**Breadth of largest leaf**	**Stem**	**Remarks**
Seedling n	Sd	1 to 2	≤ 1 cm	Absent	
Juvenile	Jv	2 to 3	>1, ≤ 2 cm	Absent	
Adult vegetative	Adv	more than 2	> 2 cm	Absent	
Adult reproductive	Adr	more than 2	> 2 cm	Present	Flowering and fruiting
Dormant	Dr				Tag found but above ground parts not found
Dead	Dd				Uprooted or dormant for two consecutive years

An annual transition matrix based on June/July censuses was constructed for each population by calculating the following demographic parameters: (i) the average number of seedlings produced per reproductive adult (F, fecundity); (ii) the proportions of individuals that survived and remained in the same size class (S, stasis); (iii) the proportions that survived and grew to a larger size class (P, progression); and (iv) the proportions that survived but shrank into a smaller size class (R, retrogression). Each transition matrix was analyzed to calculate asymptotic population growth rates (λ) and stable size distributions (Caswell, 2001), and these quantities were compared across habitats and sites. The 95% confidence interval (CI) for each population growth rate value was estimated by the bootstrap percentile interval method (Caswell, 2001).

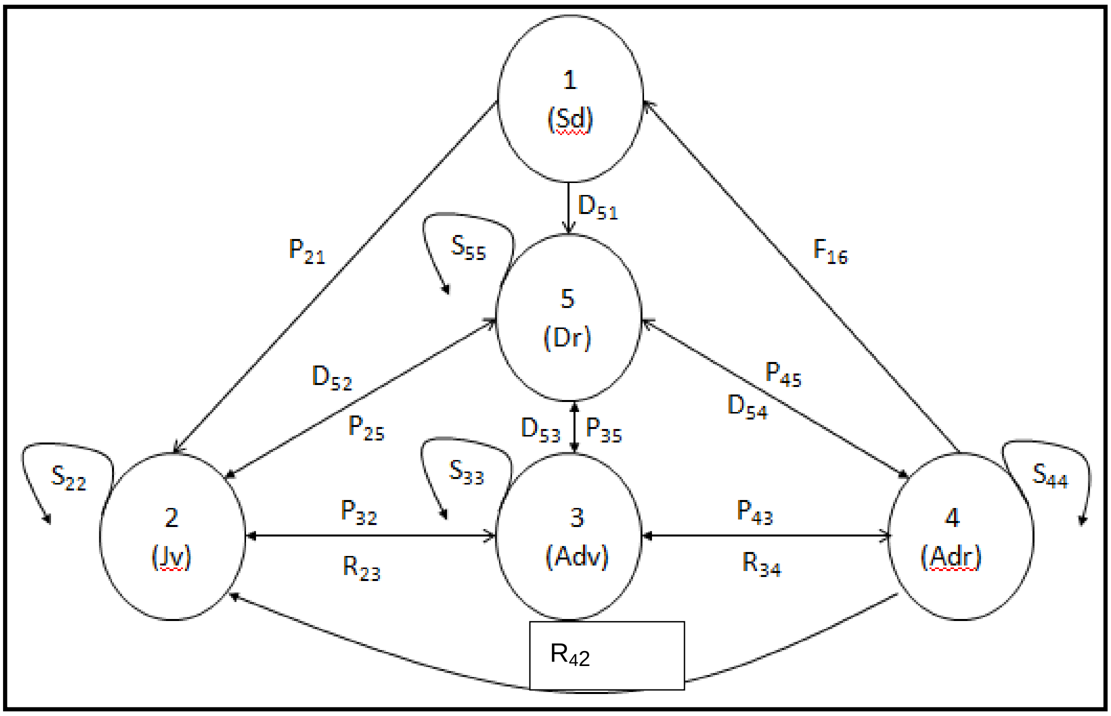


Figure 15: Life cycle graph of *Dactylorhiza hatagirea*

5.2.4 Matrix analysis

It has not always been possible to determine with certainty whether plants that have not appeared in the final year of the study are dormant or dead. However, this information was necessary to enable estimating dormancy rates and maintain sufficient data for analyses. Confirmatory excavation of tubers was not possible due to the banned conservation status of the studied species. Therefore, it is arbitrarily assumed that all plants that did not appear in the final census in 2017 were dead, which may lead to underestimation of dormancy rates and overestimation of mortality rates.

Each stage class included a minimum of 27 individuals in unprotected site and 77 individuals in protected site from which the transition probabilities could be estimated. Transition probabilities among size classes were calculated by calculating the relative frequencies of observed transition including death from year 1 to the next. Fecundities were estimated as the mean number of seedlings produced per reproductive plant.

Matrix limit properties (i.e., the dominant eigenvalues and the right and left eigenvectors corresponding to population growth rates, stable stage distributions, and stage-specific reproductive values, respectively) were obtained using the statistical software package R (https: http://www.r-project.org/). The 95% confidence intervals for λ were also computed using the same package in R.

From the right and left matrix eigenvectors the sensitivity of λ to changes in each matrix entry was computed (Caswell, 2001). From these values, elasticity matrices for the study periods (2015/2016 and 2016/2017) and population at each site were constructed. The elasticity evaluates the relative sensitivity of λ to relative changes in each matrix entry. Since the elasticity of the matrix sums to 1, each elasticity value can also be interpreted as the contribution of each matrix entry to the finite growth rate of the population (de Kroon *et al*., 1986; Silvertown *et al*., 1996; Caswell, 2001). Elasticity is therefore a useful tool from a conservation perspective, as it can identify the most vulnerable stages of a species' life cycle (de Kroon *et al*., 1986; Silvertown *et al*., 1996; Mills *et al*., 1999).

5.2.5 Matrix simulations

Numerical simulations were performed using the population projection matrix obtained for the period 2016/2017 to assess the potential impact of specific changes in specific matrix entries on population growth rate λ. Therefore, I multiplied the original fecundity estimates by various numbers from 5 to 30 to assess the impact of changing the fecundity values.

I followed the fate of all individuals of *D. hatagirea* occurring in all the populations. The number of plants per population was 199, 443 and 674 in unprotected site and 543, 989 and 956 (including seedling, juvenile plus adults) in protected site at the beginning of the experiment. Annual seedlings emergence was counted in all the plots in all the populations during 2016 and 2017. The fecundity was calculated by counting the number of seedlings produced per reproductive adult as sowing experiment was not feasible within the short duration of the study.

5.2.6 Life cycle and transition matrix model for *Dactylorhiza hatagirea*

The life cycle diagram for *D. hatagirea* (Figure 15) was translated into a projection matrix where the matrix elements a_{ij} and F_{ij} define transition from stage *j* to stage *i* within a time interval of one year.

$$\begin{bmatrix} n_{Sd} \\ \vdots \\ n_{Adr} \end{bmatrix} = \begin{bmatrix} a_{11} & \cdots & a_{15} \\ \vdots & \ddots & \vdots \\ a_{51} & \cdots & a_{55} \end{bmatrix} \cdot \begin{bmatrix} n_{Sd} \\ \vdots \\ n_{Adr} \end{bmatrix} + \begin{bmatrix} F_{Sd} & \cdots & F_{Adr} \\ \vdots & \ddots & \vdots \\ 0 & \cdots & 0 \end{bmatrix} \cdot \begin{bmatrix} n_{Sd} \\ \vdots \\ n_{Adr} \end{bmatrix}$$

The fate transition (a_{ij}) varies between 0 and 1, whereas 'fecundity' transitions (F_{ij}) can have values greater than 1. Transition matrices are described in detail by Caswell (2001). Analysis of a projection matrix at asymptotic population growth yields the population's finite growth rate; $\lambda = n_{t+1} / n_{t}$, where n_t is the size of the population at time t. It can be used to show how the population growth rate changes with a change in any matrix element a_{ij}. Elasticity, defined as the proportional change in a_{ij}, was analyzed to compensate for differences in the absolute values of a_{ij} (deKroon *et al*., 1986). The transition frequency table describes the fate of the individuals at each state, where the transition frequencies are the numbers ('counts') of individuals involved in each transition.

The importance of fecundity on the sustainable population growth rate was measured in unprotected population. To do so fecundity was increased by a factor of 5, 10, 15, 20 and 25 and compared changes in population growth rate.

5.3 Results

Decreased plant density was recorded in unprotected while it was almost constant in protected populations (chapter 4). Tables 13 and 14 show the transition matrices for unprotected and protected grassland populations respectively obtained for 2015/2016 and 2016/2017. Along with the matrices the population finite rate of increase (λ) and the size-specific reproductive values (V) are presented. The λ values of the two matrices were lower than unity for unprotected but above unity for protected populations during both the transitions.

Distinct variation in recruitment was not recorded among the sites and years but recorded higher population growth rates in protected than in unprotected (Table 13 and 14). The protected population showed increasing population growth rate (λ >1), whereas unprotected populations showed decreasing population growth rate (λ < 1) (Table 15). The wetland population in unprotected site also showed decreasing population growth rate (Table 16). The λ value in protected site indicates that, under current conditions, approximately 10% of the individuals can be harvested without exceeding their reproductive capacity, whereas harvesting in unprotected site will lead to further decline as these populations are already suffering from an unsustainable harvesting pressure. Under these conditions the fecundity value in unprotected site must be increased 10-fold to maintain the sustainable size of the populations. The wetland population of unprotected site is even more vulnerable to extinction as it requires a 25-fold increase in fecundity to reach a population growth rate of $\lambda = 1$.

Mortality in unprotected populations was irrespective of size class and was basically caused by destructive harvesting, whereas the mortality was found closely related to size class in protected populations (Table 13 and 14). The seedling stage showed highest probability of dying among the stages in both the sites. However, the mortality rate of other stages was higher in unprotected populations as compared to protected populations (Table 13 and 14).

Number of recruits of the juvenile stage is higher in both the sites and during the years. The populations' size structures observed in 2015 and 2016 were characterized by a relatively higher frequency of juvenile plants and non-reproductive adults and a lower number of seedlings and reproductive adults in unprotected populations while the protected population is characterized by higher frequency of juveniles and reproductive adults. Non-reproductive adult individuals made up approximately 32% of the populations and approximately 3% of the individuals were dormant in unprotected populations. In protected site juveniles and reproductive adults constituted approximately 30% of the population and approximately 6% of the individuals were dormant.

Table 13: Population projection matrices of unprotected site during 2015–2016 and 2016–2017

λ = 0.826

	Life cycle stage 2015									
Life cycle stage 2016	**Sd**	**Jv**	**Adv**	**Adr**	**Dr**	**Nr**	**V**	**Dar**	**Ntr**	**Gt**
Sd					NA	0.164	1.000	2.049	0.332	2.916
Jv	0.473	0.393	0.164	0.073	NA	0.591	1.290			
Adv		0.310	0.292	0.110	NA	0.244	1.269			
Adr		0.821	0.229	0.500	NA		1.163			
Dr	0.158	0.084	0.062	0.083						
N	43	444	349	317	102					
M	0.369	0.230	0.251	0.231						

λ = 0.553

	Life cycle stage 2016									
Life cycle stage 2017	**Sd**	**Jv**	**Adv**	**Adr**	**Dr**	**Nr**	**V**	**Dar**	**Ntr**	**Gt**
Sd						0.164	1	3.68	0.110	2.296
Jv	0.140	0.171	0.103	0.072	0.236	0.424	1.290			
Adv		0.180	0.190	0.187	0.148	0.410	1.146			
Adr		0.031	0.080	0.149			1.172			
Dr	0.280	0.129	0.074	0.094			0.993			
N	24	227	280	89	125					
M	0.581	0.487	0.530	0.499	0.618					

Only non-zero entries are included to facilitate reading. Above each matrix the population growth rate is given. V = size-specific reproductive value; N = number of individuals in each size category, M = mortality. Sd = Seedling, Jv = Juvenile, Adv = Adult vegetative, Adr = Adult reproductive, Dr = Dormant, Nr = New recruited, Gt = Generation time, Dar = Damping ratio, Ntr = Net Reproductive value

Table 14: Population projection matrices of Manang 2015–2016 and 2016–2017

$\lambda = 1.166$

	Life cycle stage 2015									
Life cycle stage 2016	**Sd**	**Jv**	**Adv**	**Adr**	**Dr**	**Nr**	**V**	**Dar**	**Ntr**	**Gt**
Sd					NA	0.304	1.000	2.346	0.707	4.093
Jv	0.677	0.331	0.036	0.090	NA	0.534	1.046			
Adv		0.169	0.096	0.140	NA	0.160	0.900			
Adr		0.377	0.786	0.697			0.941			
Dr	0.323	0.123	0.082	0.074						
N	433	908	448	660						
M										

$\lambda = 1.003$

	Life cycle stage 2016									
Life cycle stage 2017	**Sd**	**Jv**	**Adv**	**Adr**	**Dr**	**Nr**	**V**	**Dar**	**Ntr**	**Gt**
Sd						0.363	1	2.020	0.410	9.419
Jv	0.602	0.497	0.110	0.120	0.224	0.508	1.204			
Adv		0.203	0.370	0.228	0.128	0.129	1.277			
Adr		0.134	0.420	0.603	0.006		1.331			
Dr	0.268	0.09	0.060	0.027			0.550			
N	96	936	662	975	175					
M	0.130	0.077	0.041	0.022	0.642					

Refer Table 13 for abbreviations

The vector of scaled reproductive values (i.e., vector v in Tables 13 and 14) shows lower values for seedlings and reproductive adults for the 2015-2016 periods. The overall effect of relative changes in sets of transition parameters, representing four demographic processes (stasis, progression, retrogression and sexual fecundity) and the six stage categories were studied. This elasticity analysis showed that the change in population growth rate was strongly affected by stasis in the juvenile stage in unprotected site and the adult reproductive stage in protected site (Table 16).

Table 15: Population projection matrices of unprotected wetland 2015–2016 and 2016–2017

λ = 0.847

	Life cycle stage 2015									
Life cycle stage 2016	**Sd**	**Jv**	**Adv**	**Adr**	**Dr**	**Nr**	**v**	**Dar**	**Ntr**	**Gt**
Sd					NA	0.194	1.000	4.259	0.268	4.737
Jv	0.800	0.367	0.118	0.192	NA	0.556	0.953			
Adv		0.050	0.294	0.037	NA	0.250	1.129			
Adr		0.350	0.470	0.507	NA		0.906			
Dr		0.050	0.030	0.060	NA					
N	5	60	35	83						
M	0.200	0.183	0.089	0.204						

λ = 0.780

	Life cycle stage 2016									
Life cycle stage 2017	**Sd**	**Jv**	**Adv**	**Adr**	**Dr**	**Nr**	**v**	**Dar**	**Nt**	**Gt**
Sd						0.333	1.000	1.329	0.021	8.458
Jv	0.286	0.470	0.240	0.050	0.333	0.667	1.267			
Adv		0.107	0.040	0.063			0.872			
Adr		0.046	0.080	0.582			1.339			
Dr	0.571	0.242	0.280	0.076			0.604			
N	7	65	25	80	9					
M	0.142	0.137	0.360	0.228	0.667					

Refer Table 13 for abbreviations

Comparing the habitats, the population in the wetland habitat showed decreasing population growth rate (lambda = 0.84 and 0.74 in 2015-2016 and 2016-2017, respectively) with high number of recruits of the juvenile (Table 15). I found almost similar mortality rates among the seedling and adult reproductive stages (Table 15). The elasticity analysis showed that the stasis of the adult reproductive stage contributed most to the population growth rate in 2015-2016 transition, whereas in 2016-2017 it was juvenile stage that contributed the most (Table 16).

Table 16: Elasticity Matrices of *Dactylorhiza hatagirea* in different populations in unprotected and protected site

Unprotected grassland 2015-2016	**Life cycle stage 2016**	**Life cycle stage 2015**				
		Sd	**Jv**	**Adv**	**Adr**	
	Sd	0.050	0.072	0.038	0.032	
	Jv	**0.142**	**0.187**	0.040	0.021	
	Adv		0.098	0.070	0.031	
	Adr		0.036	0.050	**0.131**	
Unprotected grassland 2016-2017	Life cycle stage 2017	Life cycle stage 2016				
		Sd	Jv	Adv	Adr	Dr
	Sd	0.030	0.053	0.049	0.009	0.027
	Jv	0.053	**0.098**	0.054	0.010	**0.100**
	Adv		**0.091**	0.089	0.024	0.056
	Adr		0.017	0.039	0.020	
	Dr	0.082	0.058	0.030	0.010	
Protected 2015-2016	Life cycle stage 2016	Life cycle stage 2015				
		Sd	**Jv**	Adv	Adr	
	Sd	0.110	**0.086**	0.014	0.070	
	Jv	**0.171**	0.081	0.002	0.030	
	Adv		0.036	0.007	0.040	
	Adr		0.082	0.059	**0.210**	
Protected 2016-2017	Life cycle stage 2017	Life cycle stage 2016				
		Sd	Jv	Adv	Adr	Dr
	Sd	0.011	0.029	0.016	0.022	0.007
	Jv	0.061	**0.140**	0.024	0.040	0.017
	Adv		0.060	0.088	0.079	0.010
	Adr		0.041	**0.103**	**0.219**	
	Dr	0.012	0.011	0.006	0.004	
Unprotected wetland 2015-2016	Life cycle stage 2016	Life cycle stage 2015				
		Sd	**Jv**	Adv	Adr	
	Sd	0.010	0.039	0.007	0.05	
	Jv	0.094	**0.160**	0.009	0.108	
	Adv		0.026	0.026	0.023	
	Adr		**0.146**	0.034	**0.268**	
Darchula wetland 2016-2017	Life cycle stage 2017	Life cycle stage 2016				
		Sd	Jv	Adv	Adr	Dr
	Sd		0.008	0.001	0.003	0.003
	Jv	0.009	**0.350**	0.036	0.018	**0.118**
	Adv		0.054	0.004	0.014	
	Adr		0.036	0.012	**0.214**	
	Dr	0.008	0.080	0.020	0.012	

Refer Table 13 for abbreviations

5.4 Discussion

Our knowledge on the orchid longevity is limited because there are relatively few long-term population studies on orchids. Experimental application of harvest simulation is not possible for the endangered species *D. hatagirea* as harvesting is banned for this species and therefore this study applies an observational approach. Further, working in the difficult terrain is a great challenge. However, to achieve the conservation of the endangered species the challenges imposed by the endangered species must be addressed. The life history of orchids is relatively well understood qualitatively, but quantifying the dynamics and extracting a conservation message remains challenging. Although the life history of orchids is qualitatively understood, quantifying their dynamics and extracting conservation messages remains a challenge**.**

Comparison of the response of *D. hatagirea* to harvesting was based on selecting two different sites with distinct harvest pressure. However, due to logistic limitations the effect of habitat was examined using a single population in unprotected site (Darchula). Despite these limitations, this study provided clear insight into the impact of habitat variation and harvesting on demographic processes and the population dynamics of *D. hatagirea*.

5.4.1 Demography

Wetland and grassland populations differed greatly in observed stage structure. Grassland populations at the unprotected sites consisted of a high proportion of juvenile-stage individuals, whereas wetland populations at this site contained similar proportions of juvenile and adult size class individuals.

The proportions of seedlings were much lower in the wetland population. Habitat-specific variation in germination traits is common to subalpine and alpine perennial herbs and is often related to spatiotemporal variation of seed availability, snowmelt time and availability of suitable sites for germination (Shimono & Kudo, 2003).

Consistent with other studies, I found a strong size dependence of fecundity and survival in both populations (Drechsler *et al.*, 1999; Méndez & Karlsson 2004, Ghimire *et al*., 2008). The higher fecundity of the grassland population might be due to higher flowering frequency and higher rate of seedling recruitment than in the wetland population. Mortality (natural) was highest for seedlings and juveniles, particularly in the grassland, and lowest for the larger adults, however the mortality due to harvest was higher in grassland population (indicated by the holes and remains of the uprooted plants recorded during survival monitoring in 2016 and 2017) than the wetland as adults here are less vigorous with small sized tubers (personal observation). The stony substrates of grassland habitats, which have vastly different soil moisture and nutrient levels, may explain why seedling mortality compared to adult plants is higher than in wetland habitats.

Orchids produce a huge number of seeds but have a low pre-reproductive survival rate. Difficulties in dispersal, failure in seed germination, and problems with establishment are factors that severely limit the survival to maturity (Shefferson *et al*., 2020). A short growing season in alpine environments is an additional factor affecting seedling establishment. Only seedlings that reach critical size at the end of the first growing season are likely to survive the winter (Shimono & Kudo, 2003). High mortality, especially in the early part of life, is characteristic of different organisms in different taxonomic groups. Thus, a better understanding of orchids can therefore also help us understand the population dynamics of other high fecundity organisms. Conversely, other well-studied organisms with similar life strategies can shed light on important aspects of orchid ecology.

The protected populations had higher proportion of adults (both vegetative and reproductive). Large adults are often numerically dominant in populations of perennial species (Cook, 1985), and populations growing in harsh environments often experience greatly reduced seedling recruitment (chapter 2). Mortality was highest in seedlings and juveniles and lowest in adults (vegetative and reproductive) in the protected population. Orchids belong to a group of highly fertile organisms, but have a correspondingly low pre-reproductive survival rate. All these organisms are essentially based on the same principle. They produce large numbers of offspring in the hope that some will reach adulthood, with much lower mortality thereafter.

5.4.2 Population growth rate

Deviation of the population growth rate (lambda) from unity is commonly used to explore how far the population dynamics is from the equilibrium. Growth rates of the grassland and wetland populations of unprotected site were significantly <1 in both years, indicating very slow population growth. Population growth rates did not differ significantly from year to year in either habitat. Other studies have also reported small year-to-year variations in population growth rates, but large variations between habitats (Menges & Dolan, 1998; Ghimire *et al*., 2008). In contrast, growth rates in the protected populations were greater than 1 in both years, indicating positive population growth.

Elasticity analysis showed that stasis of the juvenile stage had the highest contribution to the asymptotic population growth rate in both the grassland and wetland populations of unprotected site, followed by progression of seedling to juvenile and juvenile to adult vegetative and stasis of adult reproductive stage in unprotected grassland. In contrast, in the protected population, reproductive adult stasis contributed most to the asymptotic population growth rate, followed by stasis of juveniles. High survival rates of adults and the tendency of adults to remain in the same stage from year to year (stasis) are also characteristic of other perennial species (Silvertown *et al.,* 1993; Silvertown *et al*., 1996; Ghimire *et al*., 2008). Fecundity made a much smaller contribution to the population growth rate of the studied population. This study shows that large-adult stage, through its capacity for stasis, represents a critical life-history stage that strongly influences the population growth rate of the study population.

5.4.3 Effect of harvesting

Harvesting of underground plant parts can have a significant impact on the growth rate of plant populations (Rock *et al*., 2004; Ghimire *et al*., 2008; Rokaya *et al*., 2017). It is found that harvesting of tubers affected most aspects (e.g., seedling density, fruit production, tuber size) of *D. hatagirea* (chapter 4). Harvesting had a significant impact on fecundity and growth of large individuals; it also significantly reduced population growth rate in unprotected populations.

Additional disturbances that are common in unprotected site are trampling and grazing (chapter 4). These additional human disturbances may partly explain the decreasing population growth rate observed in the unprotected populations. Animal grazing can have a profound effect on orchid population dynamics. Removal of buds and shoots by grazing can significantly affect germination and flowering, and reduced photosynthetic capacity for one year can prevent plants from absorbing sufficient carbon stores for flowering the following year. (Brzosko, 2002; Gregg, 2004).

Grazing intensity is also important and, for example, Gregg (2004) found that a moderately grazed population of *Cypripedium reginae* outperformed heavily grazed population in terms of equilibrium proportions of life stages, flowering and plant size.

5.5 Implications for sustainable management and conservation

Orchids are often the subject of conservation concern (Fay *et al*., 2015). The present results have many implications for conservation management of *D. hatagirea*. Although it is difficult to assess dormancy, it is clear that the unprotected population has not increased in number or spread over the past three years. Instead, flowering and emergence decreased in this population, while the opposite was true in the protected population. Understanding how plants and populations respond to harvesting is an integral part of a concrete foundation for conservation management. Harvest impacts on populations depend on their frequency and intensity, environmental conditions, and the viability and fertility of individuals after harvest (Ticktin, 2004; Ghimire *et al*., 2008; Rokaya *et al*., 2017). The results suggest that sustainable harvesting of D. hatagirea in unprotected grassland and wetland populations can only be achieved if fecundity can be increased by more than 10-fold and 25-fold, respectively.The protected populations showed positive population growth in both years of observation and under current conditions 10% of the protected population can be harvested sustainably.

Severe overharvesting of *D. hatagirea* throughout the Himalayas threatens the stability of many natural populations (chapter 2), and therefore their cultivation and *in situ* conservation should be encouraged. However, environmental changes appear to influence how rapidly populations recover from harvest. Differences in growth rates in different habitats can have important conservation and socioeconomic implications (Ticktin & Nantel, 2004). In particular, population size, nutrient availability and management methods can affect seed production (Oostermeijer *et al*., 1998; Lennartsson & Oostermeijer, 2001; Münzbergová, 2006), germination rate (Colling *et al*., 2002; Lindborg & Ehrlén, 2002) seedling survival (Colling *et al*., 2002) and the stasis and growth of individuals (Hegland *et al*., 2001; Dahlgren & Ehrlén, 2009Peters).

Due to the legal protection of the species, experimental harvest simulation was not feasible, so instead an observational approach was used and populations in two sites with different levels of protection and harvesting were selected to study the effect of harvesting on the population dynamics of *D. hatagirea*. The typical harvesting approach is destructive as the commercial collectors harvest the tubers irrespective of developmental stage and maturation and most of the people are unaware of the ecology and regeneration potential of *D. hatagirea* in unprotected site (chapter 4). Harvesting by commercial collectors, depends not on size and maturity of the plant, but on the response of collectors to market demand and value of the species (Ghimire *et al*., 2005). It is important to note that commercial harvesting of *D. hatagirea* in unprotected populations will lead to rapid population extinction if the current situation continues for several more years. This would be a huge social and biological loss.

The results show that *D. hatagirea* is highly sensitive to tuber harvest. This hypersensitivity is attributed to low germination and recruitment rates, high seedling and juvenile mortality (natural), and heavy harvesting of adult individuals by commercial collectors regardless of maturity. Given its limited population growth and high harvesting sensitivity, *D. hatagirea* should be managed strictly to establish seedling and meet fecundity levels that lead to stable population growth in severely depleted populations such as unprotected site. Fecundity-enhancing measures, such as demarcation of livestock grazing and trail routes, would be highly beneficial in the unprotected site.

Accurate management plans for endangered species such as orchids, based on demographic responses to harvesting in various habitats, are an important part of long-term conservation strategies, which is believed to integrate ecological, social and economic factors enabling local collectors to become economically viable from sustainable harvesting of MAPs (Ghimire *et al*., 2005; Ghimire *et al*., 2008; Rokaya *et al*., 2017). Of course, increased market demand could lead to exploitation of these populations in the future, and their status therefore depends on their level of protection and the availability of alternative harvesting opportunities.

5.6 Conclusion

The unprotected populations are highly threatened by harvesting and show declining population growth rates. Laws to stop illegal harvesting should be strictly enforced in unprotected populations to protect them from further deterioration. Management practices focused on increasing the fecundity by a factor of 10 and 25 in unprotected grassland and wetland respectively are necessary to achieve stable populations. In contrast, the protected populations showed increasing population growth rate allowing sustainable harvesting of 10% of the population. The extremely severe harvesting pressures recorded for *D. hatagirea* in unprotected populations, combined with its impacts on seedling densities and population structure suggests that continued harvesting in these populations presents serious conservation concerns. Continued monitoring by both biologists and local communities is highly recommended to conserve the species.

CHAPTER 6

SUMMARY AND CONCLUSION

6.1 Summary

Medicinal and aromatic plants (MAPs) are globally threatened mainly due to anthropogenic factors like illegal, prematrue harvesting or over-harvesting for livelihood. In addition to direct harvesting, a range of other factors including habitat degradation and fragmentation, climate change, spread of invasive species, are also related to the loss of several species of MAPs. Conservation of such species is a global concern, and research focusing on their ecology and sustainable harvest has been carried out in different parts of the world. However, there is inadequate research in this field from the Himalayan region. Therefore, this study was carried with the focus on population ecology and harvesting sustainability of two important Himalayan medicinal plant species, *Aconitum spicatum* and *Dactylorhiza hatagirea*.

The findings of this research are summarized in four sections. The first section (6.1.1) draws on Chapter 2 and deals with the effect of elevation and anthropogenic disturbances on the plant density and life history traits of *A. spicatum* in north-central Nepal. This chapter also deals with the socioeconomic values of the *A spicatum*. The second section (6.1.2) draws on Chapter 3 which describes the demography of *A. spicatum* and the effect of different harvesting regimes on its population dynamics along the elevation gradient. The third section (6.1.3) draws on Chapter 4 which identifies the major factors responsible for threatening the populations of an endangered orchid, *D. hatagirea.* This chapter further highlights the importance of community participation for the conservation of local MAPs. The fourth section (6.1.4) draws on Chapter 5 which describes the demographic characteristics of *D. hatagirea* in the locally protected (Manang) and unprotected sites (Darchula). This chapter also explores the demographic response of *D. hatagirea* on different habitats (wetland and grassland).

6.1.1 Plant density and life history traits of *Aconitum spicatum*

Plant density and life history traits of *A. spicatum* were studied in three different populations (subalpine, loweralpine and alpine) along an elevation gradient in central Nepal. A total of sixty-six (3 m × 3 m) plots were laid down along the distribution range from 3000 m asl to 4200 m asl. Four transects (with six plots in each transect) were laid down in each of the three populations namely subalpine, lower alpine and alpine. Individuals were classified into four different stages, *viz*., seedling, juvenile, adult vegetative and adult reproductive on the basis of their size and reproductive status. Effects of different environmental (biotic and abiotic) variables including cover (%) of herbs, shrubs, grass, litter, lichen, bryophytes, bare ground, solid rock and scree; and antropogenic disturbances (harvesting, trampling, animal droppings and fire) on different stages of *A. spicatum* individuals were studied. Plant biomass, and vegetative and reproductive traits were studied from 15-20 randomly selected mature individuals per population.

Semi-structured interviews were conducted during 2015-2018 among 65 informants. Informations regarding on the availability of *A. spicatum*, type of collectors, reasons for collection (local use or commercial purpose), local uses, areas and season of collection, indigenous knowledge transfer practices, special tools used, qualities, prices and market system, post-harvest practices, other sources of income, changes observed in the biotope, perceived trends in populations of the species, causes of population change, threats, conservation status, protection measurements and practices that could ensure survival and sustainable management were collected from local MAP users, leaders, teachers and students in the villages, hotel owners, local tourist guides and cattle herders.

Among the environmental variables tested, harvesting, animal droppings and fire appeared to be the most important factors affecting the density of *A. spicatum*. The lowest elevation population (subalpine) had lower recruitment and reduced density which could be due to the prevailing destructive harvesting practice. Subalpine population had very poor recruitment and loss of younger plants due to higher disturbance which damages the populations despite the enhanced vegetative performance of adults and the higher number of flowers and fruits set per reproductive individual. Reduced seed mass and decreased seed viability might be the major causes of the lower recruitment in the subalpine population. The alpine population had better reproductive fitness (higher seed set per unit fruit volume, higher seed mass, and higher seed viability and fecundity). This indicates that alpine plants develop self-sustaining adaptive strategies by increasing their resource allocation in reproductive parts, especially in seeds, to cope with the harsh environment.

The reduced reproductive performance of the subalpine population indicates that the persistence and growth of *A. spicatum* at low elevations will depend on the management practices addressed by the concerned authority for the protection of younger life stages and control of over-harvesting and premature harvesting of tubers. Locals perceive that due to its poisonous nature, all the harvested tubers are sold and are of the opinion that the indiscriminate harvesting by the outsiders (especially from the neighbouring districts Dadhing and Gorkha) is the major challenge for its growth and development in that region.

6.1.2 Sustainability of harvest of *Aconitum spicatum*

To assess ecological sustainability of harvest, the population dynamics of *A. spicatum* was studied in three different populations along an elevation gradient in Annapurna Base Camp Region, north-central Nepal. The demographic data of *A. spicatum* included 4-year observations on fecundity and survival of individuals classified by size. In each population, three belt transects of 2 m × 14 m were laid down, each of which was divided into seven 2 m × 2 m subplots. Each alternate subplot was systematically assigned for harvest treatments where the local harvesters were asked to randomly apply 25%, 50% amd 75% harvest simulation. One subplot was assigned as control plot, i.e., 0% harvest. The number of individuals per subplot was insufficient to allow modelling of harvest effects without merging some of the treatments. Therefore, 0% and 25% and 50% and 75% treatment plots were merged during analysis and labelled as low harvest and high harvest intensity, respectively. All the individuals classified into different stages were tagged with unique identity numbers and all the biological detais were recored during 2015. The tagged individuals were monitored for their survival in the following three consequitive years.

Results showed that the population growth rate was more than 1 in low harvest intensity (0-25%) in all the populations in all the years (2015-2018) indicating that the harvesting of this species at low harvest intensity is sustainable. In the high harvest intensity (50-75%) the population gowth rate is less than 1 in lower alpine and subalpine populations, indicating that these populations are sensitive to high harvest intensity. However, in the alpine population the population growth rate is more than 1 for both the low harvest and high harvest intensities.

The extinction probability derived from stochastic simulation (1000 runs) in 100 years was extremely low for all the populations in both the intensities.

The deterministic projection revealed that the subalpine population is the most sensitive population among the three studied populations. Low harvest population of lower alpine can reach the low harvest population of alpine in six years. High harvest population of lower alpine can reach the low harvest of alpine in 16 years. Low harvest and high harvest populations of subalpine can reach the low harvest of alpine population in more than 20 years. The high harvest subalpine population can reach its low harvest population in 22 years and that of lower alpine in 9 years.

Elasticity analysis revealed that progression had a higher contribution to the population growth rate followed by stasis in alpine population, but almost equal contributions of progression and stasis were observed in the lower alpine and subalpine populations. Among the size classes, adult stages (larger stages) had the higher contribution to the population growth rate in all the populations.

6.1.3 Population density and structure of *Dactylorhiza hatagirea*

Population density and structure of *D. hatagirea* was studied in two sites of different protection status: (i) the unprotected site (the area received frequent and uncontrolled harvesting of MAPs) was located in the upper Chamelia valley in Api-Nampa Conservation Area (ANCA), Darchula; and (ii) the protected site (the area is locally protected and regulated for any form of MAP harvesting) was located in Gyasumdo Valley, Manang. In each of these sites three populations were sampled along an elevation gradient of 3400-4600 m. The individual plants were classified into four life stage classes (seedling, juvenile, adult vegetative and adult reproductive) based on the leaf size and the reproductive status. Reproductive traits were studied by selecting fifteen mature individuals from each population at each site. The dry biomass estimation of tubers was done from the fifteen dried tubers from the local MAP collectors. Effects of different environmental variables like cover (%) of herbs, shrubs, grass, litter, lichen, bryophytes, bare ground, solid rock and scree and the antropogenic disturbances (harvesting, trampling, grazing, animal droppings and fire) on different life stages of *D. hatagirea* individuals were studied using zero-inflated poission regression models.

Semi-structured interviews were conducted during 2015-2017 among 117 informants in the unprotected site and 69 informants in the protected site (details in chapter 3).

The population density of *D. hatagirea* at the unprotected site ranged from 0.60 to 0.79 individuals/m^2 while the density ranged from 0.70 to 2.16 individuals/m^2 at the protected site with highest densities in the mid- elevation populations. A huge decrease (approximately by one third) was observed in mean densities across the three consecutive years (2015-2017) in unprotected site only. The comparision of the tuber production across the three consecutive years (2015-2017) showed reduced tuber production in unprotected site and increased tuber production in the protected site.

Harvesting and grazing showed significant negative effects on the density of different life stages of *D. hatagirea.* Herb cover showed significant positive effect on juvenile and adult vegetative stages. The relative radiation index had a weakly negative effect on the density of the adult vegetative stage. The interaction effects of harvesting and grazing among the sites indicate that the unprotected populations showed significant negative effects of harvesting and grazing, while these effects were not distinct for the protected populations.

The informants in the protected site were found with more knowledge about different aspects of *D. hatagirea*. Ninety-three percent of the informants in protected site (n=117) and forty-three percent informants in unprotected site (n=69) were aware about the protection category, ninety-eight percent in protected and seventy-one percent in unprotected site knows the use value, nineteen percent of the informants in protected site and five percent in unprotected site were conscious of its population ecology, twenty-one percent in protected and forty-two percent in unprotected site harvest the individuals for the local use, twenty-four percent of the informants in protected site and ninety-two percent in the unprotected site claimed that the population is decreasing. Seventy-three percent of the informants in the protected site claimed that the population is almost constants since last few decades due to the strict implementation of local regulatory mechanisms for the illegal harvest. Eighty-one per cent of the informants were of the view that the major source of their livelihood is MAPs collection. In protected site none of the families rely totally of MAPs collection due to flourished tourisn in the site.

6.1.4 Population dynamics of *Dactylorhiza hatagirea*: effect of habitats and harvesting

Demography of *D. hatagirea* was studied in two sites of different protection status as described in section 6.1.3. In each site, three populations (ranging in elevation from 3400 to 4600 m), additionnaly one wetland population in unprotected site were studied during 2015-2017. All the individuals in different stages were tagged, each with its unique identity number in 2015 and monitored in 2016 and 2017. Fecundity was estimated as the number of seedlings per adult reproductive plant in 2016 and 2017. Stage-based population projection matrix models (Lefkovitch, 1965) were constructed for all individual populations using a life cycle graph to estimate demographic parameters. In addition to four different stages classified earlier, dormancy was included as a separate stage (when the plant remained alive by tuber in 2016).

Each transition matrix was used to calculate the asymptotic population growth rate (λ) and stable size distribution (Caswell, 2001), and those quantities were compared between habitats and among sites.

The population projection matrix obtained for the period 2016/2017 was used to carry out the numerical simulations to evaluate the potential impact of specific changes in particular matrix entries on the population growth rate λ. The effect of modifying the fecundity values was evaluated by multiplying the original fecundity estimate by different numbers ranging between 5 and 30.

The population growth rates (λ) were 0.826 and 0.553 in unprotected, and 1.166 and 1.003 in the protected site for the transition years 2015/2016 and 2016/2017, respectively. The wetland and grassland habitats in the unprotected site had λ values of 0.847 and 0.826, respectively, in 2015/2016 and 0.780 and 0.553, respectively, in 2016/2017.

The elasticity analysis showed that the stasis in the juvenile stage in unprotected site, and reproductive adult stage in protected site contributed most to λ. The population growth rate reached values above unity only when fecundity was increased 10-fold in unprotected grassland populations. Further, the wetland population in unprotected site required 25-fold increase in fecundity to reach the population growth rate of 1. Unprotected populations are thus highly threatened by harvesting, while the present populations at the protected site would allow approximately 10% of the population to be harvested for local use without exceeding their reproductive capacity.

6.2 Conclusions

Anthropogenic disturbances, mainly harvesting, grazing and topographic factors, contribute to the variation in density, life history traits and population growth rate of *Aconitum spicatum* and *Dactylorhiza hatagirea*. Further, sustainability of harvest varies according to the species, sites and the management practices adopted by the local community.

This research has focused on the relationship between plant density and environmental variables like cover of substrate, herbs, shrubs and anthropogenic disturbances like harvesting, grazing, trampling, animal droppings and fire. Understanding the role of such variables can help inform management practices.

Populations of *Aconitum spicatum* at different elevations respond differently to harvest with different recovery rate. The alpine population allows the harvest of 10-20 percent of the individuals for the local use maintaining the equilibirium. At low harvest intensity all the populations show asymptotic growth rate more than 1, thus the probability of extinctly is extremely low. Subalpine (lowest elevation) population is the most sensitive population to harvest and should be conserved with greater priority. Higher contribution of larger size classes (adult vegetative and adult reproductive) to population growth rate indicated that these stages are the critical stages in the life history of *A. spicatum* and thus the conservsaion priority should focus on protection of these stages. This study demonstrates that there are important differences in the ability of *A. spicatum* to regenerate after harvest along the Himalayan elevation gradient in Nepal. This study thus contributes to widen the knowledge on the population dynamics of *A. spicatum* and results can be extrapolated to other similar MAPs.

The grassland population of *D. hatagirea* under present conditions in the unprotected site, the fecundity should be increased by a factor of 10 to achieve a stable population. The wetland population is still more vulnerable to threat as it needs to increase its fecundity 25 times to reach steady state. The locally protected populations showed stable population growth rate which allow approximately 10% of the present population of *D. hatagirea* to be harvested for local use. Juvenile and adult reproductive stages are the ctical stages in the life history of *D. hatagirea* in unprotected and protected sites respectively, thus these stages should be protected with greater priority. The better performance of populations in protected site can be described as a result of the effective conservation efforts of the local community which can be extrapolated to the unprotected site. A low level of awareness about population ecology and conservation among local people are the major challenges for the sustainable development of *D. hatagirea* populations at the unprotected site.

6.3 Recommendations for further research and management

- Management strategies and actions aiming to mitigate harvesting and grazing based on scientific knowledge could improve conservation of both species.
- Create a conducive environment to participate the local communities in the management of MAPs by strengthening people’s knowledge on the population ecology and harvesting sustainability.
- Protection of the present populations of both species with regular monitoring (rotational harvest) by biologist, local community and the management authority
- Future studies exploring the role of variation in rainfall, snow melt and other climatic conditions on the population dynamics of both species is recommended.
- Future researches that investigate trade-offs between plant survival, growth and reproduction, seed fate and early establishment of these plants would be highly informative.
- Future researchers are recommended to carry out long-term studies to explore demographic parameters for other similar MAPs.

REFERENCES

Agarwal, A., Khokhar, D., & Vishwnath. (2008). *Conservation through in vitro Propagation of a Critically Endangered Medicinal Plant*, *Dactylorhiza hatagirea* (D. Don) Soo. MV Reddy, Vishwnath Ed. Wildlife biodiversity Conservation, 294-299.

Ågren, J., Ehrlén, J., & Solbreck, C. (2008). Spatio-temporal variation in fruit production and seed predation in a perennial herb influenced by habitat quality and population size. *Journal of Ecology*, *96*(2), 334-345.

Aguilar, R., Ashworth, L., Galetto, L., & Aizen, M.A. (2006). Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters*, *9*(8), 968-980.

Alahuhta, K., Crone, E., Ettinger, A., Hens, H., Jäkäläniemi, A., & Tuomi, J. (2017). Instant death, slow death and the consequences of assumptions about prolonged dormancy for plant population dynamics. *Journal of Ecology*, *105*(2), 471-483.

Almond, R.E.A., Grooten, M., & Peterson, T. (2020). *Living Planet Report 2020-Bending the Curve of Biodiversity Loss*. World Wildlife Fund.

Anderson, P.J., & Putz, F.E. (2002). Harvesting and conservation: Are both possible for the palm, Iriartea deltoidea? *Forest Ecology and Management*, *170*(1-3), 271-283.

Antonelli, A., Smith, R.J., Fry, C., Simmonds, M.S., Kersey, P.J., Pritchard, H.W., & Qi, Y.D. (2020). *State of the World’s Plants and Fungi* (Doctoral dissertation, Royal Botanic Gardens (Kew); Sfumato Foundation).

Auerbach, M., & Shmida, A.V. I. (1993). Vegetation Change Along an Altitudinal Gradient on Mt Hermon, Israel--No Evidence for Discrete Communities. *Journal of Ecology*, 25-33.

Austrheim, G., & Eriksson, O. (2001). Plant species diversity and grazing in the Scandinavian mountains-patterns and processes at different spatial scales. *Ecography*, *24*(6), 683-695.

Baral, N., & Heinen, J.T. (2007). Resources use, conservation attitudes, management intervention and park-people relations in the Western Terai landscape of Nepal. *Environmental Conservation, 34*(1), 64-72.

Baral, S.R., & Kurmi, P.P. (2006). *Compendium of Medicinal Plants in Nepal*. Rachana Sharma.

Barton, K. (2018). *Multi-model Inference*. R package version 1.42.1.

Beigh, S.Y., Nawchoo, I.A., & Iqbal, M. (2006). Cultivation and conservation of Aconitum heterophyllum: A critically endangered medicinal herb of the northwest Himalayas. *Journal of Herbs, Spices & Medicinal Plants, 11*(4), 47-56.

Belcher, B., Ruíz-Pérez, M., & Achdiawan, R. (2005). Global patterns and trends in the use and management of commercial NTFPs: Implications for livelihoods and conservation. *World Development, 33*(9), 1435-1452.

Benton, T.G., & Grant, A. (2000). Evolutionary fitness in ecology: Comparing measures of fitness in stochastic, density-dependent environments. *Evolutionary Ecology Research*, *2*(6), 769-789.

Betts, M., Wolf, C., Ripple, W. *et al.* (2017). Global forest loss disproportionately erodes biodiversity in intact landscapes. *Nature,* 547, (7644) 441–444 https://doi.org/10.1038/nature23285

Bhandary, H.R., & Shrestha, P. (1986). Ethnobotanical investigations on the poisonous plants of Manang-Mustang and adjoining area. *J. Nat. His. Mus, 10*, 133-144.

Bhatt, A., Joshi, S.K., & Gairola, S. (2005). *Dactylorhiza hatagirea* (D. Don) Soo–A west Himalayan orchid in peril. *Current Science*, *89*(4), 610–612. http://www.jstor.org/stable/24111155

Billings, W.D. (1973). Arctic and alpine vegetations: similarities, differences, and susceptibility to disturbance. *BioScience*, *23*(12), 697-704.

Bjønness, I.M. (1980). Animal husbandry and grazing, a conservation and management problem in Sagarmatha (Mt. Everest) National Park, Nepal.

Bliss, L.C. (1962). Adaptations of arctic and alpine plants to environmental conditions. *Arctic*, *15*(2), 117-144. http://www.jstor.org/stable/40506981

Bosch, M., & Waser, N.M. (1999). Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* (Ranunculaceae). *American Journal of Botany*, *86*(6), 871-879.

Bresson, C.C., Vitasse, Y., Kremer, A., & Delzon, S. (2011). To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech? *Tree Physiology*, *31*(11), 1164-1174.

Brinckmann, J.A. (2016). *Sustainable Sourcing: Markets for Certified Chinese Medicinal and Aromatic Plants*. Geneva: International Trade Centre, *22*.

Bruna, E.M., Fiske, I.J., & Trager, M.D. (2009). Habitat fragmentation and plant populations: is what we know demographically irrelevant? *Journal of Vegetation Science*, *20*(3), 569-576.

Brzosko, E. (2002). Dynamics of island populations of Cypripedium calceolus in the Biebrza river valley (north-east Poland). *Botanical Journal of the Linnean Society*, *139*(1), 67-77.

Bucini, G., & Lambin, E.F. (2002). Fire impacts on vegetation in Central Africa: a remote-sensing-based statistical analysis. *Applied Geography*, *22*(1), 27-48.

Bulafu, C.E., Mucunguzi, P., & Kakudidi, E.K. (2007). Diversity and distribution of wild terrestrial orchids of Mt Elgon Forest National Park, eastern Uganda. *African Journal of Ecology*, *45*, 21-28.

Burgener, M., & Walter, S. (2007). *Trade Measures-Tools to Promote the Sustainable Use of NWFP? An Assessment of Trade Related Instruments Influencing the International Trade in Non-wood Forest Products and Associated Management and Livelihood Strategies*. Food and Agriculture Organization of The United Nations (FAO).

Byers, D.L., & Meagher, T.R. (1997). A comparison of demographic characteristics in a rare and a common species of Eupatorium. *Ecological Applications*, *7*(2), 519-530.

Callaway, R.M., Brooker, R.W., Choler, P., et. al., (2002). Positive interactions among alpine plants increase with stress. *Nature*, *417*(6891), 844-848.

Calvo, R.N. (1990). Four-year growth and reproduction of Cyclopogon cranichoides (Orchidaceae) in South Florida. *American Journal of Botany, 77*(6), 736-741.

CAMP. (2001). *Conservation Assessment and Management Prioritization Report*. International Development Research Centre (IDRD), Canada and Ministry of Forest and Soil Conservation, Kathmandu, Nepal.

Caswell, H. (2001). *Matrix Population Models: Construction, Analysis, and Interpretation*. 2nd edn sinauer associates. Inc., Sunderland, MA.

Chambers, J.C. (1995). Disturbance, life history strategies, and seed fates in alpine herbfield communities. *American Journal of Botany, 82*(3), 421-433.

Chambers, J.C., MacMahon, J.A., & Brown, R.W. (1990). Alpine seedling establishment: The influence of disturbance type. *Ecology*, *71*(4), 1323-1341.

Chardon, N.I., Wipf, S., Rixen, C., Beilstein, A., & Doak, D.F. (2018). Local trampling disturbance effects on alpine plant populations and communities: Negative implications for climate change vulnerability. *Ecology and Evolution*, *8*(16), 7921-7935.

Charitonidou, M., Stara, K., Kougioumoutzis, K., & Halley, J.M. (2019). Implications of salep collection for the conservation of the Elder-flowered orchid (Dactylorhiza sambucina) in Epirus, Greece. *Journal of Biological Research-Thessaloniki*, *26*(1), 1-13.

Chen, Y., Yang, X., Yang, Q., Li, D., Long, W., & Luo, W. (2014). Factors affecting the distribution pattern of wild plants with extremely small populations in Hainan Island, China. *PLoS One*, *9*(5), e97751.

Chhetri, P. (2014). *Livelihood Strategies of People in the Himalayan Region of Nepal: A Study in the Villages of Eastern Manang*.

CITES. (2020). *Convention on International Trade in Endangered Species of Wild Fauna and Flora: The CITES Appendices*. Accessed on August 14, 2020 from https://cites.org/eng/app/appendices.php.

Colling, G., Matthies, D., & Reckinger, C. (2002). Population structure and establishment of the threatened long-lived perennial Scorzonera humilis in relation to environment. *Journal of Applied Ecology, 39*(2), 310-320.

Cook, R.E. (1985). *Growth and Development in Clonal Plant Populations*.

Cornwell, W.K., Pearse, W.D., Dalrymple, R.L., & Zanne, A.E. (2019). What we (don't) know about global plant diversity. *Ecography*, *42*(11), 1819-1831.

Coulson, T., Mace, G.M., Hudson, E., & Possingham, H. (2001). The use and abuse of population viability analysis. *Trends in Ecology & Evolution*, *16*(5), 219-221.

Crone EE, Ellis MM, Morris WF, Stanley A, Bell T, Bierzychudek P, Ehrlén J, Kaye TN, Knight TM, Lesica P, Oostermeijer G, Quintana-Ascencio PF, Ticktin T, Valverde T, Williams JL, Doak DF, Ganesan R, McEachern K, Thorpe AS, Menges, ES. (2013). Ability of matrix models to explain the past and predict the future of plant populations. *Conservation Biology*. 27(5):968-978. doi: 10.1111/cobi.12049. Epub 2013 Apr 8. PMID: 23565966.

Dahlgren, J.P., & Ehrlén, J. (2009). Linking environmental variation to population dynamics of a forest herb. *Journal of Ecology*, 666-674.

Dai, L., Guo, X., Ke, X., Zhang, F., Li, Y., Peng, C., Shu, K., Li, Q., Lin, gb L., Cao,G., & Du, Y. (2019). Moderate grazing promotes the root biomass in Kobresia meadow on the northern Qinghai-Tibet Plateau. *Ecology and Evolution*, *9*(16), 9395-9406.

Das, S.P. (2004). *Indian Orchids in Indigenous Medicine System*. Orchid's diversity and conservation. The Rapinat Herbarium, St John's College, Tiruchirappalli.

de Kroon, H., Plaisier, A., van Groenendael, J., & Caswell, H. (1986). Elasticity: The relative contribution of demographic parameters to population growth rate. *Ecology*, *67*(5), 1427-1431.

Dhiman, N., Sharma, N.K., Thapa, P., Sharma, I., Kumar Swarnkar, M., Chawla, A., Shankar, R., & Bhattacharya, A. (2019). De novo transcriptome provides insights into the growth behaviour and resveratrol and trans-stilbenes biosynthesis in *Dactylorhiza hatagirea* - An endangered alpine terrestrial orchid of western Himalaya. *Scientific Reports*, 9(1), 1-13, https://doi.org/10.1038/s41598-019-49446-w

Di Marco, M., Venter, O., Possingham, H.P., & Watson, J.E. (2018). Changes in human footprint drive changes in species extinction risk. *Nature Communications*, *9*(1), 1-9.

Dinnétz, P., & Nilsson, T. (2002). Population viability analysis of Saxifraga cotyledon, a perennial plant with semelparous rosettes. *Plant Ecology, 159*(1), 61-71.

Djordjević, V., & Tsiftsis, S. (2020). The role of ecological factors in distribution and abundance of terrestrial orchids. Orchids phytochemistry, biology and horticulture, reference series in phytochemistry. *Fundamentals and Applications*, 1-71.

Djordjević, V., Tsiftsis, S., Lakušić, D., Jovanović, S., & Stevanović, V. (2016). Factors affecting the distribution and abundance of orchids in grasslands and herbaceous wetlands. *Systematics and Biodiversity*, *14*(4), 355-370.

Djordjević, V., Tsiftsis, S., Lakušić, D., Jovanović, S., Jakovljević, K., & Stevanović, V. (2020). Patterns of distribution, abundance and composition of forest terrestrial orchids. *Biodiversity and Conservation*, *29*(14), 4111-4134.

DNPWC. (2015). *Management Plan for Api Nampa Conservation Area, Darchula (2015-2019)*. Kathmandu, Nepal: Government of Nepal Ministry of Forests and Soil Conservation, Department of National Parks and Wildlife Conservation.

Doak, D.F., Waddle, E., Langendorf, R.E. *et al*., (2021). A critical comparison of integral projection and matrix projection models for demographic analysis. *Ecological Monographs*, *91*(2), e01447.

Dobriyal, A.K., Vinteshwar, P.B., Neeraj, K., & Bisht, M.S. (2002). *An Appraisal of the Fishery Potential of River Nayar with a Note on its Improvement Possibilities*. Coldwater Fish Genetic Resources and Their Conservation, *7,* 235-242.

DPR. (2012). *Plants of Nepal: Fact sheet*. Department of Plant Resources (DPR), Ministry of Forest and Soil Conservation, Government of Nepal, Kathmandu, Nepal.

Drechsler, M., Lamont, B.B., Burgman, M.A., Akcakaya, H.R., Witkowski, E.T., & Supriyadi, Y. (1999). Modelling the persistence of an apparently immortal Banksia species after fire and land clearing. *Biological Conservation*, *88*(2), 249-259.

Ehrlich, P.R. (1988). The loss of diversity. *Biodiversity*, 21-27.

Ellner, S.P., Fieberg, J., Ludwig, D., & Wilcox, C. (2002). Precision of population viability analysis. *Conservation Biology*, *16*(1), 258-261.

Endels, P., Adriaens, D., Verheyen, K., & Hermy, M. (2004). Population structure and adult plant performance of forest herbs in three contrasting habitats. *Ecography*, *27*(2), 225-241.

Endress, B.A., Gorchov, D.L., & Berry, E.J. (2006). Sustainability of a non-timber forest product: effects of alternative leaf harvest practices over 6 years on yield and demography of the palm Chamaedorea radicalis. *Forest Ecology and Management*, *234*(1-3), 181-191.

Faast, R., Facelli, J.M., & Austin, A.D. (2011). Seed viability in declining populations of Caladenia rigida (Orchidaceae): Are small populations doomed? *Plant Biology*, *13,* 86-95.

Fan, D.M., & Yang, Y.P. (2009). Altitudinal variations in flower and bulbil production of an alpine perennial, *Polygonum viviparum* L. (Polygonaceae). *Plant Biology*, *11*(3), 493-497.

Fay, M.F. (2018). Orchid conservation: How can we meet the challenges in the twenty-first century? *Botanical Studies*, *59*(1), 1-6.

Fay, M.F., Pailler, T., & Dixon, K.W. (2015). Orchid conservation: Making the links. *Annals of Botany*, *116*(3), 377-379.

Franco, M., & Silvertown, J. (1996). Life history variation in plants: an exploration of the fast-slow continuum hypothesis. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *351*(1345), 1341-1348.

Freckleton, R.P., Matos, D.S., Bovi, M.L.A., & Watkinson, A.R. (2003). Predicting the impacts of harvesting using structured population models: The importance of density-dependence and timing of harvest for a tropical palm tree. *Journal of Applied Ecology*, *40*(5), 846-858.

Gaoue, O.G., Horvitz, C.C., Ticktin, T., Steiner, U.K., & Tuljapurkar, S. (2013). Defoliation and bark harvesting affect life-history traits of a tropical tree. *Journal of Ecology, 101*(6), 1563-1571.

Garbarino, M., Lingua, E., Marzano, R., Urbinati, C., Bhuju, D., & Carrer, M. (2014). Human interactions with forest landscape in the Khumbu valley, Nepal. *Anthropocene, 6*, 39-47.

García, M.B., Pico, F.X., & Ehrlén, J. (2008). Life span correlates with population dynamics in perennial herbaceous plants. *Amer. J. Bot. 95*, 258-262.

Gargano, D., Gullo, T., & Bernardo, L. (2009). Do inefficient selfing and inbreeding depression challenge the persistence of the rare *Dianthus guliae* Janka (Caryophyllaceae)? Influence of reproductive traits on a plant's proneness to extinction. *Plant Species Biology, 24*(2), 69-76.

Ghimire, S.K., Awasthi, B., Rana, S., Rana, H.K., Bhattarai, R., & Pyakurel, D. (2016). Export of medicinal and aromatic plant materials from Nepal. *Botanica Orientalis: Journal of Plant Science, 10*, 24-32.

Ghimire, S.K., Gimenez, O., Pradel, R., McKey, D., & Aumeeruddy-Thomas, Y. (2008). Demographic variation and population viability in a threatened Himalayan medicinal and aromatic herb Nardostachys grandiflora: matrix modelling of harvesting effects in two contrasting habitats. *Journal of Applied Ecology*, *45*(1), 41-51.

Ghimire, S.K., McKey, D., & Aumeeruddy-Thomas, Y. (2005). Conservation of Himalayan medicinal plants: Harvesting patterns and ecology of two threatened species, Nardostachys grandiflora DC and Neopicrorhiza scrophulariiflora (Pennell) Hong. *Biological Conservation*, *124*(4), 463-475.

Ghimire, S.K., Sah, J.P., Shrestha, K.K., & Bajracharya, D. (1999). Ecological study of some high altitude medicinal and aromatic plants in the Gyasumdo valley, Manang, Nepal. *Ecoprint, 6*(1), 17-25.

Ghimire, S.K., Subedi, C.K., Budha-Magar, S., Adhikari, M., Pandey, T.R., Awasthi, B., Thapa-Magar, S., Paudeyl, M.R., Ghimire, K.M., Shrestha, B.B., Bhatta, G.D., Joshi, L.R., Poudel, A., Chapagain, D.J., & Gurung, J. (2021). *Flora of Kailash Sacred Landscape Nepal: An Annotated Checklist*.

Ghorbani, A., Gravendeel, B., Naghibi, F., & de Boer, H. (2014). Wild orchid tuber collection in Iran: A wake-up call for conservation. *Biodiversity and Conservation*, *23*(11), 2749-2760.

Giri, D., & Tamta, S. (2012). Propagation and conservation of *Dactylorhiza hatagirea* (D. Don) Soo, an endangered alpine orchid. *African Journal of Biotechnology*, *11*(62), 12586-12594.

GoN. (2011). Protected plants of Nepal: Its amendments. Ministry of Forests and Soil Conservation, Kathmandu, Nepal. *Nepal Gaz*, *60*, 38-45.

GoN. (2015). Notice of Ministry of Forests and Soil Conservation. *Nepal Gaz*., 65, 26-3.

GoN. (2018). Notice of Ministry of Forests and Environment. *Nepal Gaz*., 68, 34-3...

Govaerts, R., Nic Lughadha, E., Black, N., Turner, R., & Paton, A. (2021). The world checklist of vascular plants, a continuously updated resource for exploring global plant diversity. *Scientific Data*, *8*(1), 1-10.

Gregg, K.B. (2004). Recovery of Showy Lady's Slippers (*Cypripedium reginae* Walter) from Moderate and Severe Herbivory by White-tailed Deer (*Odocoileus virginianus* Zimmerman). *Natural Areas Journal*, *24*(3), 232-241.

Havens, K., Kramer, A.T., & Guerrant Jr, E.O. (2014). Getting plant conservation right (or not): The case of the United States. *International Journal of Plant Sciences*, *175*(1), 3-10.

Hegland, S.J., Van Leeuwen, M., & Oostermeijer, J.G.B. (2001). Population structure of Salvia pratensis in relation to vegetation and management of Dutch dry floodplain grasslands. *Journal of Applied Ecology*, *38*(6), 1277-1289.

Hens, H., Pakanen, V.M., Jäkäläniemi, A., Tuomi, J., & Kvist, L. (2017). Low population viability in small endangered orchid populations: Genetic variation, seedling recruitment and stochasticity. *Biological Conservation*, *210*, 174-183.

Hinsley, A., De Boer, H.J., Fay, M.F., Gale, S.W., Gardiner, L.M., Gunasekara, R.S., Kumar, P., Masters, S., Metusala, D., Roberts, D.L., Veldman, S., Wong, S., &

Phelps, J. (2018). A review of the trade in orchids and its implications for conservation, *Botanical Journal of the Linnean Society*, *186*(4), 435-455. https://doi.org/10.1093/botlinnean/box083

Hinsley, A., Nuno, A., Ridout, M., John, F.A.V.S., & Roberts, D.L. (2017). Estimating the extent of CITES noncompliance among traders and end-consumers; lessons from the Global Orchid Trade. *Conservation Letters, 10*, 602-609. https://doi.org/10.1111/conl.12316

Hobbs, R.J., Mallik, A.U., & Gimingham, C.H. (1984). Studies on fire in Scottish heathland communities: III. Vital attributes of the species. *The Journal of Ecology*, 963-976.

Hodkinson, I.D. (2005). Terrestrial insects along elevation gradients: Species and community responses to altitude. *Biological Reviews*, *80*(3), 489-513.

Hong Qian, Jian Zhang, & Jingchao Zhao. (2022). How many known vascular plant species are there in the world? An integration of multiple global plant databases[J]. *Biodiversity Science*, *30*(7), 22254.

Horvitz, C.C., & Schemske, D.W. (1995). Spatiotemporal variation in demographic transitions of a tropical understory herb: Projection matrix analysis. *Ecological Monographs*, *65*(2), 155-192.

Huai, H., Wen, G., Xu, W., & Bai, W. (2013). Effects of commercial harvesting on population characteristics and rhizome yield of Anemone altaica. *Economic Botany*, *67*(1), 41-50.

Humphreys, A.M., Govaerts, R., Ficinski, S.Z., Lughadha, E.N., & Vorontsova, M.S. (2019). Global dataset shows geography and life form predict modern plant extinction and rediscovery. *Nature Ecology & Evolution*, *3*(7), 1043-1047.

Hutchings, M.J. (2010). The population biology of the early spider orchid Ophrys sphegodes Mill. III. Demography over three decades. *Journal of Ecology, 98*(4), 867-878.

Hutchings, M.J., Robbirt, K.M., Roberts, D.L., & Davy, A.J. (2018). Vulnerability of a specialized pollination mechanism to climate change revealed by a 356-year analysis. *Botanical Journal of the Linnean Society*, *186*(4), 498-509.

IPBES. (2019). *Global Assessment Report on Biodiversity and Ecosystem Services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services*. E.S. Brondizio, J. Settele, S. Díaz, & H.T. Ngo (Eds.). Germany: IPBES Secretariat, Bonn,. 1148 pp. https://doi.org/10.5281/zenodo.3831673

IPNI. (2022). *International Plant Names Index*. Published on the Internet http://www.ipni.org, The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Herbarium. [Retrieved 15 December 2022].

IUCN. (2020). *The IUCN Red List of Threatened Species*. Version 2020-3. *3*, Accessed on March 30, 2021 from: https://www.iucnr edlist.org.

Jacquemyn, H., Brys, R., Hermy, M., & Willems, J.H. (2005). Does nectar reward affect rarity and extinction probabilities of orchid species? An assessment using historical records from Belgium and the Netherlands. *Biological Conservation*, *121*(2), 257-263.

Jacquemyn, H., Brys, R., Hermy, M., & Willems, J.H. (2007). Long-term dynamics and population viability in one of the last populations of the endangered *Spiranthes spiralis* (Orchidaceae) in the Netherlands. *Biological Conservation*, *134*(1), 14-21.

Kala, C.P. (2000). Status and conservation of rare and endangered medicinal plants in the Indian trans-Himalaya. *Biological Conservation*, *93*(3), 371-379.

Kala, C.P. (2005). Indigenous uses, population density, and conservation of threatened medicinal plants in protected areas of the Indian Himalayas. *Conservation Biology*, *19*(2), 368-378.

Katerere, D.R., & Luseba, D. (eds.). (2010). *Ethnoveterinary Botanical Medicine: Herbal Medicines for Animal Health*. CRC Press.

Kaye, T.N., Pendergrass, K.L., Finley, K., & Kauffman, J.B. (2001). The effect of fire on the population viability of an endangered prairie plant. *Ecological Applications*, *11*(5), 1366-1380.

Kéry, M., & Gregg, K.B. (2003). Effects of life-state on detectability in a demographic study of the terrestrial orchid Cleistes bifaria. *Journal of Ecology*, *91*(2), 265-273.

Kéry, M., Matthies, D., & Schmid, B. (2003). Demographic stochasticity in population fragments of the declining distylous perennial *Primula veris* (Primulaceae). *Basic and Applied Ecology*, *4*(3), 197-206.

Kindlmann, P., Kull, T., Whigham, D.F., & Willems, J. (2006). Ecology and population dynamics of terrestrial orchids: An introduction. *Folia Geobotanica*.

Kizu, H., Kaneko, E.I., & Tomimori, T. (1999). Studies on Nepalese crude drugs. XXVI. Chemical constituents of Panch Aunle, the roots of *Dactylorhiza hatagirea* D. Don. *Chemical and Pharmaceutical Bulletin*, *47*(11), 1618-1625.

Klimas, C.A., Cropper Jr, W.P., Kainer, K.A., & de Oliveira Wadt, L.H. (2012). Viability of combined timber and non-timber harvests for one species: A *Carapa guianensis* case study. *Ecological Modelling*, *246*, 147-156.

Körner, C. (2003). *Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems*, 2nd edn Springer. Berlin.

Körner, C., & Kèorner, C. (1999). Alpine plant life: Functional plant ecology of high mountain ecosystems.

Kreziou, A., de Boer, H., & Gravendeel, B. (2015). Harvesting of salep orchids in north-western Greece continues to threaten natural populations. *Oryx*, *50*(3), 393-396.

Kudo, G., & Hirao, A.S. (2006). Habitat-specific responses in the flowering phenology and seed set of alpine plants to climate variation: Implications for global-change impacts. *Population Ecology*, *48*(1), 49-58.

Kull, T., & Hutchings, M.J. (2006). A comparative analysis of the decline in the distribution ranges of orchid species in Estonia and the United Kingdom. *Biological Conservation*, *129*, 31-39. https://doi. org/10.1016/j.biocon.2005.09. 046

Kunwar, R.M., Rimal, B., Sharma, H.P., Poudel, R.C., Pyakurel, D., Tiwari, A., Thapa-Magar, S., Karki, G., Bhandari, G.S., Pandey, P., & Bussmann, R.W. (2021). Distribution and habitat modeling of *Dactylorhiza hatagirea* (D. Don) Soo, Paris polyphylla Sm. and Taxus species in Nepal Himalaya. *Journal of Applied Research on Medicinal and Aromatic Plants*, *20*, 100274.

Lama, Y.C., Ghimire, S.K., & Aumeeruddy-Thomas, Y. (2001). *Medicinal Plants of Dolpo*. Amchis' Knowledge and Conservation, WWF Nepal Program, Katmandu.

Lande, R. (1998). Anthropogenic, ecological and genetic factors in extinction and conservation. *Population Ecology*, *40*(3), 259-269.

Laurance, W.F., & Bierregaard, R.O. (Eds.). (1997). *Tropical Forest Remnants: Ecology, Management, and Conservation of Fragmented Communities*. University of Chicago Press.

Lefkovitch, L.P. (1965). The study of population growth in organisms grouped by stages. *Biometrics,* 1-18.

Lehtilä, K., Syrjänen, K., Leimu, R., Garcia, M.B., & Ehrlén, J. (2006). Habitat change and demography of *Primula veris*: Identification of management targets. *Conservation Biology*, *20*(3), 833-843.

Lennartsson, T., & Oostermeijer, J.G.B. (2001). Demographic variation and population viability in Gentianella campestris: Effects of grassland management and environmental stochasticity. *Journal of Ecology,* 451-463.

Lesica, P., & Crone, E.E. (2007). Causes and consequences of prolonged dormancy for an iteroparous geophyte, Silene spaldingii. *Journal of Ecology,* 1360-1369.

Lesica, P., & Steele, B.M. (1994). Prolonged dormancy in vascular plants and implications for monitoring studies. *Natural Areas Journal*, *14,* 209-212.

Liancourt, P., Le Bagousse-Pinguet, Y., Rixen, C., & Dolezal, J. (2017). SGH: stress or strain gradient hypothesis? Insights from an elevation gradient on the roof of the world. *Annals of Botany*, *120*(1), 29-38.

Light, M.H., Kell, S.P., & Jackson, P.W. (2003). The role of education and training in orchid conservation: An overview and critique. *Orchid Conservation,* 357-382.

Lindborg, R., & Ehrlén, J. (2002). Evaluating the extinction risk of a perennial herb: Demographic data versus historical records. *Conservation Biology*, *16*(3), 683-690.

Liu, H., & Koptur, S. (2003). Breeding system and pollination of a narrowly endemic herb of the Lower Florida Keys: Impacts of the urban-wildland interface. *American Journal of Botany*, *90*(8), 1180-1187.

Ma, W., Shi, P., Li, W., He, Y., Zhang, X., Shen, Z., & Chai, S. (2010). Changes in individual plant traits and biomass allocation in alpine meadow with elevation variation on the Qinghai-Tibetan Plateau. *Science China Life Sciences*, *53*(9), 1142-1151.

Manandhar, N.P. (2002). *Plants and People of Nepal*. Portland, Oregon: Timber Press Inc.

Margules, C.R., & Pressey, R.L. (2000). Systematic conservation planning. *Nature*, *405*(6783), 243-253.

McCormick, M.K., & Jacquemyn, H. (2014). What constrains the distribution of orchid populations? *New Phytologist*, *202*(2), 392-400.

McKechnie, I.M., & Sargent, R.D. (2013). Do plant traits influence a species' response to habitat disturbance? A meta-analysis. *Biological Conservation*, *168*, 69-77.

McKinney, M.L. (2002). Urbanization, biodiversity, and conservation. The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *BioScience*, *52*(10), 883-890.

Méndez, M., & Karlsson, P.S. (2004). Between-population variation in size-dependent reproduction and reproductive allocation in *Pinguicula vulgaris* (Lentibulariaceae) and its environmental correlates. *Oikos*, *104*(1), 59-70.

Menges, E.S. (2000). Applications of population viability analyses in plant conservation. *Ecological Bulletins*, *48*, 73-84. http://www.jstor.org/stable/20113249

Menges, E.S. (2000). Population viability analyses in plants: Challenges and opportunities. *Trends in Ecology & Evolution*, *15*(2), 51-56.

Menges, E.S., & Dolan, R.W. (1998). Demographic viability of populations of Silene regia in midwestern prairies: Relationships with fire management, genetic variation, geographic location, population size and isolation. *Journal of Ecology*, *86*(1), 63-78.

Merow, C., Dahlgren, J.P., Metcalf, C.J.E., Childs, D.Z., Evans, M.E.K., Jongejans, E., Record, S., Rees, M., Salguero-Gomez, R., & McMahon, S.M. (2014). Advancing population ecology with integral projection models: A practical guide. *Methods in Ecology and Evolution*, *5*(2), 99-110.

Miao, Y.C., Lang, X.D., Zhang, Z.Z., & Su, J.R. (2014). Phylogeography and genetic effects of habitat fragmentation on endangered T axus yunnanensis in southwest C hina as revealed by microsatellite data. *Plant Biology*, *16*(2), 365-374.

Mills, L.S., Doak, D.F., & Wisdom, M.J. (1999). Reliability of conservation actions based on elasticity analysis of matrix models. *Conservation Biology*, *13*(4), 815-829.

Mincheva, I., & Kozuharova, E. (2018). Is the traditional use of “salep” in the Bulgarian Rhodopes hazardous for the wild populations of terrestrial orchids. *Flora Mediterranea*, *28*, 399-418.

MoFE. (2019). *Forest Act 2019*. Kathmandu: Ministry of Forests and Environment (MoFE), Government of Nepal.

MoFSC. (1993). *Forest Act 1993*. Kathmandu: Ministry of Forests and Soil Conservation (MoFSC), Government of Nepal.

Molau, U. (1993). Relationships between flowering phenology and life history strategies in tundra plants. *Arctic and Alpine Research*, *25*(4), 391-402.

Mollie, E.B., Kasper Kristensen, K., Koen, J.V.B., Arni, M., Casper, W.B., Anders, N., Hans, J.S., Martin, M., & Benjamin, M.B. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*, 378-400. DOI 10.32614/RJ-2017-066.

Mondragón Chaparro, D., & Ticktin, T. (2011). Demographic effects of harvesting epiphytic bromeliads and an alternative approach to collection. *Conservation Biology*, *25*(4), 797-807.

Mora, C., Tittensor, D.P., Adl, S., Simpson, A.G., & Worm, B. (2011). How many species are there on Earth and in the ocean? *PLoS Biology*, *9*(8), e1001127.

Morris, W.F., & Doak, D.F. (2002). *Quantitative Conservation Biology*. Sinauer, Sunderland, Massachusetts, USA.

Münzbergová, Z. (2006). Effect of population size on the prospect of species survival. *Folia Geobotanica*, *41*(2), 137-150.

Mustajärvi, K., Siikamäki, P., Rytkönen, S., & Lammi, A. (2001). Consequences of plant population size and density for plant-pollinator interactions and plant performance. *Journal of Ecology,* 80-87.

Nautiyal, B.P., Prakash, V., Bahuguna, R., Maithani, U., Bisht, H., & Nautiyal, M.C. (2002). Population study for monitoring the status of rarity of three Aconite species in Garhwal Himalaya. *Tropical Ecology*, *43*(2), 297-303.

Nic Lughadha, E., Govaerts, R., Belyaeva, I., Black, N., Lindon, H., Allkin, R., Magill, R.E., & Nicolson, N. (2016). Counting counts: Revised estimates of numbers of accepted species of flowering plants, seed plants, vascular plants and land plants with a review of other recent estimates. *Phytotaxa*, *272*, 82.

Nielsen, A., Totland, Ø., & Ohlson, M. (2007). The effect of forest management operations on population performance of *Vaccinium myrtillus* on a landscape-scale. *Basic and Applied Ecology*, *8*(3), 231-241.

NTNC. (2016). *Technical Report on Biodiversity Monitoring in Bhimthang Valley*. National Trust for Nature Conservation (NTNC), Khumaltar, Lalitpur, Nepal.

Öckinger, E., Dannestam, Å., & Smith, H.G. (2009). The importance of fragmentation and habitat quality of urban grasslands for butterfly diversity. *Landscape and Urban Planning*, *93*(1), 31-37.

Ohlson, M., & Grønli, K.E. (2006). Recruitment and growth in *Aconitum septentrionale* and *Actaea spicata* in relation to microbial soil communities manipulated by additions of glucose and nutrients. *Flora-Morphology, Distribution, Functional Ecology of Plants*, *201*(3), 215-226.

Oke, T.R. (1987). *Boundary Layer Climates* (2^{nd} edn.), New York, Methuen and Co.

Olmsted, I., & Alvarez-Buylla, E.R. (1995). Sustainable harvesting of tropical trees: Demography and matrix models of two palm species in Mexico. *Ecological Applications*, *5*(2), 484-500.

Olsen, C.S. (1998). The trade in medicinal and aromatic plants from central Nepal to northern India. *Economic Botany, 52*(3), 279-292.

Olsen, C.S., & Helles, F. (1997). Medicinal plants, markets, and margins in the Nepal Himalaya: Trouble in paradise. *Mountain Research and Development*, 363-374.

Olsen, C.S., & Larsen, H.O. (2003). Alpine medicinal plant trade and Himalayan mountain livelihood strategies. *The Geographical Journal, 169*, 243-254

Oostermeijer, J.G.B., Luijten, S.H., Křenová, Z.V., & Den Nijs, H.C. (1998). Relationships between population and habitat characteristics and reproduction of the rare *Gentiana pneumonanthe* L. *Conservation Biology, 12*(5), 1042-1053.

Pant, B., & Raskoti, B.B. (2013). *Medicinal Orchids of Nepal*. Himalayan Map House.

Pauli, H., Gottfried, M., Lamprecht, A., Niessner, S., Rumpf, S.B., Winkler, M., ... & Grabherr, G. (2015). *The GLORIA Field Manual-standard Multi-Summit Approach, Supplementary Methods and Extra Approaches*.

Pellegrino, G., & Bellusci, F. (2014). Effects of human disturbance on reproductive success and population viability of Serapias cordigera (Orchidaceae). *Botanical Journal of the Linnean Society*, *176*(3), 408-420.

Peres, C.A., Baider, C., Zuidema, P.A., Wadt, L.H.O., Kainer, K.A., Gomes-Silva, D.A.P., Salomão, R.P., Simões, L.L., Franciosi, E.R.N., Valverde, F.C., Gribel, R., Shepard Jr, G.H., Kanashiro, M., Coventry, P., Yu, D.W., Watkinson, A.R., & Freckleton, R.P. (2003). Demographic threats to the sustainability of Brazil nut exploitation. *Science*, *302*(5653), 2112-2114.

Peterson, A., Bartish, I.V., & Peterson, J. (2008). Effects of population size on genetic diversity, fitness and pollinator community composition in fragmented populations of Anthericum liliago L. *Plant Ecology*, *198*(1), 101-110.

Pimm, S.L., Jenkins, C.N., Abell, R., Brooks, T.M., Gittleman, J.L., Joppa, L.N., Raven, P.H., Roberts, C.M., & Sexton, J.O. (2014). The biodiversity of species and their rates of extinction, distribution, and protection. *Science*, 30; *344*(6187). : 1246752. doi: 10.1126/science.1246752. PMID: 24876501.

Popli, D., & Sood, H. (2017). *Elicitation of Dactylorhin-E and Studying Anticancerous Potential of Dactylorhiza hatagirea (D. Don).*

Poudeyal, M.R., Meilby, H., Shrestha, B.B., & Ghimire, S.K. (2019). Harvest effects on density and biomass of *Neopicrorhiza scrophulariiflora* vary along environmental gradients in the Nepalese Himalayas. *Ecology and Evolution*, *9*(13), 7726-7740.

Pouliot, M., Pyakurel, D., & Smith-Hall, C. (2018). High altitude organic gold: The production network for Ophiocordyceps sinensis from far-western Nepal. *Journal of Ethnopharmacology*, *218*, 59-68.

Putz, F.E., Blate, G.M., Redford, K.H., Fimbel, R., & Robinson, J. (2001). Tropical forest management and conservation of biodiversity: An overview. *Conservation Biology*, *15*(1), 7-20.

Pyakurel, D., Sharma, I.B., & Smith-Hall, C. (2018). Patterns of change: The dynamics of medicinal plant trade in far-western Nepal. *Journal of Ethnopharmacology*, *224*, 323-334.

Pyakurel, D., Smith-Hall, C., Bhattarai-Sharma, I. (2019). Trade and conservation of Nepalese medicinal plants, fungi, and lichen. *Econ. Bot.*, *73*, 505-521. https://doi.org/10.1007/s12231-019-09473-0

R Development Core Team. (2017). *A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available at http://www.Rproject.org/

Ramsay, P.M., & Oxley, E.R.B. (1997). The growth form composition of plant communities in the *Ecuadorian páramos*. *Plant Ecology*, *131*(2), 173-192.

Ranpal, S. (2009). *An Assessment of Status and Antibacterial Properties of Dactylorhiza hatagirea in Annapurna Conservation Area* (*A Case Study of Paplekharka, Lete VDC, Mustang*). I Tribhuvan University Institute of Forestry, 3.

Raskoti, B.B. (2009). *Orchids of Nepal*. B.B. Raskoti, & R. Ale.

Rasmussen, H.N. (1995). *Terrestrial Orchids: From Seed to Mycotrophic Plant*. Cambridge University Press.

Rasmussen, H.N., & Rasmussen, F.N. (2018). The epiphytic habitat on a living host: reflections on the orchid-tree relationship. *Botanical Journal of the Linnean Society*, *186*(4), 456-472.

Ratsirarson, J., Silander Jr, J.A., & Richard, A.F. (1996). Conservation and management of a threatened Madagascar palm species, *Neodypsis decaryi*, Jumelle. *Conservation Biology*, *10*(1), 40-52.

Réale, D., Garant, D., Humphries, M.M., Bergeron, P., Careau, V., & Montiglio, P.O. (2010). Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1560), 4051-4063.

Reed, J. M., Mills, L. S., Dunning, J. B., Jr, Menges, E. S., McKelvey, K. S., Frye, R., Beissinger, S. R., Anstett, M. C., & Miller, P. (2002). Emerging Issues in Population Viability Analysis. *Conservation Biology: the journal of the Society for Conservation Biology*, *16*(1), 7–19. https://doi.org/10.1046/j.1523-1739.2002.99419.x

Rees, M., Childs, D.Z., & Ellner, S.P. (2014). Building integral projection models: A user's guide. *Journal of Animal Ecology*, *83*(3), 528-545.

Reintal, M., Tali, K., Haldna, M., & Kull, T. (2010). Habitat preferences as related to the prolonged dormancy of perennial herbs and ferns. *Plant Ecology*, *210*(1), 111-123.

Reiter, N., Vlcek, K., O’Brien, N., Gibson, M., Pitts, D., Brown, G.R., Bower, C.C., & Phillips, R.D. (2017). Pollinator rarity limits reintroduction sites in an endangered sexually deceptive orchid (*Caladenia hastata*): Implications for plants with specialized pollination systems. *Botanical Journal of the Linnean Society*, *184*(1), 122-136. https://doi.org/10.1093/botlinnean/box017

Renison, D., Chartier, M.P., Menghi, M., Marcora, P.I., Torres, R.C., Giorgis, M., Hansen, I., & Cingolani, A.M. (2015). Spatial variation in tree demography associated to domestic herbivores and topography: Insights from a seeding and planting experiment. *Forest Ecology and Management*, *335*, 139-146.

Robinon, M.R., & Zhang, X. (2011). *The World Medicine Situation* (*Traditional Medicines: Global Situation, Issues and Challenges*). Geneva. World Health Organization, Geneva, Switzerland.

Rock, J.H., Beckage, B., & Gross, L.J. (2004). Population recovery following differential harvesting of *Allium tricoccum* Ait. in the southern Appalachians. *Biological Conservation*, *116*(2), 227-234.

Roff, DA. (1992). *Evolution of Life Histories: Theory and Analysis*. London: Champman & Hall.

Rokaya, M.B., Münzbergová, Z., & Dostálek, T. (2017). Sustainable harvesting strategy of medicinal plant species in Nepal-results of a six-year study. *Folia Geobotanica*, *52*(2), 239-252.

Runk, J.V. (1998). Productivity and sustainability of a vegetable ivory palm (*Phytelephas aequatorialis*, Arecaceae) under three management regimes in northwestern Ecuador. *Economic Botany*, *52*(2), 168-182.

Salick, J., Ghimire, S.K., Fang, Z., Dema, S., & Konchar, K.M. (2014). Himalayan alpine vegetation, climate change and mitigation. *Journal of Ethnobiology*, *34*(3), 276-293.

Saunders, D.A., Hobbs, R.J., & Margules, C.R. (1991). Biological consequences of ecosystem fragmentation: A review. *Conservation Biology*, *5*(1), 18-32.

Schemske, D.W., Husband, B.C., Ruckelshaus, M.H., Goodwillie, C., Parker, I.M., & Bishop, J.G. (1994). Evaluating approaches to the conservation of rare and endangered plants. *Ecology*, *75*(3), 585-606.

Schmidt, I.B., Mandle, L., Ticktin, T., & Gaoue, O.G. (2011). What do matrix population models reveal about the sustainability of non-timber forest product harvest? *Journal of Applied Ecology*, *48*(4), 815-826.

Schulze, E.D., Beck, E., Buchmann, N., Clemens, S., Müller-Hohenstein, K., & Scherer-Lorenzen, M. (2019). Interactions between plants, plant communities and the abiotic and biotic environment. In *Plant Ecology* (pp. 689-741). Springer, Berlin, Heidelberg.

Scrucca, L. (2004). qcc: An R package for quality control charting and statistical process control. *dim (pistonrings), 1*(200), 3.

Semwal, D.P., Saradhi, P.P., Nautiyal, B.P., & Bhatt, A.B. (2007). Current status, distribution and conservation of rare and endangered medicinal plants of Kedarnath Wildlife Sanctuary, Central Himalayas, India. *Current Science*, 1733-1738.

Shackleton, C.M., & Shackleton, S.E. (2006). Household wealth status and natural resource use in the Kat River valley, South Africa. *Ecological Economics*, *57*(2), 306-317.

Shackleton, C.M., Pandey, A.K., & Ticktin, T. (eds.). (2015). *Ecological Sustainability for Non-timber Forest Products: Dynamics and Case Studies of Harvesting*. Routledge.

Shefferson, R.P., Jacquemyn, H., Kull, T., & Hutchings, M.J. (2020). The demography of terrestrial orchids: Life history, population dynamics and conservation. *Botanical Journal of the Linnean Society*, *192*(2), 315-332.

Shefferson, R.P., Kull, T., & Tali, K. (2005). Adult whole-plant dormancy induced by stress in long-lived orchids. *Ecology, 86*(11), 3099-3104.

Shefferson, R.P., Kull, T., Hutchings, M.J., Selosse, M.A., Jacquemyn, H., Kellett, K.M., Menges, E.S., Primack, R.B., Tuomi, J., Alahuhta, K., Hurskainen, S., Alexander, H.M., Anderson, D.S., Brys, R., Brzosko, E., Dostálik, S., Gregg, K., Ipser, Z., Jäkäläniemi, A., Jersáková, J., & Whigham, D.F. (2018). Drivers of vegetative dormancy across herbaceous perennial plant species. *Ecology letters, 21*(5), 724–733. https://doi.org/10.1111/ele.12940

Shefferson, R.P., Kull, T., Hutchings, M.J., Selosse, M.A., Jacquemyn, H., Kellett, K.M., & Whigham, D.F. (2018). Drivers of vegetative dormancy across herbaceous perennial plant species. *Ecology Letters*, *21*(5), 724-733.

Shefferson, R.P., Sandercock, B.K., Proper, J., & Beissinger, S.R. (2001). Estimating dormancy and survival of a rare herbaceous perennial using mark-recapture models. *Ecology*, *82*(1), 145-156.

Sheldon, J.W., Balik, M., & Laird, S. (1998). *Plant Talk*, 29-31.

Shimono, A., & Kudo, G. (2003) Intraspecific variation in seedling emergence and survival of *Potentilla matsumurae* (Rosaceae) between alpine fellfield and snowbed habitats. *Annals of Botany*, *91*, 21- 29.

Shrestha, B., Tsiftsis, S., Chapagain, D.J., Khadka, C., Bhattarai, P., Kayastha Shrestha, N., Kolanowaska, M.A., & Kindlmann, P. (2021). Suitability of habitats in Nepal for *Dactylorhiza hatagirea* now and under predicted future changes in climate. *Plants*, *10*(3), 467.

Shrestha, B., Tsiftsis, S., Chapagain, D.J., Khadka, C., Bhattarai, P., Kayastha Shrestha, N., & Kindlmann, P. (2021). Suitability of habitats in Nepal for *Dactylorhiza hatagirea* now and under predicted future changes in climate. *Plants*, *10*(3), 467.

Shrestha, B.B., & Jha, P.K. (2010). Life history and population status of the endemic Himalayan Aconitum naviculare. *Mountain Research and Development*, *30*(4), 353-364.

Shyaula, S.L. (2011). Phytochemicals, traditional uses and processing of Aconitum species in Nepal. *Nepal Journal of Science and Technology*, *12*, 171-178.

Silvertown, J., Franco, M., & Menges, E. (1996). Interpretation of elasticity matrices as an aid to the management of plant populations for conservation. *Conservation Biology*, *10*(2), 591-597.

Silvertown, J., Franco, M., Pisanty, I., & Mendoza, A. N. A. (1993). Comparative plant demography--relative importance of life-cycle components to the finite rate of increase in woody and herbaceous perennials. *Journal of Ecology*, 465-476.

Singh, A., & Duggal, S. (2009). Medicinal orchids-an overview. *Ethnobotanical Leaflets*, *2009*(3), 3.

Smithson, A. (2006). Pollinator limitation and inbreeding depression in orchid species with and without nectar rewards. *New Phytologist*, *169*(2), 419-430.

Snyder, R.E. (2003). How demographic stochasticity can slow biological invasions. *Ecology, 84*, 1333-1339.

Sood, S.K., Rana, S., & Lakhanpal, T.N. (2005). *Ethnic Aphrodisiac Plants*. Scientific Publishers.

Srivastava, N., Sharma, V., Kamal, B., & Jadon, V. (2010). Aconitum: need for sustainable exploitation (with special reference to Uttarakhand). *International Journal of Green Pharmacy*, *4*(4), 220.

Stachurska-Swakoń, A., Cieślak, E., & Ronikier, M. (2011). Genetic variability of small isolated populations of *Cicerbita alpina* (L.) Wallr. (Asteraceae) in the Beskid Mały Mts (southern Poland). *Polish Journal of Ecology, 59*(2), 279-288.

Steffan-Dewenter, I., Klein, A.M., Gaebele, V., Alfert, T., & Tscharntke, T. (2006). Bee diversity and plant-pollinator interactions in fragmented landscapes. *Specialization and Generalization in Plant-pollinator Interactions*, 387-410.

Stevens, S. (2003). Tourism and deforestation in the Mt Everest region of Nepal. *Geographical Journal*, *169*(3), 255-277.

Štípková, Z., & Kindlmann, P. (2015). Extent and reasons for meadows in South Bohemia becoming unsuitable for orchids. *European Journal of Environmental Sciences, 5*(2).

Subedi, A., Kunwar, B., Choi, Y., Dai, Y., van Andel, T., Chaudhary, R. P., de Boer, H. J., & Gravendeel, B. (2013). Collection and trade of wild-harvested orchids in Nepal. *Journal of ethnobiology and ethnomedicine*, *9*(1), 64. https://doi.org/10.1186/1746-4269-9-64

Subedi, B.P., & Chapagain, P.S. (2011). Tourism development in upper Manang valley of Annapurna region. *Nepal Tourism and Development Review, 1*(1), 56-68.

Svenning, J.C., & Macía, M.J. (2002). Harvesting of Geonoma macrostachys Mart. leaves for thatch: An exploration of sustainability. *Forest Ecology and Management*, *167*(1-3), 251-262.

Swarts, N.D., & Dixon, K.W. (2009) Terrestrial orchid conservation in the age of extinction. *Annals of Botany*, *104*, 543-556. http://dx.doi.org/10.1093/aob/mcp025

Teoh, E.S. (2019). The challenge: Orchid conservation. In *Orchids as Aphrodisiac, Medicine or Food* (pp. 363-376). Springer, Cham.

Thakur, D., Rathore, N., Sharma, M.K., Parkash, O., & Chawla, A. (2021). Identification of ecological factors affecting the occurrence and abundance of *Dactylorhiza hatagirea* (D. Don) Soo in the Himalaya. *Journal of Applied Research on Medicinal and Aromatic Plants*, *20*, 100286.

Thakur, M., & Dixit, V.K. (2007). Aphrodisiac activity of *Dactylorhiza hatagirea* (D. Don) Soo in male albino rats. *Evidence-Based Complementary and Alternative Medicine*, *4*(S1), 29-31.

Thomas, Y., Karki, M., Gurung, K., & Parajuli, D. (2005). *Himalayan Medicinal and Aromatic Plants: Balancing Use and Conservation*. Ministry of Forests and Soil Conservation, Kathmandu.

Ticktin, T. (2004). The ecological implications of harvesting non-timber forest products. *Journal of Applied Ecology*, *41*(1), 11-21.

Ticktin, T. (2015). The ecological sustainability for non-timber forest products harvest: Principles and methods. In C.M. Shackleton, A.K. Pandey, & T. Ticktin (Eds.), *Ecological Sustainability for Non Timber Forest Products; Dynamics and Case Studies of Harvesting* (pp. 31-52). Routledge.

Ticktin, T., & Nantel, P. (2004). Dynamics of harvested populations of the tropical understory herb *Aechmea magdalenae* in old-growth versus secondary forests. *Biological Conservation*, *120*(4), 461-470.

Timsina, B., Kindlmann, P., Münzbergová, Z., & Rokaya, M.B. (2021). Six-Year Demographic study of the terrestrial orchid, *Crepidium acuminatum*: Implications for conservation. *Frontiers in Ecology and Evolution*, 684.

Tomimatsu, H., & Ohara, M. (2010). Demographic response of plant populations to habitat fragmentation and temporal environmental variability. *Oecologia*, *162*(4), 903-911.

Tonnabel, J., Schurr, F.M., Boucher, F., Thuiller, W., Renaud, J., Douzery, E.J., & Ronce, O. (2017). Life-history traits evolved jointly with climatic niche and disturbance regime in the genus Leucadendron (Proteaceae). *The American Naturalist*, *191*(2), 220-234.

Totland, Ø., & Nyléhn, J. (1998). Assessment of the effects of environmental change on the performance and density of *Bistorta vivipara*: The use of multivariate analysis and experimental manipulation. *Journal of Ecology*, *86*(6), 989-998.

Tremblay, R.L., Ackerman, J.D., Zimmerman, J.K., & Calvo, R.N. (2005). Variation in sexual reproduction in orchids and its evolutionary consequences: A spasmodic journey to diversification. *Biological Journal of the Linnean Society*, *84*(1), 1-54.

Tsiftsis, S., Tsiripidis, I., & Trigas, P. (2011). Identifying important areas for orchid conservation in Crete. *European Journal of Environmental Sciences*, *1*(2).

Upreti, D.K., Divakar, P.K., & Nayaka, S. (2005). Commercial and ethnic use of lichens in India. *Economic Botany*, *59*(3), 269-273.

Uys, R.G., Bond, W.J., & Everson, T.M. (2004). The effect of different fire regimes on plant diversity in Southern African grasslands. *Biological Conservation*, *118*, 489-499. DOI 10.1016/j.biocon.2003.09.024.

Vaidya, B.N. (2019). Nepal: A Global Hotspot for Medicinal Orchids. In: Joshee, N., Dhekney, S., Parajuli, P. (Eds.). *Medicinal Plants* (pp. 35-80). Springer, Cham. https://doi.org/10.1007/978-3-030-31269-5_3

Vaidya, B.N. (2019). Nepal: A global hotspot for medicinal orchids. In *Medicinal Plants*. Springer, Cham.

Vakhrameeva, M.G., Tatarenko, I.V., Varlygina, T.I., Totosyan, G.K., & Zagulskii, M.N. (2008). *Orchids of Russia and Adjacent Countries*. ARG Gantner Verlag KG.

Valverde, T., & Silvertown, J. (1998). Variation in the demography of a woodland understorey herb (*Primula vulgaris*) along the forest regeneration cycle: Projection matrix analysis. *Journal of Ecology*, *86*(4), 545-562.

Van der Cingel, N.A. (1995). An atlas of orchid pollination: European orchids. *Balkem*.

Verheyen, K., & Hermy, M. (2004). Recruitment and growth of herb-layer species with different colonizing capacities in ancient and recent forests. *Journal of Vegetation Science, 15*(1), 125-134.

Vetaas, O.R. (1992a). Gradients in field-layer vegetation on an arid misty mountain plateau in the Sudan. *Journal of Vegetation Science*, *3*(4), 527-534.

Vetaas, O.R. (1992b). Micro-site effects of trees and shrubs in dry savannas. *Journal of Vegetation Science*, *3*(3), 337-344.

Vij, S.P. (1995). Orchid genetic diversity in India: Conservation and commercialization. In Proc. *5th Asia Pacific Orchid Conference and Show, Fukuoka* (pp. 20–39).

Vij, S.P. (2001). Orchids and tissue culture: Current status. *Role of Plant Tissue Culture in Biodiversity Conservation and Economic Development.* S.K. Nandi, L.M.S. Palni & A. Kumar (eds.), Gyanodaya Prakashan, Nainital, India, 491-502.

Wani, I.A., Kumar, V., Verma, S., Tasleem Jan, A., & Rather, I.A. (2020). *Dactylorhiza hatagirea* (D. Don) Soo: A critically endangered perennial orchid from the North-West Himalayas. *Plants*, *9*(12), 1644.

Warghat, A.R., Bajpai, P.K., Srivastava, R.B., & Chaurasia, O.P. (2014). In vitro protocorm development and mass multiplication of an endangered orchid, *Dactylorhiza hatagirea*. *Turkish Journal of Botany*, *38*(4), 737-746.

Warghat, A.R., Bajpai, P.K., Srivastava, R.B., Chaurasia, O.P., & Sood, H. (2013). Population genetic structure and conservation of small fragmented locations of *Dactylorhiza hatagirea* in Ladakh region of India. *Scientia Horticulturae*, *164,* 448-454.

Watanabe, T., Rajbhandari, K.K., Malla, K.J., & Yahara, S. (2005). *A Handbook of Medicinal Plants of Nepal*.

Webber, P.J., & May, D.E. (1977). The magnitude and distribution of belowground plant structures in the alpine tundra of Niwot Ridge, Colorado. *Arctic and Alpine Research*, *9*(2), 157-174.

Weiner, J., & Thomas, S.C. (1986). Size variability and competition in plant monocultures. *Oikos*, 211-222.

Wolf, J.H., & Konings, C.J. (2001). Toward the sustainable harvesting of *epiphytic bromeliads*: A pilot study from the highlands of Chiapas, Mexico. *Biological Conservation*, *101*(1), 23-31.

World Health Organization. (2002). *The World Health Report 2002: Reducing Risks, Promoting Healthy Life*. World Health Organization.

World Health Organization. (2006). *The World Health Report 2006: Working Together for Health*. World Health Organization.

Wraith, J., Norman, P., & Pickering, C. (2020). Orchid conservation and research: An analysis of gaps and priorities for globally Red Listed species. *Ambio*, 1-11.

Xia, J., Lu, J., Wang, Z.X., Hao, B.B., Wang, H.B., & Liu, G.H. (2013). Pollen limitation and Allee effect related to population size and sex ratio in the endangered *Ottelia acuminata* (Hydrocharitaceae): Implications for conservation and reintroduction. *Plant Biology*, *15*(2), 376-383.

Yeung, E.C. (2017). A perspective on orchid seed and protocorm development. *Botanical Studies*, *58*(1), 1-14.

Zeigler, S.L., Che-castaldo, J.P., & Neel, M.C. (2013). Actual and potential use of population viability analyses in recovery of plant species listed under the US Endangered Species Act. *Conservation Biology*, *27*(6), 1265-1278.

Zhang, A., Cornwell, W., Li, Z., Xiong, G., Fan, D., & Xie, Z. (2019). Strong restrictions on the trait range of co-occurring species in the newly created riparian zone of the Three Gorges Reservoir Area, China. *Journal of Plant Ecology*, *12*(5), 825-833.

APPENDICES

Appendix I: Questionnaires for ethnoecological survey

This survey is being carried out by Central Department of Botany, Tribhuvan University, Kathmandu, Nepal to assess local ecological knowledge and practices of the indigenous society in the Apinampa Conservation Area, Darchula, Bhimthang area of Manang and Annapurna Base Camp region of Kaski. Ms. Deep Jyoti Chapagain (PhD student of the department) and field assistants will be doing this questionnaire survey. The information given will not be used on other purposes except academic uses.

Socio-economic information

Name : ______________________________

Age : ______________________________

Sex : ______________________________

Address : ______________________________

Land holding : ______________________________

Occupation : ______________________________

Number of members in the family : ______________________________

Livestock : ______________________________

Average income/month : ______________________________

Source of income : ______________________________

Percentage contribution by NTFPs out of total income : ______________________

1. Do you collect any NTFPs for your household use or for sale in the market? If yes who (adult, females, children) mostly collect?

   a. Yes   b. No

   i. Adult male   ii. Adult female

   iii. Both male & female   iv. Children   v. All

2. What species do you collect mainly? Please specify in order of collection most frequently collected to less frequently collected:

Order	**Name of species**	**Month of collection & duration**	**Collection site**	**Distance from your home**	**Method of collection**
1					
2					
3					
4					
5					
6					

3. What are the traditional uses of these species?

Species	**Part collected**	**Traditional uses**
Dactylorhiza hatagirea		
Aconitum spicatum		

4. What was the amount of these MAPs you sold and price you got last year?

Species	**Quantity (kg)**	**Price/kg NPR**	**Total income**	**Buyer and location you sold**
D. hatagirea				
A. spicatum				

5. For what purpose did you use the money you earn from these MAPs (*D. hatagirea* and *A. spicatum*) sell? How much does it contribute in your household expenses in percentage?

6. How do you collect these species?

Species	**Method of collection**	**Method of processing**	**Method of storage**
D. hatagirea			
A. spicatum			

7. Who taught this knowledge of these MAPs collection & use?

8. Do you leave some parts, seeds or small plants in the ground or collect all the plants you find?

9. Do you think it is important to leave some seed, parts or small plants for future use?

10. What is your observation about the availability of these species?

Species	**Availability decreasing**	**Availability increasing**	**No changes**	**Abundant/sparse/ or rear**	**Do not know**
D. hatagirea					
A. spicatum					

11. What are the causes for increase or decrease on the availability of these MAPs?

    a. Over harvesting b. Over grazing
    c. Climate change d. If others please specify…

12. Does this availability trend have negatively impacted on your income? If so how much income in average are you losing? Or in quantity?
13. What needs to be done for the conservation of these species? Do you have any suggestion?

Species	Suggestions for conservation of the species
D. hatagirea	
A. spicatum	

14. What obstacles are you facing on collection and sell of these MAPs?
15. What differences are you observing in the collection of these MAPs after the establishment of Apinampa Conservation Area?
16. How did you acquire all this knowledge about these MAPs?
17. Do you think the knowledge is being transferred to new generation too?
18. Do farmers cultivate any MAPs?
19. If so which species are grown?
20. Do you also grow in your farm land?
21. Do you use these MAPs for medicinal value?

Species	Disease treated	Method of preparation	Dose	Others uses
D. hatagirea				
A. spicatum				

22. Have you noticed change in flowering and harvesting season of these MAPs?
23. If yes, please elaborate:

Species	Month and duration before	Month & duration now a days	Remarks
D. hatagirea			
A. spicatum			

24. What could be the reason for these changes?
25. Are you aware about the MAPs species that are banned by the Government for collection?

26. Who look after the regulation of these MAPs?

a. Local/village committee b. District level authority

c. Park head quarter members d. Others

27. How do they provide permission for collection?

a. Year wise rotation b. Yearly

c. Half yearly d. Others

28. Do you think their regulation is satisfactory?

29. How many species (MAPs) are traded from this district?

30. Do you have any ideas about the total volume of MAPS exported annually from district?

31. In which trade rank *D. hatagirea* and *A. spicatum* belong?

32. Do you have the ideas on the following features of *A. spiactum* and *D. hatagirea* in your locality?

	A. spicatum	***D. hatagirea***
No. of known populations		
Characteristics of different populations		
Habitat types		
Population size		
Abundance		
Life cycle stages		
Flowering time		
Fruiting time		
Seed production per plant		
Reproductive biology		
Yield of tuber per plant		
Seasonality of growth		

33. Which category of tuber is traded?

a. Mature b. Immature

c. Others

Thank you

Appendix II: Summary of environmental variables (topographic and substrate) characterizing three populations each of locally unprotected and locally protected sites of *Dactylorhiza hatagirea* in Api-Nampa Conservation Area (ANCA), Western Nepal and Manang, Central Nepal. Estimates of the topographic and substrate variables are specified as mean ± SE; ranges are also stated for topographic variables

Topographic variables	**Locally unprotected site (Darchula)**			**Locally protected site (Manang)**		
	Tadapani	**Nwagidanda**	**Kalidhunga**	**Bhimthang**	**Ponker hill**	**Salpodanda**
Elevation (m asl)	3604.91± 9.09	3799.41± 3.52	3975.62± 7.44	3712.59± 4.67	4045.80± 10.60	4436.50± 12.40
	(3,460-3,750)	(3,750-3,860)	(3,855-4,090)	(3,620-3,790)	(3,880-4,200)	(4,205-4,600)
Slope (degree)	17.20±0.87	26.06±1.38	10.04±0.58	25.50±1.80	31.09±1.64	36.46±1.58
	(2-25)	(6-65)	(1-22)	(4-63)	(5-56)	(7-65)
Aspect (degree)	162.30±4.66	222±4.56	231.83±5.89	119.59±5.96	166.80±3.78	164.67±4.18
	(90-248)	(132-342)	(20-320)	(30-250)	(100-260)	(104-280)
Relative radiation index (RRI)	0.92±0.00	0.88±0.01	0.89±0.00	0.81±0.01	0.90±0.00	0.89±0.00
	(0.77- 0.99)	(0.38-0.98)	(0.78-0.99)	(0.58-0.99)	(0.55-0.99)	(0.61-0.99)
Substrate variables						
Herb cover (%)	75.10±1.80	53.80±3.22	61.58±2.70	26.32±1.71	17.13±1.54	46.23±2.40
Grass cover (%)	2.76±0.36	15.82±1.90	15.66±2.08	1.87±0.14	11.84±1.49	11.97±0.80
Moss cover (%)	2.41±0.16	6.13±0.52	4.58±0.47	28.49±1.57	27.57±1.86	16.10±1.38
Lichen cover (%)	0.11±0.02	1.20±0.17	1.16±0.11	5.70±0.40	9.67±0.63	4.10±0.36
Litter cover (%)	2.92±0.17	4.47±0.46	2.51±0.16	7.66±0.91	12.13±0.74	8.37±0.74
Rock cover (%)	4.50±1.09	15.10±2.26	4.99±0.98	20.42±1.98	18.11±2.26	12.10±1.76
Scree cover (%)	0.16±0.07	0.20±0.11	1.41±0.50	2.19±0.36	1.69±0.34	0.22±0.10
Bare ground cover (%)	12.10±1.30	3.37±0.44	8.21±1.18	7.17±1.44	2.02±0.53	1.21±0.21

Appendix III: Set of models prepared using glmmTMB for the distribution analysis of *A. spicatum*

Model 1

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + (1|Plot)

Model 2

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b(Population) (1|Plot)

Model 3

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b(Population)+ c_1Herb cover$_{ij}$+ (1|Plot)

Model 4

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b(Population)+ c_1Herb cover$_{ij}$+ c_2 Shrub cover $_{ij}$+ (1|Plot)

Model 5

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a) + b(Population)+ c_1Herb cover $_{ij}$ + c_2 Shrub cover $_{ij\,+}$ c_3Harvesting $_{ij}$+ (1|Plot)

Model 6

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b(Population)+ c_1Herb cover $_{ij}$ + c_2Harvesting $_{ij}$+ (1|Plot)

Model 7

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b(Population)+ c_1 Shrub cover $_{ij+}$ c_2Harvesting $_{ij\ j}$+ (1|Plot)

Model 8

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b(Population) + c_1 RRI + (1|Plot)

Model 9

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b(Population) + c_1Harvesting + (1|Plot)

Model 10

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b(Population) + c_1Herb_cover $_{ij}$ + c_2 Shrub cover $_{ij\,+}$ c_3 Harvesting $_{ij}$ + c_4RRI $_{ij}$ + (1|Plot)

Model 11

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b(Population) + c_1Harvesting $_{ij}$ + c_2RRI $_{ij}$ + (1|Plot)

Model 12

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + (1|Plot) + b(Population) + c_1Herb_cover $_{ij}$ + c_2RRI $_{ij}$

Model 13

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b(Population) + c_1Shrub_cover $_{ij}$ + c_2RRI $_{ij}$ + (1|Plot)

Model 14

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b(Population) + c_1Herb_cover $_{ij}$ + c_2Harvesting $_{ij}$ + c_3RRI $_{ij}$ + (1|Plot)

Model 15

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b(Population) + c_1Shrub_cover $_{ij}$ + c_2Harvesting $_{ij}$ + c_3RRI $_{ij}$ + (1|Plot)

Model 16

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b(Population) + + c_1RRI $_{ij}$ $_+$$c_2$Herb_cover $_{ij}$ + c_3 Shrub cover $_{ij}$ $_+$ c_4 Harvesting $_{ij}$ + c_5Trampling $_{ij}$ $_+$ c_6 Animal drooping $_{ij+}$ c_7 Fire $_{ij}$ + (1|Plot)

Model 17

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b(Population) + c_1 Harvesting $_{ij}$ + c_2Trampling $_{ij\ +}$ c_3 Animal drooping $_{ij+}$ c_4 Fire $_{ij}$ + (1|Plot)

Model 18

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b (Population) + c_1 Harvesting $_{ij}$ + c_2Trampling $_{ij\ +}$ c_3 Animal drooping $_{ij+}$ c_4 Fire $_{ij}$ + c_6 Herb $_{ij}$ + (1|Plot)

Model 19

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b (Population) + c_1Herb_cover $_{ij\ +}$ c_2 Harvesting $_{ij}$ + c_3Trampling $_{ij\ +}$ c_4 Animal drooping $_{ijj}$ + (1|Plot)

Model 20

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b (Population) + c_1Herb_cover $_{ij}$ + c_2 Harvesting $_{ij}$ + c_3Trampling $_{ij}$ + (1|Plot)

Model 21

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b (Population) + c_1Harvesting $_{ij}$ + c_2Trampling $_{ij}$ + (1|Plot)

Model 22

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b (Population) + c_1Fire $_{ij\,+}$ c_2 Harvesting $_{ij}$ + c_3Trampling $_{ij}$ + (1|Plot)

Model 23

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b (Population) + c_1Animal drooping $_{ij}$ + c_2 Trampling $_{ij\,+}$ c_3 Fire $_{ij}$ + (1|Plot)

Model 24

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b (Population) + c_1Animal drooping $_{ij}$ + c_2 Fire $_{ij}$ + (1|Plot)

Model 25

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b (Population) + c_1Trampling $_{ij}$ + c_2 Fire + (1|Plot)

Model 26

Density $_{ij}$ (of a particular stage of *A. spicatum*) = a + b (Population) + c_1Harvesting $_{ij}$ + c_2Fire$_{ij}$ + (1|Plot)

Where, a, b (Population) and $c_1 \ldots c_{13}$ are fixed model parameters, i = 1…66 is the plot (random effect) and j = 1…5 is the sub-plot. Population has three categories (subalpine, lower alpine and alpine). Density is expressed as the number of individuals in a given stage category counted within a 1 m^2 sub-plot.

Appendix IV: Model selecton table for the distribution of different stages of *Aconitum spicatum*

I. Seedling stage

Model Selection table for Seedling stage

SN	Models	logLik	AICc	delta	Weight
1	glmm. mzip6Sd	-116.93	248.2	0	0.168
2	glmm. mzip14Sd	-115.9	248.2	0.03	0.165
3	glmm. mzip5Sd	-116.29	249	0.82	0.111
4	glmm. mzip10Sd	-115.51	249.6	1.37	0.084
5	glmm. mzip7Sd	-117.76	249.9	1.65	0.074
6	glmm. mzip9Sd	-118.97	250.2	1.98	0.062
7	glmm. mzip19Sd	-115.82	250.2	1.98	0.062
8	glmm. mzip20Sd	-116.93	250.3	2.09	0.059
9	glmm. mzip11Sd	-118.15	250.7	2.43	0.05
10	glmm. mzip15Sd	-117.25	250.9	2.73	0.043
11	glmm. mzip26Sd	-118.78	251.9	3.68	0.027
12	glmm. mzip18Sd	-115.74	252.2	3.95	0.023
13	glmm. mzip21Sd	-118.93	252.2	3.99	0.023
14	glmm. mzip17Sd	-116.87	252.3	4.08	0.022
15	glmm. mzip16Sd	-114.14	253.3	5.04	0.013
16	glmm. mzip22Sd	-118.75	254	5.74	0.01
17	glmm. mzip12Sd	-122.05	258.4	10.23	0.001
18	glmm. mzip3Sd	-123.25	258.8	10.54	0.001
19	glmm. mzip24Sd	-122.27	258.9	10.66	0.001
20	glmm. mzip4Sd	-122.43	259.2	10.98	0.001
21	glmm. mzip23Sd	-122.27	261	12.76	0
22	glmm. mzip13Sd	-123.45	261.2	13.03	0
23	glmm. mzip8Sd	-124.54	261.3	13.13	0
24	glmm. mzip2Sd	-125.58	261.4	13.14	0
25	glmm. mzip25Sd	-124.75	263.8	15.63	0
26	glmm. mzip1Sd	-129.86	265.8	17.58	0

II. Juvenile stage

Model Selection table for Juvenile stage

SN	Models	logLik	AICc	delta	weight
1	glmm. mzip9Juv	-156.05	324.4	0	0.164
2	glmm. mzip21Juv	-155.03	324.4	0.06	0.16
3	glmm. mzip19Juv	-153.54	325.6	1.28	0.087
4	glmm. mzip17Juv	-153.54	325.6	1.28	0.087
5	glmm. mzip11Juv	-155.81	326	1.6	0.074

6	glmm. mzip6Juv	-156.03	326.4	2.05	0.059
7	glmm. mzip20Juv	-155.01	326.5	2.11	0.057
8	glmm. mzip22Juv	-155.03	326.5	2.15	0.056
9	glmm. mzip15Juv	-155.07	326.6	2.23	0.054
10	glmm. mzip5Juv	-155.12	326.7	2.33	0.051
11	glmm. mzip18Juv	-153.53	327.8	3.4	0.03
12	glmm. mzip14Juv	-155.79	328	3.66	0.026
13	glmm. mzip10Juv	-155.05	328.7	4.31	0.019
14	glmm. mzip24Juv	-157.32	329	4.62	0.016
15	glmm. mzip23Juv	-156.37	329.2	4.83	0.015
16	glmm. mzip2Juv	-159.66	329.5	5.15	0.012
17	glmm. mzip8Juv	-159.33	330.9	6.55	0.006
18	glmm. mzip16Juv	-153	331	6.62	0.006
19	glmm. mzip25Juv	-158.39	331.1	6.78	0.006
20	glmm. mzip3Juv	-159.66	331.6	7.22	0.004
21	glmm. mzip13Juv	-158.76	331.9	7.5	0.004
22	glmm. mzip4Juv	-158.88	332.1	7.76	0.003
23	glmm. mzip12Juv	-159.32	333	8.64	0.002
24	glmm. mzip1Juv	-164.97	336	11.65	0
25	glmm. mzip26Juv	-284.21	582.8	258.41	0
26	glmm. mzip7Juv	-284.26	582.9	258.51	0

III. Adult vegetative stage

Model Selection table for Adult vegetative stage

SN	Models	logLik	AICc	delta	weight
1	glmm. mzip24Adv	-281.59	577.5	0	0.214
2	glmm. mzip19Adv	-279.83	578.2	0.7	0.151
3	glmm. mzip17Adv	-280.05	578.7	1.15	0.12
4	glmm. mzip23Adv	-281.11	578.7	1.15	0.12
5	glmm. mzip18Adv	-279.59	579.9	2.36	0.066
6	glmm. mzip9Adv	-284.28	580.8	3.31	0.041
7	glmm. mzip2Adv	-285.41	581	3.48	0.038
8	glmm. mzip21Adv	-283.44	581.2	3.71	0.034
9	glmm. mzip11Adv	-283.65	581.6	4.13	0.027
10	glmm. mzip8Adv	-284.82	581.9	4.38	0.024
11	glmm. mzip16Adv	-278.52	582	4.5	0.023
12	glmm. mzip26Adv	-284.21	582.8	5.25	0.016
13	glmm. mzip7Adv	-284.26	582.9	5.35	0.015
14	glmm. mzip6Adv	-284.28	582.9	5.39	0.014
15	glmm. mzip3Adv	-285.4	583.1	5.54	0.013

16	glmm. mzip22Adv	-283.38	583.2	5.69	0.012
17	glmm. mzip20Adv	-283.44	583.3	5.8	0.012
18	glmm. mzip15Adv	-283.52	583.5	5.98	0.011
19	glmm. mzip13Adv	-284.67	583.7	6.17	0.01
20	glmm. mzip14Adv	-283.64	583.7	6.21	0.01
21	glmm. mzip12Adv	-284.8	584	6.44	0.009
22	glmm. mzip25Adv	-284.84	584	6.51	0.008
23	glmm. mzip5Adv	-284.26	585	7.45	0.005
24	glmm. mzip4Adv	-285.36	585.1	7.55	0.005
25	glmm. mzip10Adv	-283.5	585.6	8.05	0.004
26	glmm. mzip1Adv	-309.07	624.2	46.69	0

IV. Adult reproductive stage

Model Selection table for Adult reproductive stage

SN	Models	logLik	AICc	delta	weight
1	glmm. mzip24Adr	-376.42	767.2	0	0.441
2	glmm. mzip23Adr	-376.06	768.6	1.37	0.222
3	glmm. mzip17Adr	-375.6	769.8	2.57	0.122
4	glmm. mzip26Adr	-378.67	771.7	4.5	0.046
5	glmm. mzip18Adr	-375.59	771.9	4.68	0.043
6	glmm. mzip25Adr	-379.06	772.5	5.27	0.032
7	glmm. mzip22Adr	-378.37	773.2	6	0.022
8	glmm. mzip19Adr	-378.02	774.6	7.41	0.011
9	glmm. mzip16Adr	-374.85	774.7	7.5	0.01
10	glmm. mzip11Adr	-380.59	775.5	8.33	0.007
11	glmm. mzip9Adr	-381.91	776.1	8.89	0.005
12	glmm. mzip7Adr	-380.91	776.2	8.97	0.005
13	glmm. mzip15Adr	-379.99	776.4	9.24	0.004
14	glmm. mzip8Adr	-382.1	776.5	9.27	0.004
15	glmm. mzip14Adr	-380.15	776.7	9.56	0.004
16	glmm. mzip2Adr	-383.52	777.2	10.05	0.003
17	glmm. mzip6Adr	-381.46	777.3	10.08	0.003
18	glmm. mzip21Adr	-381.51	777.4	10.17	0.003
19	glmm. mzip12Adr	-381.57	777.5	10.3	0.003
20	glmm. mzip13Adr	-381.58	777.5	10.31	0.003
21	glmm. mzip5Adr	-380.68	777.8	10.62	0.002
22	glmm. mzip10Adr	-379.73	778	10.82	0.002
23	glmm. mzip3Adr	-382.98	778.2	11.04	0.002
24	glmm. mzip20Adr	-381.12	778.7	11.5	0.001
25	glmm. mzip4Adr	-382.32	779	11.8	0.001
26	glmm. mzip1Adr	-402.01	810.1	42.91	0

Appendix V: Observed and expected (stable) stage distributions for different harvesting treatments and populations of *Aconitum spicatum*

Transition periods	**Harvesting**	**Stage class**	**Population**					
			Alpine		**Lower alpine**		**Sub alpine**	
			Observed	**Stable**	**Observed**	**Stable**	**Observed**	**Stable**
2015-2016	0-25%	Seedling	0.107	0.313	0.058	0.306	0.041	0.276
		Juvenile	0.253	0.200	0.104	0.158	0.049	0.108
		Adult vegetative	0.076	0.130	0.341	0.158	0.270	0.225
		Adult reproductive	0.262	0.250	0.358	0.223	0.459	0.187
		Dormant	0.302	0.107	0.139	0.154	0.180	0.205
		Keyfitz’s Δ		**0.260**		**0.303**		**0.317**
	50-75%	Seedling	0.075	0.319	0.081	0.326	0.098	0.201
		Juvenile	0.302	0.207	0.048	0.170	0.137	0.067
		Adult vegetative	0.098	0.135	0.097	0.216	0.235	0.361
		Adult reproductive	0.168	0.252	0.500	0.221	0.490	0.133
		Dormant	0.358	0.087	0.274	0.067	0.039	0.238
		Keyfitz’s Δ		**0.366**		0.486		0.427
2016-2017	0-25%	Seedling	0.017	0.067	0.059	0.272	0.044	0.292
		Juvenile	0.187	0.159	0.080	0.130	0.052	0.144
		Adult vegetative	0.239	0.390	0.266	0.252	0.274	0.199
		Adult reproductive	0.357	0.041	0.372	0.196	0.311	0.189
		Dormant	0.199	0.343	0.223	0.151	0.319	0.177
		Keyfitz’s Δ		0.345		0.263		0.339
	50-75%	Seedling	0.012	0.124	0.068	0.149	0.071	0.303
		Juvenile	0.214	0.271	0.095	0.110	0.054	0.330
		Adult vegetative	0.271	0.368	0.149	0.495	0.375	0.138
		Adult reproductive	0.373	0.077	0.459	0.090	0.304	0.175
		Dormant	0.130	0.160	0.230	0.157	0.196	0.053
		Keyfitz’s Δ		0.297		0.443		0.509
2017-2018	0-25%	Seedling	0.019	0.250	0.054	0.276	0.038	0.383
		Juvenile	0.130	0.309	0.081	0.257	0.046	0.116
		Adult vegetative	0.424	0.142	0.314	0.151	0.277	0.220
		Adult reproductive	0.100	0.183	0.362	0.186	0.331	0.251
		Dormant	0.327	0.116	0.189	0.130	0.308	0.031
		Keyfitz’s Δ		0.493		0.398		0.414
	50-75%	Seedling	0.009	0.061	0.061	0.158	0.077	0.290
		Juvenile	0.208	0.103	0.098	0.182	0.096	0.131
		Adult vegetative	0.467	0.473	0.317	0.452	0.346	0.322
		Adult reproductive	0.131	0.022	0.280	0.085	0.365	0.159
		Dormant	0.185	0.341	0.244	0.121	0.115	0.098
		Keyfitz’s Δ		0.214		0.318		0.248

Keyfitz’s Δ is a standard measure of the distance between two probability vectors, which values range between 0 (when the vectors are identical) to 1 (when the vectors are different) [Caswell 2001]. Here Keyfitz’s Δ was used to compare stable stage-class distributions with observed stage class distributions. distributions, obtained from the left eigenvectors of the transition matrices, were compared with observed stage class distributions

Appendix VI: Set of models prepared using glmmTMB for the distribution analysis of *Dactylorhiza hatagirea*

Model 1

Density $_{ij}$ (of a particular stage of *D. hatagirea*) = a +b (population) + c_1Harvesting $_{ij}$ + c_2Herbcover $_{ij}$ + (1|Plot)

Model 2

Density $_{ij}$ (of a particular stage of *D. hatagirea*) = a + b (Population) +c_1Harvesting $_{ij}$ + (1|Plot)

Model 3

Density $_{ij}$ (of a particular stage of *D. hatagirea*) = a + b (Population) + c_1RRI $_{ij}$+ c_2Harvesting $_{ij\,+}$ (1|Plot)

Model 4

Density $_{ij}$ (of a particular stage of *D. hatagirea*) = a + b (Population) + c_1Herb cover $_{ij}$+ c_2 RRI $_{ij}$+ c_3 Harvesting $_{ij\,+}$ (1|Plot)

Model 5

Density $_{ij}$ (of a particular stage of *D. hatagirea*) = a) + b (Population)+ c_1Harvesting $_{ij}$ + c_2 Trampling $_{ij\,+}$ c_3 Animal dropping $_{ij}$ + (1|Plot)

Model 6

Density $_{ij}$ (of a particular stage of *D. hatagirea*) = a + b(Population)+ c_1Herb cover $_{ij}$ + c_2Harvesting $_{ij}$+ c_3 Trampling $_{ij+}$ c_4 Animal dropping $_{ij+}$ (1|Plot)

Model 7

Density $_{ij}$ (of a particular stage of *D. hatagirea*) = a + b(Population)+ c_1Herb cover $_{ij}$ + c_2Harvesting $_{ij}$+ c_3 Trampling $_{ij}$ + (1|Plot)

Model 8

Density $_{ij}$ (of a particular stage of *D. hatagirea*) = a + b (Population) + c_1 Harvesting $_{ij}$ + c_2Trampling $_{ij}$ + (1|Plot)

Model 9

Density $_{ij}$ (of a particular stage of *D. hatagirea*) = a + b (Population) + c_1 Animal dropping $_{ij}$ + c_2 Trampling $_{ij}$ + (1|Plot)

Model 10

Density $_{ij}$ (of a particular stage of *D. hatagirea*) = a + b (Population) + c_1Animal dropping $_{ij}$ + (1|Plot)

Where, a (intercept), b (Population) and $c_1 \ldots c_3$ are fixed model parameters, i = 1…144 is the plot (included as a random effect) and j = 1…4 is the sub-plot. Population has six categories (three at each site). Density is expressed as the number of individuals in a given stage category counted within a 1 m^2 sub-plot.

Appendix VII: Conferences participation

- Chapagain, D.J., Mielby, H. & Ghimire, S.K. (2016). *Current Status of a Threatened Medicinal Plant Dactylorhiza hatagirea (D. Don) Soo in Api-Nampa Conservation Area North West Nepal.* Conference paper presented in Seventh National Conference on Science and Technology, Kathmandu, Nepal.

- Chapagain, D.J., Mielby, H. & Ghimire, S.K. (2016). *Status of a Threatened Medicinal Plant Allium wallichii Kunth in Annapurna Conservation Area Central Nepal* presented in Second Graduate Conference on Environment and Sustainable Development, Kathmandu, Nepal.

- Chapagain, D.J., Mielby, H. & Ghimire, S.K. (2017). *Sustainability of Harvest of Commercially Threatened Medicinal Plant Aconitum spicatum (Briihl) Stapf in Central Nepal* presented in International Conference on Biodiversity, Climate Change Assessment and Impacts on livelihoods, Kathmandu, Nepal.

- Chapagain, D.J., Mielby, H. & Ghimire, S.K. (2017). *Sustainability of Harvest of Commercially Threatened Medicinal Plant Aconitum spicatum (Briihl) Stapf in Central Nepal* presented in 5th International Conference on Wild Harvest Governance and Livelihoods in Asia, Kathmandu, Nepal.

Poster Presentation

- Chapagain, D.J., Mielby, H. & Ghimire, S.K. (2017). *Conference on Plant Conservation and Sustainable Development Goals.* Missouri Botanical Garden, USA.

Appendix VIII: Publications (Main papers and other outputs during the PhD periods)

This PhD has four main papers

Main papers

I. **Chapagain, D.J.**, Meilby, H. & Ghimire, S.K. (2019). Plant density and life history traits of *Aconitum spicatum* in North- Central Nepal: Effects of elevation and anthropogenic disturbances. *PeerJ* 7:e7574 http://doi.org/10.7717/ peerj.7574 (attached at the end of the thesis)

II. **Chapagain, D.J.**, Meilby, H., Baniya, C.B., Magar, S.B. & Ghimire, S.K. (2021). *Illegal Harvesting and Livestock Grazing Threaten the Endangered Orchid Dactylorhiza hatagirea (D. Don) Soo in Nepalese Himalaya* (attached at the end of the thesis)

III. **Chapagain, D.J.**, Meilby, H. & Ghimire, S.K. (Unpublished manuscript). *Population Dynamics of An Endangered Orchid Dactylorhiza hatagirea (D. Don) Soó in Nepalese Himalaya: Effect of Habitats and Harvesting*.

IV. **Chapagain, D.J.**, Meilby, H. & Ghimire, S.K. (Unpublished manuscript). *Sustainability of Harvest of Commercially Threatened Medicinal Herb Aconitum spicatum in Nepalese Himalaya*.

Supportive publications during PhD

I. Smith-Hall, C., Pouliot, M., Pyakurel, D., Fold, N., Chapagain, A., Ghimire, S., Meilby, H., Kmoch, L., **Chapagain, D.J.**, Das, A., Jun, H., Nepal, K., Poudeyak, M.R. & Larsen, H.O. (2018). *Data Collection Instruments and Procedures for Investigating National Level Trade in Medicinal and Aromatic Plants - The Case of Nepal*. University of Copenhagen, Department of food and Resource Economics, IFRO Documentation 2018/2.

II. Shrestha, B., Tsiftsis, S., **Chapagain, D. J.**, Khadka, C., Bhattarai, P., Shrestha, N.K., Kolanoswska, M.A. & Kindlmann, P. (2021). Suitability of habitats in Nepal for *Dactylorhiza hatagirea* now and under predicted future changes in climate. *Plants, 10*, 467. https://doi.org/10.3390/plant s1003 0467

III. Ghimire, S.K., Subedi, C.K., Budha-Magar, S., Adhikari, M., Pandey, T.R., Awasthi, B., Thapa-Magar, S., Paudeyal, M.R., Ghimire, K.M., Shrestha, B.B., Bhatt, G.D., Joshi, L.R., Paudel, A., **Chapagain D.J**. & Gurung, J. (2021). *Flora of Kailash Sacred Landscape Nepal: An Annotated Checklist. Volume 1* (Gymnosperms and Angiosperms: Ephedraceae– *Buxaceae*). Research Centre for Applied Science and Technology (RECAST), Tribhuvan University, Kathmandu, Nepal.

Appendix IX: Courses attended during PhD

I. *Biodiversity and Environmental Manangement* (November 15, 2015-May 15, 2016), Central Department of Botany, Tribhuvan University, Kirtipur, Nepal.

II. *An Introductory Course to PhD Students* (March 26-31, 2017), University of Copenhagen, Copenhagen, Denmark.

III. *Art of Scientific Writing* (March-May, 2017), University of Copenhagen, Copenhagen, Denmark.

IV. *An Introductory Course to MATLAB for Multivariate Analysis* (May 3-11, 2017), University of Copenhagen, Copenhagen, Denmark.

V. *Training on Research Methodology* (June 20-26, 2017), conducted by Central Departmet of Botany, Kirtipur, Nepal.

Plant density and life history traits of *Aconitum spicatum* in North-central Nepal: effects of elevation and anthropogenic disturbances

Deep J. Chapagain[1,2], Henrik Meilby[2] and Suresh K. Ghimire[1]

[1] Central Department of Botany, Tribhuvan University, Kathmandu, Nepal
[2] Department of Food and Resource Economics, University of Copenhagen, Copenhagen, Denmark

ABSTRACT

Increasing cross-border trade of medicinal and aromatic plants (MAPs) has put heavy pressure on a considerable number of species in the Himalayas. One of the threatened species in Nepal is *Aconitum spicatum*. Unfortunately for this species and for many others, our knowledge on population ecology and performance across the distribution range is insufficient, hindering the formulation of species-specific management plans. We therefore studied density and population structure of *A. spicatum* and assessed variation in its life history traits among three populations (subalpine, lower alpine and alpine) along an elevation gradient (3,000–4,200 m a.s.l.) in Annapurna Conservation Area, north-central Nepal. The results show that human disturbances and topographic factors contributed to the variation in density and life history traits. The overall density ranged between 0.56 ± 0.09 (Mean ± SE) and 2.48 ± 0.24 plants/m^2 with highest mean density in the lower alpine and lowest in the subalpine population. The subalpine population was also characterized by lower investment in reproductive structures with lowest seed mass and low seed viability and fecundity. Among the environmental variables tested, harvesting, animal droppings and fire appeared to be the most important factors affecting density of different life stages of *A. spicatum*. The prevailing harvesting pattern is destructive as it involves uprooting of the whole plant and this appears to be a main reason for low recruitment and reduced density of the subalpine population. The level of disturbance decreased with increasing elevation. In terms of reproductive effort, the alpine population performed best. Our results indicate that the viability of *A. spicatum* populations depends on controlling over-harvesting and pre-mature harvesting of tubers and protecting younger life stages from grazing, trampling and fire. We therefore recommend that when formulating management guidelines, measures aiming to mitigate such anthropogenic disturbances should be considered.

Subjects Biodiversity, Conservation Biology, Ecology, Plant Science, Population Biology
Keywords Population density, Tuber biomass, Sensitivity, Mixed Zero Inflated Poisson

Submitted 11 February 2019
Accepted 29 July 2019
Published 10 September 2019

Corresponding author
Deep J. Chapagain,
chapagaindeep@gmail.com

Academic editor
Renato Benesperi

Additional Information and Declarations can be found on page 17

DOI **10.7717/peerj.7574**

INTRODUCTION

The study of variation in life history traits of a species is crucial to understanding the role of particular types of ecological pressure in shaping population demographics

How to cite this article Chapagain DJ, Meilby H, Ghimire SK. 2019. Plant density and life history traits of *Aconitum spicatum* in North-central Nepal: effects of elevation and anthropogenic disturbances. *PeerJ* 7:e7574 http://doi.org/10.7717/peerj.7574

(*Roff, 1992*; *Réale et al., 2010*; *Tonnabel et al., 2017*). Variation in environmental conditions can affect recruitment dynamics (*Franco & Silvertown, 1996*) and population growth rates (*Stearns & Hoekstra, 2000*; *Caswell, 2001*). Himalayan alpine plants respond to environmental and climate change variables including biogeography, elevation and precipitation (*Salick et al., 2014*). Increasing anthropogenic activities have also created and continue to create a range of novel environments in mountain ecosystems (*Zhang et al., 2019*). Along the elevation gradient in mountain ecosystems, large changes in environmental factors occur over short distances, leading to huge variation in the selection pressure imposed on plant life-history strategies and traits (*Bresson et al., 2011*). Particularly, topographic variation affects microclimatic conditions, which may contribute to variation in life history traits. These variations are coupled with disturbance regimes, like harvesting, fire and grazing which themselves are structured along elevation gradients. The concentration of these anthropogenic disturbances, especially in alpine and subalpine ecosystems may put high-altitude plants under stress. To cope with such stressful conditions, high-altitude plants develop self-sustaining adaptation strategies (*Körner, 2003*), including a long floral axis, more globular flowers (*Molau, 1993*), persistent sepals, larger root systems (*Körner, 1999*), vegetative apices or perennating buds at safe depth below ground (*Körner, 2003*), higher proportion of belowground biomass (*Webber & May, 1977*) and increased number and size of seeds (*Körner, 1999*), which contribute to enhance the persistence of their populations.

Population density and individual-level traits, like plant size and reproductive performance (*Totland & Nyléhn, 1998*; *Nielsen, Totland & Ohlson, 2007*; *Fan & Yang, 2009*) are considered the most important basic parameters needed to assess the capacity of a plant species to maintain its population. Reproductive traits are especially important for a species to establish and to persist following disturbance. Rates of recruitment vary and depend on, e.g., seed number, seed mass, seed viability, growing season, and disturbance characteristics (*Chambers, MacMahon & Brown, 1990*). Understanding the variation in such traits is important for the assessment of the long-term persistence of populations growing in habitats disturbed by human activities. Habitat disturbance often has negative effects on plant reproductive success (*Chambers, 1995*). Cattle grazing, trampling and fire, for example, may lead to lower fruit set and subsequent recruitment (*McKechnie & Sargent, 2013*; *Renison et al., 2015*). However, fire is deemed to be a prerequisite for recruitment, growth and development of plant species in some ecosystems (*Bucini & Lambin, 2002*) and has in some cases been shown to promote higher species diversity (*Hobbs, Mallik & Gimingham, 1984*).

Harvesting of medicinal and aromatic plants (MAPs) is a customary subsistence practice in the Himalayan region. Over 300 taxa of MAPs are traded from Nepal with a total annual amount of 10,770 tons and a value of USD 60.09 million (*Ghimire et al., 2016*; *Government of Nepal, 2015*; *Government of Nepal, 2018*). Trans-boundary trade of Himalayan MAPs has increased to meet the growing demand from international pharmaceutical companies and has led to heavy and indiscriminate harvesting of some species. *Aconitum spicatum* (family Ranunculaceae) is among the ten most traded medicinal plants from the Himalayan region of Nepal (*Olsen & Larsen, 2003*). It is vulnerable in Nepal, mainly due to over-harvesting

(*Bhattarai, Tandon & Ved, 2002*). Considering the high trade value of *A. spicatum*, the government of Nepal has nominated it as one of thirty herbs of national priority for development, research and cultivation (*Department of Plant Resources, 2012*). Despite the commercial interest and high conservation value of *A. spicatum*, detailed studies describing its ecological, morphological and reproductive characteristics are largely lacking within its entire range. Scientific information on how the different disturbance regimes (including harvesting) shape the life history parameters (reproduction and growth) and determine population density and structure of important Himalayan MAPs like *A. spicatum* across ecological gradients is important for the development of management strategies to maintain viable populations (*Ticktin, 2004*; *Ghimire, McKey & Aumeeruddy-Thomas, 2005*). In this paper, we aim to analyze whether impacts of anthropogenic disturbance on population performance of *A. spicatum* depend on ecological factors associated with elevation. Thus, examining populations located within three consecutive elevation ranges, the goal of this study was to answer the following questions: (i) do the impacts of anthropogenic disturbance on population structure and density of *A. spicatum* vary along an elevation gradient? (ii) How do plant size and reproductive performance of *A. spicatum* vary along an elevation gradient?

MATERIALS AND METHODS

Study area

The study was carried out in upper Modi River Valley (N28°29.455′and E083°53.546′to N28°31.806′and E083°52.537′) within Annapurna Conservation Area (ACA) in north-central Nepal (Fig. 1). The study area is characterized by temperate and alpine climate. The vegetation consists of upper temperate coniferous and mixed broad-leaved forests at lower elevations (>3,000–3,500 m), sub-alpine mixed forests dominated by *Abies spectabilis* and *Betula utilis* at and below the tree line (>3,500–4,000 m), lower alpine thickets mainly of dwarf bushes of *Rhododendron* spp. at and above the tree line (*ca.* 4,000 m), and alpine meadows and grasslands above 4,000 m. Snowfall starts in October and the area remains snow-covered for up to six months, until March. High seasonal differences in temperature and rainfall, short growing season and heavy snowfall are the main constraints to plant growth at higher elevations. The study area comprises the most popular trekking routes of Nepal, and each year approximately 30,000 tourists visit the area. Trekking tourism can be considered one of the most important factors affecting the structure of forest ecosystems at high elevation in the Himalayan region (*Biønness, 1980*; *Stevens, 2003*; *Garbarino et al., 2014*). There are 14 hotels along the trekking route passing through the study area, and the nearest villages (Chhomrong and Sinwa) are inhabited by 62 households. Along the upper Modi Valley there are two cattle sheds ('goths' in Nepali), each with about 500 cattle. The livelihood of local people is based on subsistence agriculture, pastoralism, seasonal trade, and more importantly, tourism. Interviews with local residents indicated that people from the neighboring Gorkha and Dhading districts of Nepal, recently started harvesting highly valued MAPs, including *A. spicatum* from the ACA region (D Chapagain, pers. obs., 2018).

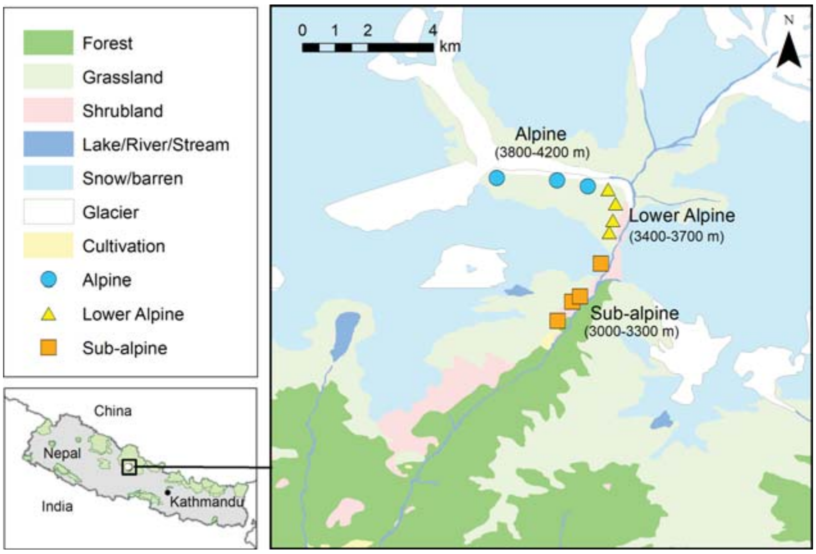


Figure 1 **Map showing study area (N28°29.455′ and E083°53.546′ to N28°31.806′ and E083°52.537′, Annapurna Conservation Area) and the locations of the *Aconitum spicatum* populations sampled.** Source of land cover map: Department of Survey, Government of Nepal.

Full-size DOI: 10.7717/peerj.7574/fig-1

Study species

A. spicatum is a vulnerable (*Bhattarai, Tandon & Ved, 2002*) medicinal plant species with a distribution restricted to the Himalayas of Nepal, India, Bhutan and China, at elevations between 2,900 and 4,200 m above sea level (asl) (*Ghimire et al., 1999*). In the study area, fragmented populations of *A. spicatum* occur between 3,000–4,200 m asl. It is a tall perennial herb with an erect stem reaching up to two m in height and bearing a crown of blue zygomorphic flowers with numerous stamens. It bears 1–70 flowers (mean ± SE 10.64 ± 1.29) and 1–110 fruits (mean ± SE 14.03 ± 1.53) per plant. Each fruit is an aggregate of five to six follicles. Each fruit produces an average of 41 seeds; thus an individual plant bears about 574 seeds on average. The plant produces paired dark-brown tubers, which are best harvested as soon as the above-ground parts die in autumn (*Ghimire et al., 1999*). The aerial part of the plant is annual but the tubers are perennial. The mother tuber dies every year, but before this happens it produces a number of sprouting buds, which grow into one to five daughter tubers (direct field observation). The plant shows low seed germination potential and the seeds can stay dormant for a long time (*Srivastava et al., 2010*), and even tubers show frequent dormancy (D Chapagain, pers. obs., 2018). The plant is highly poisonous, and the tubers contain alkaloids, mainly aconitine, hypaconitine and mesaconitine, which are reported to have antibacterial, antipyretic, analgesic and enzyme inhibition properties (*Srivastava et al., 2010*). Tubers of *A. spicatum* are used in traditional medicines to cure fever, headache and infections in lungs and intestines and to heal cuts and wounds (*Tsarong, 1994*; *Lama, Ghimire & Aumeeruddy-Thomas, 2001*).

Sampling

This research is part of a long-term study carried out between 2015 and 2018 to understand the population dynamics of *A. spicatum* in ACA (research permit issued by Department of National Park and Wildlife Conservation (DNPWC), ref. No. 93/070/071). During the summer of 2015, sampling was carried out in three sites at elevations ranging from 3,000 m to 4,200 m. Sampling covered the whole elevation range of *A. spicatum* in the study area as the transects were established from the lowest to the highest point of its distribution. In each 100 m elevation band, a transect with six plots (three m × three m) was established with plots located at a minimum plot-to-plot distance of 10 m. Each plot was divided into nine subplots of one m × one m, five of which (center and four corner subplots) were systematically selected for detailed biological measurements. In total, we laid out 66 (three m × three m) plots, with 330 (one m × one m) sub-plots. In each plot, we recorded latitude and longitude using an eTrex Garmin GPS device and cross-checked the elevation using a calibrated altimeter. Slope and aspect were measured using clinometer and compass, respectively. In each subplot, the cover (%) of shrubs, herbs, grasses, lichens, bryophytes, litter, bare ground, solid rock and scree were estimated using standard methods (*Pauli et al., 2015*).

Individuals of *A. spicatum* were classified into four stage classes on the basis of their size and reproductive status and were counted. The stage classes were: seedlings (Sd; stem girth $\leq$ one mm measured at five cm from soil surface, leaf number = 1–2), juveniles (Jv; stem girth = 1–<three mm, leaf number $\geq$ 2–<5), vegetative adults (Adv; stem girth $\geq$ three mm, leaf number $\geq$ 2) and reproductive adults (Adr; flowering or fruiting individuals). In each subplot, the count of plants at different stages was used to calculate population structure and density. Animal droppings, trampling, harvesting and fire were considered as anthropogenic disturbances. A five-point ordinal scale (0–4) was applied to describe the level of disturbance. A score of '0' corresponded to no disturbance, while '4' corresponded to heavy disturbance.

We grouped four elevation bands together and defined three wider ecotone populations, namely subalpine (3,000–3,300 m), lower alpine (3,400–3,700 m) and alpine (3,800–4,200 m). We uprooted twenty of the most vigorous plants from each of these populations to record plant biomass and to measure vegetative traits (plant height, stem girth, number of leaves, leaf size and length of floral axis) and reproductive traits (number of buds, flowers and fruits). The fresh weight of aboveground and belowground plant parts was measured in the field. Afterwards, the plants were air dried, packed in paper bags and brought to the laboratory for dry biomass estimation. Seed number and seed mass estimations were based on random selection of 15 mature reproductive individuals from each population during the seed maturation period in October. From each such individual, we collected five fruits, recorded their diameter, length and fresh weight in the field, and then packed the fruits separately in paper bags for subsequent dry biomass estimation.

We carried out seed sowing experiments in October 2015 and 2016. In each year, we introduced mature seeds ($n = 100$) into each of five randomly selected one m × one m subplots, located in a similar habitat adjacent to the study plots. Dark brown seeds which sank in water were considered mature and were selected for the sowing experiment. The seeds sown during 2015 failed to germinate. However, the seeds sown in 2016 germinated and established in 2017. Seedlings that recruited and survived were recorded in 2017. The data resulting from these experiments along with the information on seed production were used to estimate fecundity. Fecundity was calculated as the percentage of established seedlings from 100 seeds multiplied by the average number of viable seeds per individual in each population.

Laboratory study

We oven dried (at 60 °C for 72 h) all the air-dried plant materials and measured their biomass to an accuracy of 0.001 g. Similarly, we counted the number of seeds per fruit and per individual plant, and measured the seed mass (expressed as dry mass of 50 seeds) per individual. We tested seed viability by soaking the seeds in water, cutting them into halves and subsequently dipping them in a 10 percent solution of 2, 3, 5 Triphenyl Tetrazolium Chloride (TTC) for a minimum of 12 h in total darkness. We examined pink coloration of the embryo under a microscope, indicating viability of the seed (*Beigh, Nawchoo & Iqbal, 2006*).

STATISTICAL ANALYSIS

Population structure and density

Given the clustered sampling design with five sub-plots per plot, we analyzed the variation of *A. spicatum* density using a mixed-modeling approach including a random effect of plot. Over-dispersion of the count data was confirmed by using the qcc package (*Scrucca, 2004*), and the analysis of the counts for different stages was made using the glmmTMB package (*Mollie et al., 2017*) in R, version 3.5.3 (*R Development Core Team, 2017*).

Direct field observations confirmed the plant as rare and sparsely distributed at the study site. Hence, methods especially designed to cater for count data with excess zeros were considered from the project's early stages. Simple transformations, such as square root and log, were not capable of making our data appropriate for modeling using standard statistical assumptions of normality. The abundance of zero counts prevented these transformations from having the desired effect of symmetrizing the data and eliminating dependence between the mean and variance. Based on the Akaike Information Criterion (AIC), the best fit among several model alternatives was obtained using Mixed Zero-Inflated Poisson (mixed ZIP) models (*Hall, 2000*). Hence, we used mixed ZIP models to quantify the relationship between environmental factors and the abundance of plants at different stages of development using explanatory variables such as population (a given elevation range), relative radiation index (RRI), cover of shrubs, cover of herbs, harvesting, trampling, animal dropping and fire. All models included a random effect of the plot. We prepared twenty-six sets of candidate models using the glmmTMB package (see Appendix S1) and finally prepared an average model based on a set of 16 best candidate models (selected on the basis of delta AIC) using the MuMIn package (*Barton, 2018*) in R. The 16 models with AIC values less than 258.4 for seedling stage, 330.9 for juvenile stage, 583.3 for adult vegetative stage and 777.3 for adult reproductive stage were used to prepare the average model by MuMIn (see Appendix S2).

The full models were in all cases expressed as:

Density$_{ij}$ (of a particular stage of *A. spicatum*) = a + b (Population) + c_1 RRI$_{ij}$ + c_2 Herb cover$_{ij}$ + c_3 Shrub cover$_{ij}$ + c_4 Harvesting$_{ij}$ + c_5 Animal dropping$_{ij}$ + c_6 Trampling$_{ij}$ + c_7 Fire$_{ij}$ where, a, b(Population) and c_1 … c_7 are fixed model parameters, $i = 1 \ldots 66$ is the plot (included as a random effect) and j=1…5 is the sub-plot. Population had three categories (subalpine, lower alpine and alpine). Density was expressed as the number of individuals in a given stage category counted within a one m^2 sub-plot. A relative radiation index (RRI) was used as a proxy of microclimate at the site. RRI is a relative measure of the exposure to solar radiation at noon at a specific location and was calculated for each plot as a function of aspect, latitude, and slope. RRI was calculated as: RRI = $\cos(180°\text{-}\Omega) \times \sin(\beta) \times \sin(\Phi) + \cos(\beta) \times \cos(\Phi)$, where Ω = aspect (slope azimuth in degrees), Φ = latitude (degrees) and β = slope inclination (degrees) (*Oke, 1987*; *Vetaas, 1992*). The preliminary analysis showed that the shrub, litter, bryophyte and lichen cover were closely correlated. Similarly, herb and grass cover, and scree and bare ground cover were closely correlated. It was therefore decided to exclude litter, bryophyte, lichen, grass and scree cover in subsequent analyses.

Plant size and reproductive performance

Unlike plant density and structure, other life history traits were studied using sample individuals selected at the population level (see above). As the measurements were done on individual plants, we sampled 20 replicates for each of the size-based and reproductive trait variables. Before doing the analysis we examined whether data for each of these variables met the assumptions of parametric tests. Except for plant height and biomass, no other variables met the standard assumptions of normality and homogeneity of variance. Therefore, we applied Kruskal–Wallis tests to examine the variation of these traits among the three populations. In the case of plant height and biomass, logarithmic transformation of the data gave statistical normality, so in these cases we applied one-way ANOVA. A double logarithmic allometric model was prepared to describe the relationship between plant height and the biomass of tubers. We further performed multiple comparison tests after ANOVA and Kruskal Wallis to compare the significant differences among the populations. All tests were conducted using the R version 3.5.3 (*R Development Core Team, 2017*).

RESULTS

Population structure and density

We found significant differences in the densities of juvenile, and vegetative and reproductive adults among the three populations (Table 1). Similarly, we found differences in the observed stage distributions among them (Fig. 2). All the populations revealed lower proportions of plants in smaller size classes (seedlings and juveniles) than in larger size classes (vegetative and reproductive adults). The variation in density of *A. spicatum* across elevations was hump-shaped and density reached its maximum in the lower alpine population with a mean density of 2.48 ± 0.24 plants/m^2. The lowest density (0.56 ± 0.09 plants/m^2) was found in the subalpine population.

Table 1 **Population density (m^{-2}) for different life stages of *A. spicatum* in three populations in Annapurna Conservation Area, Central Nepal.** Densities are stated as mean ± SE. χ^2 and p-values were based on Kruskal–Wallis test, $df = 2$, $n = 66$. Values for each stage class among the populations with same superscript letter do not vary significantly at $p = 0.05$ level based on multiple comparison test after Kruskal Wallis.

Population	Seedling	Juvenile	Adult vegetative	Adult reproductive	Total
Subalpine (3,000–3,300 m)	0.05 ± 0.02	0.07 ± 0.03^{a}	0.10 ± 0.03^{a}	0.34 ± 0.06^{a}	0.56 ± 0.09
Lower alpine (3,400–3,700 m)	0.13 ± 0.03	0.30 ± 0.06^{b}	0.81 ± 0.12^{b}	1.24 ± 0.13^{b}	2.48 ± 0.24
Alpine (3,800–4,200 m)	0.2 ± 0.06	0.24 ± 0.13^{b}	0.78 ± 0.21^{b}	0.93 ± 0.17^{b}	2.16 ± 0.48
χ^2 value	5.18	10.33	34.79	34.17	45.23
p-value	0.08	<0.01	<0.0001	<0.0001	<0.0001

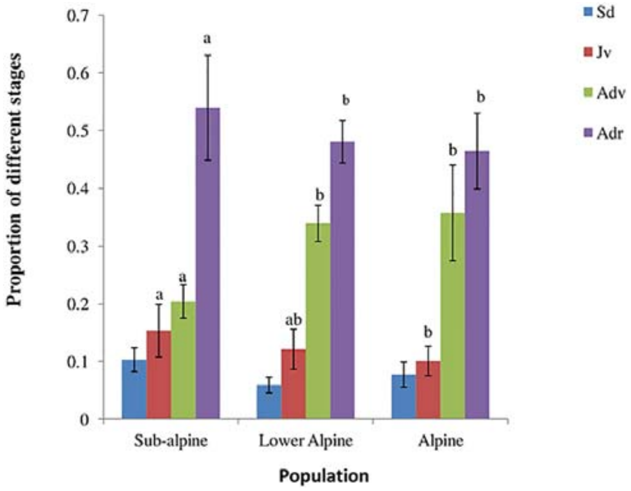


Figure 2 **Population structure of *A. spicatum* in Annapurna Conservation Area, Central Nepal (Sd, seedlings; Jv, juveniles; Adv, adult vegetative; Adr, adult reproductive).** Bars with same letter for each stage class among populations do not vary significantly at $p = 0.05$. Sd (ns).

Full-size DOI: 10.7717/peerj.7574/fig-2

Variation in plant size and reproductive performance

The plants showed the greatest vegetative vigor in terms of plant height and stem girth in the subalpine population (Table 2). Similarly, the subalpine population showed the highest total numbers of flowers and fruits per plant (Table 2).

The number of daughter tubers per individual tended to be higher in higher-elevation populations though the difference was not statistically significant (Table 2). The dry mass of daughter tubers, and that of below- and aboveground plant parts were significantly lower in the alpine than in the other populations (Table 2). In lower alpine and subalpine populations, the average biomass of belowground plant parts exceeded that of the aboveground parts. The dry biomass ratios for plant parts below and above ground, and for aerial vegetative and reproductive parts, varied significantly among the studied populations (Table 2). The dry biomass ratios for plant parts below and above ground were highest for plants from the subalpine population, and the ratio of dry biomass of

Table 2 Variation in life history traits (vegetative traits and reproductive outputs) of *A. spicatum* among three populations in Annapurna Conservation Area, Central Nepal. Values are mean ± SE ($n = 60$). *Population comparisons were based on either [1]Kruskal–Wallis tests or [2]one-way ANOVA. Values for each trait among the populations with same superscript letter do not vary significantly at $p = 0.05$ level based on multiple comparison tests after[1]Kruskal Wallis and [2]ANOVA.

Traits	Subalpine (3,000–3,300 m)	Lower alpine (3,400–3,700 m)	Alpine (3,800–4,200 m)	χ2/*F* value*	*P*-value
Leaf number[1]	12.05 ± 0.33^{a}	10.9 ± 0.46^{a}	6.35 ± 0.18^{b}	40.22	<0.001
Plant height (cm)[2]	156.43 ± 5.62^{a}	151.28 ± 5.29^{a}	81.77 ± 4.20^{b}	67.47	<0.001
Stem girth (mm)[1]	10.04 ± 0.95^{a}	9.60 ± 0.68^{a}	6.62 ± 0.25^{b}	12.51	0.001
Length of floral axis (cm)[1]	19.67 ± 0.86^{a}	19.13 ± 1.42^{a}	10.01 ± 0.60^{b}	33.18	<0.001
Average leaf area (cm^2)[1]	175.36 ± 14.21^{a}	190.98 ± 10.98^{a}	91.57 ± 4.95^{b}	31.26	<0.001
No. of buds per plant[1]	29.77 ± 10.03^{a}	9.14 ± 2.89^{ab}	6.00 ± 1.19^{b}	12.48	0.001
No. of flowers per plant[1]	15.85 ± 4.44	10.61 ± 2.03	6.20 ± 0.71	5.29	0.070
No. of fruits per plant[1]	22.15 ± 6.80^{a}	17.5 ± 4.56^{a}	4.12 ± 0.67^{b}	10.72	0.004
Total reproductive parts per plant[1]	39.85 ± 7.31	26.75 ± 5.07	16.45 ± 1.71	4.89	0.086
Number of daughter tubers per plant[1]	1.6 ± 0.19	1.85 ± 0.25	1.80 ± 0.27	0.45	0.799
Volume of daughter tuber (mm^3)[1]	272.66 ± 25.97^{a}	246.41 ± 41.94^{a}	109.67 ± 12.29^{b}	25.88	<0.001
Dry biomass of a daughter tuber (g)[2]	17.88 ± 2.83^{a}	12.18 ± 0.82^{a}	4.58 ± 0.55^{b}	32.58	<0.001
Total dry biomass of daughter tubers per plant (g)[2]	21.92 ± 3.47^{a}	16.09 ± 1.32^{a}	5.60 ± 0.86^{b}	27.02	<0.001
Total dry biomass of mother tubers per plant (g)[2]	8.04 ± 1.03^{a}	7.85 ± 0.79^{a}	1.87 ± 0.21^{b}	40.29	<0.001
Total belowground dry biomass (including daughter and mother tubers) per plant (g)[2]	29.97 ± 4.39^{a}	23.95 ± 1.83^{a}	7.86 ± 0.95^{b}	36.14	<0.001
Total above ground dry biomass per plant (g)[2]	$23.61 \pm 2.\ 90^{a}$	21.95 ± 1.46^{a}	9.95 ± 1.05^{b}	16.22	<0.001
Ratio of dry biomass of belowground and aboveground parts[2]	1.34 ± 0.12^{a}	1.13 ± 0.07^{a}	0.86 ± 0.07^{b}	7.00	<0.01
Ratio of dry biomass of reproductive and aerial vegetative parts[2]	0.21 ± 0.03^{a}	0.27 ± 0.04^{a}	0.46 ± 0.05^{b}	9.61	<0.001

reproductive and aerial vegetative parts was highest in the alpine population (Table 2). The double-logarithmic allometric model revealed a significant relationship between plant height and total dry mass of tubers (both daughter and mother tubers) ($P < 0.0001$, Fig. 3). Overall, compared to the subalpine and lower alpine populations, the alpine population was generally characterized by stunted growth, low biomass, fewer and smaller leaves and fewer and smaller reproductive structures.

The volume of fruits varied significantly among the populations ($\chi^2 = 51.58$, $P < 0.0001$) with highest mean value (19.19 ± 0.49 cm^3) for the lower alpine population and lowest mean (14.41 ± 0.33 cm^3) for the alpine population (Table 3). We found similar results for the number of seeds per fruit ($\chi^2 = 17.85$, $P < 0.0001$). The number of seeds per unit volume of fruit was found to vary significantly among the three populations with highest mean value for the alpine population ($\chi^2 = 7.74$, $P = 0.0208$). Similarly, we found higher seed mass per 50 seeds in the alpine than in the lower alpine and subalpine populations ($F = 5.69$, $P = 0.006$) (Table 3). Seed viability and fecundity were highest in the alpine and lowest in the subalpine population (Table 3). The seed viability showed statistically significant differences among the populations ($F = 10.69$, $P < 0.0001$).

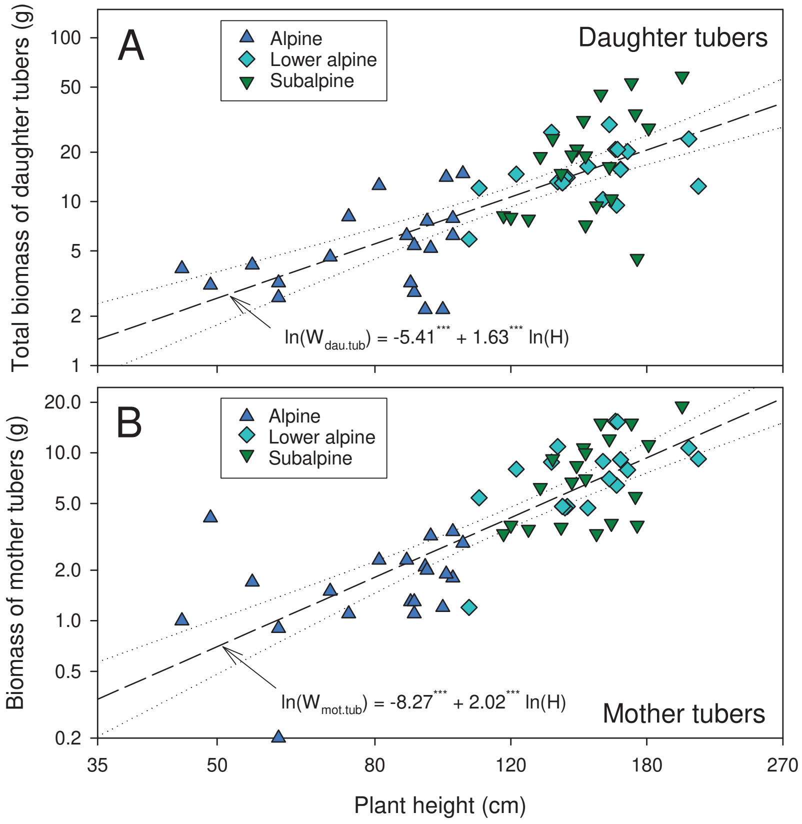


Figure 3 **Relationship between plant height and biomass of daughter tubers (A) and mother tubers (B) of *A. spicatum*.** Level of significance:***: $p < 0.0001$.

Full-size DOI: 10.7717/peerj.7574/fig-3

Table 3 **Variation in reproductive performance of *A. spicatum* among three populations in Annapurna Conservation Area, Central Nepal.** Values are mean ± SE ($n = 225$). *Population comparisons were based on either [1]Kruskal-Wallis tests or [2]one-way ANOVA. Values for each trait among the populations with same superscript letter do not vary significantly at $p = 0.05$ level based on multiple comparison tests after [1]Kruskal Wallis and [2]ANOVA.

Reproductive performance traits	Subalpine (3,000–3,300 m)	Lower alpine (3,400–3,700 m)	Alpine (3,800–4,200 m)	χ^2/F value*	*P*-value
Fresh weight of fruit and seed (g)[1]	0.45 ± 0.01^{a}	0.55 ± 0.02^{b}	0.57 ± 0.01^{b}	20.98	<0.0001
Dry weight of fruit and seed (g)[1]	0.08 ± 0.004^{a}	0.09 ± 0.004^{ab}	0.1 ± 0.003^{b}	9.64	0.008
Volume of fruit (cm^3)[1]	18.91 ± 0.75^{a}	19.19 ± 0.49^{a}	14.41 ± 0.33^{b}	51.58	<0.0001
No. of seeds per fruit[1]	37.21 ± 1.76^{a}	47.16 ± 1.95^{b}	36.63 ± 2.59^{a}	17.85	<0.0001
No. of seeds per unit volume of fruit[1]	2.08 ± 0.10^{a}	2.44 ± 0.07^{b}	2.60 ± 0.18^{ab}	7.74	0.0208
Dry weight of seeds per fruit (g)[1]	0.03 ± 0.002	0.03 ± 0.003	0.04 ± 0.003	1.34	0.511
Seed mass of 50 seeds (g)[2]	0.04 ± 0.004^{a}	0.04 ± 0.004^{a}	0.06 ± 0.002^{b}	5.69	0.006
Seed viability (%)[2]	16.07 ± 0.99^{a}	17.14 ± 1.05^{a}	22.5 ± 0.64^{b}	10.69	<0.0001
Fecundity	2.59	2.71	2.9		

Table 4 Summary of environmental variables (substrate and topographic) characterizing the three study populations of *A. spicatum* in Annapurna Conservation Area, Central Nepal. Estimates for substrate and topographic variables are specified as mean ± SE; for topographic variables ranges are also stated.

Substrate variables	**Subalpine (3,000–3,300 m)**	**Lower alpine (3,400–3,700 m)**	**Alpine (3,800–4,200 m)**
Shrub cover (%)	7.74 ± 1.23	11.28 ± 2.02	0.78 ± 0.51
Herb cover (%)	62.4 ± 2.51	60.2 ± 1.96	49.44 ± 3.21
Grass cover (%)	6.92 ± 1.04	8.43 ± 1.19	30.5 ± 2.89
Bryophyte cover on soil (%)	6.02 ± 0.41	7.96 ± 0.76	5.1 ± 0.46
Lichen cover on soil (%)	1.18 ± 0.10	0.89 ± 0.10	1.06 ± 0.11
Litter cover (%)	6.07 ± 0.45	11.45 ± 1.15	7.56 ± 0.80
Solid rock cover (%)	16.62 ± 2.59	7.65 ± 1.10	5.34 ± 1.15
Scree cover (%)	0.00 ± 0.00	0.63 ± 0.20	0.00 ± 0.00
Bare ground cover (%)	0.80 ± 0.28	2.78 ± 0.41	1.00 ± 0.27
Lichen cover under vascular plants (%)	1.05 ± 0.10	0.88 ± 0.13	1.04 ± 0.14
Bryophyte cover under vascular plants (%)	9.63 ± 0.74	11.72 ± 1.01	5.92 ± 0.65
Bryophyte cover on rock (%)	24.93 ± 3.34	17.92 ± 2.67	7.49 ± 1.66
Lichen cover on rock (%)	2.37 ± 0.50	1.06 ± 0.15	4.73 ± 1.18
Bare rock cover (%)	20.88 ± 3.15	41.86 ± 3.84	22.22 ± 3.70
Topographic variables			
Elevation (m a.s.l.)	3,204.16 ± 9.74 (3,000–3,300)	3,584.16 ± 11.15 (3,400–3,700)	3,968.66 ± 11.80 (3,800–4,200)
Slope (degrees)	24.5 ± 1.37 (3–55)	19.25 ± 1.03 (3–39)	11.94 ± 0.94 (3–40)
Relative radiation index (RRI)	0.85 ± 0.01 (0.71–0.99)	0.88 ± 0.01 (0.78–0.99)	0.85 ± 0.01 (0.59–0.96)

Response of density of different life stages to environmental variables (mixed ZIP models)

The three studied populations varied in substrate and topographic conditions (Table 4). The subalpine population was located in herb-dominated pasture with loamy soil. The lower alpine population was in a shrub dominated pasture with high bryophyte, litter and scree cover. It had sandy loam soil. The alpine population was found in a grass-dominated pasture with silty soil. The subalpine population was affected more by anthropogenic pressure compared to the other two populations (Fig. 4).

Mixed zero-inflated Poisson (mixed ZIP) regression models revealed that harvesting and herb cover had significant negative effects on seedling density. For juvenile density, harvesting had a significant negative effect. The other variables tested did not show significant effects on seedling and juvenile density. In the case of adult stages (both vegetative and reproductive adults) population showed a significant effect on density. The mixed ZIP model analysis further showed that, compared to the alpine population and taking cover and disturbance variables into account, the subalpine population exhibited a significantly lower density of vegetative and reproductive adult stages. Interestingly, animal droppings and fire had a significant positive effect on adult density (Table 5). The zero–inflation model component was significant for juvenile and adult reproductive stages but no covariates showed any significant effect on this component.

Table 5 **Mixed Zero-Inflated Poisson regression models for the density (m^{-2}) of seedling, juvenile, adult vegetative and adult reproductive stages of *A. spicatum*.** Densities of different stages were modeled as functions of population, relative radiation index (RRI), shrub cover (%), herb cover (%) and disturbance (animal droppings, trampling, harvesting and fire, which were assessed using an ordinal 0–4 scale). Zi is the intercept in the zero-inflation component; all other parameters refer to the count component of the model. Parameter estimates with standard errors in brackets for Full average and Conditional average models.

		ZI component	Count component									
	Average model	Zi	Intercept	Lower alpine	Subalpine	Shrub cover	Herb cover	RRI	Harvesting	Trampling	Animal droppings	Fire
Seedling	Full	−0.382	−1.703	−0.185	−0.303	0.003	−0.008	1.247	−0.955**	0.011	0.022	0.007
		(1.003)	(2.109)	(0.410)	(0.555)	(0.006)	(0.007)	(2.349)	(0.332)	(0.131)	(0.072)	(0.063)
	Conditional	−0.382	−1.703	−0.185	−0.303	0.010	−0.012*	3.491	−0.955**	0.053	0.185	0.081
		(1.003)	(2.109)	(0.411)	(0.555)	(0.008)	(0.006)	(2.759)	(0.332)	(0.281)	(0.116)	(0.191)
Juvenile	Full	0.941***	−0.131	0.083	−0.620	0.001	−0.000	0.248	−0.536*	−0.187	0.044	0.001
		(0.280)	(1.110)	(0.380)	(0.573)	(0.005)	(0.003)	(1.188)	(0.252)	(0.269)	(0.093)	(0.086)
	Conditional	0.941**	−0.131	0.083	−0.620	0.011	0.000	1.390	−0.561*	−0.369	0.182	0.007
		(0.280)	(1.109)	(0.380)	(0.573)	(0.009)	(0.005)	(2.510)	(0.229)	(0.276)	(0.102)	(0.189)
Adult	Full	0.121	0.206	0.152	−1.685**	−0.000	0.000	0.138	−0.097	−0.104	0.123	−0.029
Vegetative		(0.198)	(0.589)	(0.215)	(0.375)	(0.001)	(0.002)	(0.630)	(0.125)	(0.155)	(0.088)	(0.085)
	Conditional	0.121	0.206	0.152	−1.685**	−0.002	0.003	1.749	−0.173	−0.183	0.165**	−0.047
		(0.198)	(0.589)	(0.215)	(0.375)	(0.007)	(0.003)	(1.478)	(0.121)	(0.167)	(0.060)	(0.104)
Adult	Full	−1.007**	0.035	0.171	−0.826**	0.000	−0.000	0.044	−0.025	−0.046	0.107	0.178*
reproductive		(0.312)	(0.329)	(0.181)	(0.235)	(0.00)	(0.000)	(0.334)	(0.059)	(0.096)	(0.064)	(0.082)
	Conditional	−1.007**	−0.035	0.171	−0.826***	0.004	−0.000	1.485	−0.088	−0.099	0.124*	0.186*
		(0.312)	(0.329)	(0.181)	(0.235)	(0.004)	(0.002)	(1.255)	(0.083)	(0.121)	(0.052)	(0.074)

Notes.
Levels of significance: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

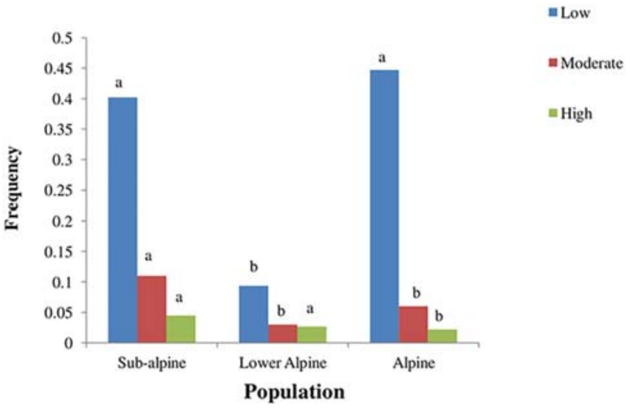


Figure 4 **Frequency of different levels of anthropogenic disturbances in three populations of *A. spicatum* in Annapurna Conservation Area, Central Nepal.** Mean of animal droppings, trampling, harvesting and fire intensity. Scale: 0–1 = low, 2 = moderate, 3–4 = high. Bars with same letter for each disturbance class among populations do not vary significantly at $p = 0.05$ level based on multiple comparison test after Kruskal Wallis.

Full-size DOI: 10.7717/peerj.7574/fig-4

DISCUSSION

Variation in population density and structure

The mean density of *A. spicatum* in the populations examined is comparable with previous findings. For example, densities of 0.34 (*Kala, 2005*) to 3.71 (*Nautiyal et al., 2002*) plants/m^2 in *A. balfourii*, 2.57 (*Nautiyal et al., 2002*) to 3.1 (*Semwal et al., 2007*) plants/m^2 in *A. heterophyllum*, 2.7 plants/m^2 in *A. rotundifolium* (*Kala, 2005*), and 2 plants/m^2 in *A. violaceum* (*Kala, 2000*; *Kala, 2005*) have been reported from the Indian Himalayas. However, much higher densities have also occasionally been observed. For example, *Nautiyal et al. (2002)* recorded a density of 6.7 plants/m^2 in *A. violaceum* in Gharwal Himalaya, India, which was attributed to limited human exploitation due to habitat inaccessibility. Similarly, *Shrestha & Jha (2010)* reported a mean density of 7.3 plants/m^2 in *A. naviculare* in Manang, Nepal, which was described as a result of subjective sampling due to the patchy distribution of the species.

In the high mountain ecosystem, elevation represents a complex gradient closely correlated with numerous other environmental variables, such as temperature, precipitation, soil texture and nutrients, substrate stability, and disturbance (*Auerbach & Shmida, 1993*; *Ramasay & Oxley, 1997*). All these factors affect plant life in a number of ways. Our mixed ZIP model showed that the subalpine population exhibited a significantly lower density of adult vegetative and adult reproductive plants than did the lower alpine and alpine populations. The higher plant density in lower alpine and alpine populations might partly be due to lower human pressure (Fig. 4) in these habitats. In addition, the smaller size of individuals in higher-elevation populations also makes it possible for a higher number of individuals to share a given amount of space. With increasing elevation the number of competing species of similar or larger size decreases. This causes a small area to hold a large number of individuals, thus increasing the density. The shift from an environment characterized by high degree of competition to a more facilitative environment favors this higher density (*Liancourt et al., 2017*). *Bosch & Waser (1999)* found that the pollinator visitation is higher in dense populations of plants than in sparse populations, which may further increase seed set and ultimately the population density.

The low proportions of younger life stages (seedlings and juveniles) and correspondingly high proportion of adults (vegetative and reproductive) in the studied populations clearly indicate that the recruitment potential of *A. spicatum* is generally rather low. The recruitment was further reduced in the subalpine population. The small seed size, and low seed viability and fecundity recorded in the subalpine population compared to those of the lower alpine and alpine populations support that the subalpine population is suffering from lack of sufficient natural regeneration, consequently lowering the population density. Studies have shown that disturbance characteristics determine the success of different reproductive strategies (*Chambers, 1995*). Anthropogenic disturbance, like harvesting and trampling can reduce seed viability and fecundity in plants from mountain ecosystems (*Tonnabel et al., 2017*) and thereby reduce the population density.

Response of density of different life stages to environmental variables

The mixed ZIP model showed significant negative impact of harvesting on both seedling and juvenile plant density, and population (elevation) had a significant negative effect on adult density. Based on conversations with collectors and key informants, it appears that traditional healers prefer to harvest *A. spicatum* at younger stages, believing that relatively young tubers have high medicinal efficacy. However, commercial collectors prefer mostly the large adult plants. Removal of the entire plant before seed maturation reduces the possibility of seed development for future regeneration (*Sheldon, Balik & Laird, 1997*). The negative effects of such damages caused by collectors may be further enhanced by infrequent sexual regeneration and possibly also by limited availability of suitable sites for regeneration in subalpine and alpine grasslands (*Austrheim & Eriksson, 2001*). The mixed ZIP model analysis further showed that herb cover had a significant negative impact (at the 10% level) on the density of seedlings, which may be a consequence of herb cover reducing the space available for seedling establishment, increasing the competition and thereby affecting growth and development.

Although frequent trampling by sheep, wild animals and humans damage plants and prevents them from flowering, developing seeds to maturity, and dispersing their seeds (*Chardon et al., 2018*), and seedlings of *A. spicatum* may be particularly sensitive in this regard, as they have soft stems with only one to two leaves, we found positive effects of some disturbances on density of adults. Mixed ZIP model revealed positive significance of animal droppings and fire on adult (both vegetative and reproductive) density. Direct observation also support the result as the *A. spicatum* individuals seemed to show preference for growing in the proximity of cattle sheds where the soil had higher concentration of animal droppings. *Ohlson & Grønli (2006)* also reported a significant positive effect on life history parameters in *A. septentrionale* growing in experimental plot supplied with higher concentrations of nutrients. The tolerance of adult stages of *A. spicatum* to fire in the present study is comparable with the findings of *Uys, Bond & Everson (2004)* who showed the tolerance of many forb species to fire.

Variation in plant size and reproductive performance

A. spicatum has developed an astonishing range of life history traits in response to highly specific ecological environments in subalpine, lower alpine and alpine habitats. A high degree of variation with respect to different traits was found among the populations (Table 2). The decreasing trend of plant height, stem girth, leaf area, length of floral axis and plant biomass observed in plants from subalpine to alpine populations (Table 2) showed that the size of individuals generally tends to decrease with increasing elevation. *A. spicatum* in the subalpine (lower elevation) population exhibited better performance in terms of high vegetative vigor and production of higher number of flowers and fruits per individual. *Shrestha & Jha (2010)*, studying *A. naviculare,* also reported higher vigor for the individuals examined in a lower elevation population. Plants may perform better simply because of favorable abiotic conditions (*Billings, 1973*; *Körner, 2003*), such as temperature and moisture (*Körner, 2003*). Plant performance is also enhanced in habitats with better abiotic conditions through facilitation (positive interactions), a common phenomenon at high altitudes, which helps plants to cope with the harsh environment (*Callaway et al., 2002*). Thus, the higher vegetative vigor of *A. spicatum* observed at lower elevation is presumably related to the presence of appropriate abiotic conditions in which plants exhibit enhanced growth (*Shrestha & Jha, 2010*). The harsh environmental conditions at higher elevation generally impose constraints on plant growth (*Körner, 2003*). Similar trends of decreasing plant vigor with increasing elevation have been observed in other herbs, such as *Silene acaulis*, in which disturbance has been described as beneficial for the performance of plants at lower elevation (*Chardon et al., 2018*).

The decreasing number of flowers and fruits set that we observed along the elevation gradient is likely related to plant size but could also be due to the time of flowering, which is influenced by the ambient temperature and the timing of snow melt (*Kudo & Hirao, 2006*). Flower production is often correlated with plant size, which generally increases with resource availability and decreases with increasing plant density (*Weiner & Thomas, 1986*). Moreover, flower and fruit production are shaped by the interactions between abiotic and biotic environment (*Ma et al., 2010*; *Agren, Ehrlén & Solbreck, 2008*). For example, population density negatively affects fruit production of individual plants through its effect on flower number (*Agren, Ehrlén & Solbreck, 2008*). Disturbances like trampling frequently break the inflorescence, and repeated disturbances year after year also reduce the reproductive potential of the individuals, thus limiting the production of flowers and fruits (*Chardon et al., 2018*).

Like many high-elevation plants (e.g., *Arenaria kansuensis, Rhodiola quadrifida, Tribulus terrestris* etc.), *A. spicatum* also allocated a large amount of biomass to its underground structure and, as a result, aboveground reproductive organs are reduced and roots are enlarged (*Ma et al., 2010*). A high proportion of belowground biomass has often been interpreted as an adaptive response to severe environmental conditions (*Webber & May, 1977*). Particularly for high-elevation plants, subterranean organs are dedicated to produce annual aerial parts shortly after snow melt and, thus, high elevation plants are found to invest more in belowground parts as an adaptive strategy.

Despite the lower number of flowers and fruits set per individual in alpine populations, in terms of reproductive allocation, these plants performed far better than subalpine and lower alpine populations. The ratio of dry biomass of reproductive to aerial vegetative parts was higher in plants from the alpine population than in plants from subalpine and lower alpine populations, and this clearly indicates that plants from very high elevation invest more in reproduction. The subalpine population had larger fruits than the two other populations and, as a result, they produced higher number of seeds per fruit. Contrary to this, though producing the smallest number of fruits, the plants from the alpine population, compared to the two other populations from lower elevation, set higher number of seeds per unit volume of fruits, and these were seeds with a higher seed mass. Seed production by species from high elevations depend on environmental conditions (short, cool growing seasons) and is temporally and spatially variable (*Chambers, 1995*). The increasing seed mass with increasing elevation can be explained by more resources being allocated to the individual seed in alpine populations, thereby increasing their viability and fecundity to cope with a stressful environment. The increase in seed mass together with a reduced number of seeds per individual in populations from higher elevations may indicate that *A. spicatum* allocates more resources to the seeds and may reflect a strategy that helps increasing fitness and long-term population persistence in harsh alpine environments.

CONCLUSION

Human disturbance and topographical factors are related to plot-level microclimatic conditions, which contribute to the variation in density and the studied life history traits. Among the environmental variables tested, harvesting, animal droppings and fire appeared to be the most important (proxy) factors affecting the density of *A. spicatum*. The population at the lowest elevation was exposed to the highest anthropogenic pressure, the level of which decreased with increasing elevation. The prevailing harvesting pattern was destructive as it involved uprooting of the whole plant and was probably a main reason for the lower recruitment and reduced density in the subalpine population. The enhanced vegetative performance of adults and the higher number of flowers and fruits set per reproductive individual in the subalpine population cannot compensate for the lower recruitment and loss of younger plants due to high disturbance. The lower recruitment in the subalpine population was presumably also related to reduced seed mass, and decreased seed viability. In terms of reproductive effort (higher seed set per unit fruit volume, higher seed mass, and higher seed viability and fecundity), the alpine populations were performing better. This confirms that alpine plants develop self-sustaining adaptive strategies by increasing their resource allocation in reproductive parts, especially in seeds, to cope with the harsh environment. As the subalpine population was suffering from reduced reproductive performance, the persistence and growth of *A. spicatum* at low elevations will depend on how effectively management will address the protection of younger life stages and control over-harvesting and premature harvesting of tubers. Although the adult individuals were found tolerant to animal droppings and fire, the viability of *A. spicatum* populations not only depends on controlling over harvesting and premature harvesting but also on mitigating other common anthropogenic disturbances, like grazing and trampling, to ensure completion of flowering, fruiting and dispersal of viable seeds. We therefore recommend that when formulating management guidelines, measures aiming to mitigate such anthropogenic disturbances should be considered. As science-based harvesting and conservation strategies have not yet been developed for *A. spicatum* and other Himalayan MAPs, and harvest and trade are conducted on ad hoc basis, we hope that this study will contribute to the conservation of MAPs by helping government authorities to formulate scientifically informed conservation management strategies for *A. spicatum* and other similar MAPs.

ACKNOWLEDGEMENTS

We thank the officials of Annapurna Conservation Area Project for logistic support. We are highly indebted to the local people who helped us in establishing the sample plots. We thank Shanta Buda Magar for her assistance during the field work. We would like to thank Department of Survey, Government of Nepal for the land cover map of the study area. We are highly thankful to Naresh Subedi and Robbie Hart for their critical comments on an earlier version of the manuscript. We thank the two anonymous reviewers for their careful and constructive comments on the manuscript.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

The study was supported by the Danish Ministry of Foreign Affairs, Grant No. 13-07KU, Rufford Small Grant (RSG-19960-1), and USAID-funded Hariyo Ban Program of WWF Nepal (EN02). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
Danish Ministry of Foreign Affairs: 13-07KU.
Rufford Small Grant: RSG-19960-1.
USAID-funded Hariyo Ban Program of WWF Nepal: EN02.

Competing Interests

The authors declare that there are no competing interests.

Author Contributions

- Deep J. Chapagain conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft, collected the data and ran the data digitalization and curation.

- Henrik Meilby analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Suresh K. Ghimire conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

Field works were approved by the Department of National Park and Wildlife Conservation, Nepal (ref. No. 93/070/071).

Data Availability

The following information was supplied regarding data availability:

Raw data are available in Datasets S1–S3.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.7574#supplemental-information.

REFERENCES

Agren J, Ehrlén J, Solbreck C. 2008. Spatio-temporal variation in fruit production and seed predation in a perennial herb influenced by habitat quality and population size. *Journal of Ecology* **96**:334–345 DOI 10.1111/j.1365-2745.2007.01334.x.

Auerbach M, Shmida A. 1993. Vegetation change along an altitudinal gradient on Mt Hermon, Israel–No evidence for discrete communities. *Journal of Ecology* **81**:25–33 DOI 10.2307/2261221.

Austrheim G, Eriksson O. 2001. Plant species diversity and grazing in the Scandinavian mountains- patterns and processes at different spatial scales. *Ecography* **24**:683–695 DOI 10.1034/j.1600-0587.2001.240607.x.

Barton K. 2018. MuMIn: multi-model inference. R package version 1.42.1. Vienna: The R Foundation for Statistical Computing. *Available at https://CRAN.R-project.org/package=MuMIn*.

Beigh SY, Nawchoo IA, Iqbal M. 2006. Cultivation and conservation of *Aconitum heterophyllum*: a critically endangered medicinal herb of the Northwest Himalayas. *Journal of Herbs, Spices & Medicinal Plants* **11**:47–56 DOI 10.1300/J044v11n04_06.

Bhattarai N, Tandon V, Ved DK. 2002. Highlights and outcomes of the Conservation Assessment and Management Planning (CAMP) Workshop, Pokhara, Nepal. In: Bhattarai N, Karki M, eds. *Sharing local and national experience in conservation of medicinal and aromatic plants in South Asia. Proceedings of the Regional Workshop held at Pokhara, Nepal, 21-23 January 2001*. New Delhi, India: Medicinal and Aromatic Plants Program in Asia (MAPPA), International Development Research Centre (IDRC), South Asian Regional Office, 46–53.

Billings WD. 1973. Arctic and alpine vegetations: similarities, differences, and susceptibility to disturbance. *BioScience* **23**:697–704 DOI 10.2307/1296827.

Biønness IM. 1980. Animal husbandary and grazing, a conservation and management problem in Sagarmatha (Mt. Everest), National Park, Nepal. *Norsk Geografisk Tidsskrift* **34**:59–76 DOI 10.1080/00291958008621916.

Bosch M, Waser NM. 1999. Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* (Ranunculaceae). *American Journal of Botany* **86**:871–879 DOI 10.2307/2656707.

Bresson CC, Vitasse Y, Kremer A, Delzon S. 2011. To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech? *Tree Physiology* **00**:1–11 DOI 10.1093/treephys/tpr084.

Bucini G, Lambin EF. 2002. Fire impacts on vegetation in Central Africa: a remote-sensing-based statistical analysis. *Applied Geography* **22**:27–48 DOI 10.1016/S0143-6228(01)00020-0.

Callaway RM, Brooker RW, Choler P, Kikvidze Z, Lortie CJ, Michalet R, Paolini L, Pugnaire FI, Newingham B, Aschehoug ET, Armas C, Kikodze D, Cook BJ. 2002. Positive interactions among alpine plants increase with stress. *Nature* **417**:844–848 DOI 10.1038/nature00812.

Caswell H. 2001. *Matrix population models: construction, analysis and interpretation*. Sunderland: Sinauer Associates.

Chambers JC. 1995. Disturbance, life history strategies, and seed fates in alpine herbfield communities. *American Journal of Botany* **82**:421–433 DOI 10.1002/j.1537-2197.1995.tb12647.x.

Chambers JC, MacMahon JA, Brown RW. 1990. Alpine seedling establishment: the influence of disturbance type. *Ecology* **71**:1323–1341 DOI 10.2307/1938270.

Chardon NI, Wipf S, Rixen C, Beilstein A, Doak DF. 2018. Local trampling disturbance effects on alpine plant populations and communities: negative implications for climate change vulnerability. *Ecology & Evolution* **8**:7921–7935 DOI 10.1002/ece3.4276.

Department of Plant Resources. 2012. *Plants of Nepal: fact sheet*. Kathmandu, Nepal: Department of Plant Resources (DPR), Ministry of Forest and Soil Conservation, Government of Nepal.

Fan DM, Yang YP. 2009. Altitudinal variations in flower and bulbil production of an alpine perennial, *Polygonum viviparum* L. (Polygonaceae). *Plant Biology* **11**:493–497 DOI 10.1111/j.1438-8677.2008.00188.x.

Franco M, Silvertown J. 1996. Life history variation in plants: an exploration of the fast-slow continuum hypothesis. *Philosophical Transactions: Biological Sciences* **351**:1341–1348 DOI 10.1098/rstb.1996.0117.

Garbarino M, Lingua E, Marzano R, Urbinati C, Bhuju D, Carrer M. 2014. Human interactions with forest landscape in the Khumbu valley, Nepal. *Anthropocene* **6**:39–47 DOI 10.1016/j.ancene.2014.05.004.

Ghimire SK, Awasthi B, Rana S, Rana HK, Bhattarai R, Pyakurel D. 2016. Export of medicinal and aromatic plant materials from Nepal. *Botanica Orientalis—Journal of Plant Science* **10**:24–32 DOI 10.3126/botor.v10i0.21020.

Ghimire SK, McKey D, Aumeeruddy-Thomas Y. 2005. Conservation of Himalayan medicinal plants: harvesting patterns and ecology of two threatened species, *Nardostachys grandiflora* D.C. and *Neopicrorhiza scrophulariiflora* (Pennell) Hong. *Biological Conservation* **124**:463–475 DOI 10.1016/j.biocon.2005.02.005.

Ghimire SK, Sah JP, Shrestha KK, Bajracharya D. 1999. Ecological study of some high altitude medicinal and aromatic plants in the Gyasumdo valley, Manang, Nepal. *Ecoprint* **6**:17–25.

Hall DB. 2000. Zero-inflated poisson and binomial regression with random effects: a case study. *Biometrics* **56**:1030–1039 DOI 10.1111/j.0006-341X.2000.01030.x.

Hobbs RJ, Mallik AU, Gimingham CH. 1984. Studies on fire in Scottish heathland communities: III. Vital attributes of the species. *Journal of Ecology* **72**:963–976 DOI 10.2307/2259544.

Kala CP. 2000. Status and conservation of rare and endangered medicinal plants in the Indian trans-Himalaya. *Biological Conservation* **93**:371–379 DOI 10.1016/S0006-3207(99)00128-7.

Kala CP. 2005. Indigenous uses, population density and conservation of threatened medicinal plants in protected areas of Indian Himalayas. *Conservation Biology* **19**:368–378 DOI 10.1111/j.1523-1739.2005.00602.x.

Körner C. 1999. Alpine plants: stressed or Adapted? In: Press MC, Scholes JD, Barker MG, eds. *Physiological plant ecology*. Oxford: Blackwell Science Ltd 297–311.

Körner C. 2003. *Alpine plant life*. 2nd edition. Berlin: Springer-Verlag.

Kudo G, Hirao AS. 2006. Habitat-specific responses in flowering phenology and seed set of alpine plants for climate variation: implications for global- change impacts. *Population Ecology* **48**:49–58 DOI 10.1007/s10144-005-0242-z.

Lama YC, Ghimire SK, Aumeeruddy-Thomas Y. 2001. *Medicinal plants of Dolpo: Amchis' knowledge and conservation*. Kathmandu: WWF Nepal.

Liancourt P, Pinguet YLB, Rixen C, Dolezal J. 2017. SGH: stress or strain gradient hypothesis? Insights from an elevation gradient on the roof of the world. *Annals of Botany* **120**:29–38 DOI 10.1093/aob/mcx037.

Ma WL, Shi PL, Li WH, He YT, Zhang XZ, Shen ZX, Chai SY. 2010. Changes in individual plant traits and biomass allocation in alpine meadow with elevation variation on the Qinghai-Tibetan Plateau. *Science China Life Sciences* **53**:1142–1151 DOI 10.1007/s11427-010-4054-9.

McKechnie IM, Sargent RD. 2013. Do Plants traits influence a species' response to habitat disturbance? *A Meta-Analysis Biological Conservation* **168**:69–77.

Molau U. 1993. Relationships between flowering phenology and life history strategies in tundra plants. *Arctic and Alpine Research* **25**:391–402 DOI 10.2307/1551922.

Mollie EB, Kasper Kristensen K, Koen JVB, Arni M, Casper WB, Anders N, Hans JS, Martin M, Benjamin MB. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal* **9**:378–400 DOI 10.32614/RJ-2017-066.

Nautiyal BP, Prakash V, Bahuguna R, Maithani U, Bisht H, Nautiyal MC. 2002. Population study for monitoring the status of rarity of three Aconite species in Garhwal Himalaya. *Tropical Ecology* **43**:297–303.

Government of Nepal. 2015. Notice of ministry of forests and soil conservation. *Nepal Gazette* **65**:26–3.

Government of Nepal. 2018. Notice of ministry of forests and environment. *Nepal Gazette* **68**:34–3.

Nielsen A, Totland Ø, Ohlson M. 2007. The effect of forest management operations on population performance of *Vaccinium myrtillus* on a landscape-scale. *Basic and Applied Ecology* **8**:231–241 DOI 10.1016/j.baae.2006.05.009.

Ohlson M, Grønli KE. 2006. Recruitment and growth in *Aconitum septentrionale* and *Actaea spicata* in relation to microbial soil communities manipulated by additions of glucose and nutrients. *Flora* **201**:215–226 DOI 10.1016/j.flora.2005.08.002.

Oke TR. 1987. *Boundary layer climates.* New York: Methuen & Co. Ltd.

Olsen CS, Larsen HO. 2003. Alpine medicinal plant trade and Himalayan mountain livelihood strategies. *The Geographical Journal* **169**:243–254 DOI 10.1111/1475-4959.00088.

Pauli H, Gottfried M, Lamprecht A, Niessner S, Rumpf S, Winkler M, Steinbauer K, Grabherr G. 2015. *The GLORIA field manual—standard multi-summit approach, supplementary methods and extra approaches.* Vienna: GLORIA-Coordination, Austrian Academy of Sciences and University of Natural resources and Life Sciences.

R Development Core Team. 2017. A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. *Available at http://www.R-project.org/*.

Ramasay PM, Oxley ERB. 1997. The growth form composition of plant communities in the ecuadorian p'aramos. *Plant Ecology* **131**:173–192 DOI 10.1023/A:1009796224479.

Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio PO. 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions: Biological Sciences* **365**:4051–4063 DOI 10.1098/rstb.2010.0208.

Renison D, Chartier MP, Menghi M, Marcora PI, Torres RC, Giorgis M, Hensen I, Cingolani AM. 2015. Spatial variation in tree demography associated to domestic herbivores and topography: insights from a seeding and planting experiment. *Forest Ecology and Management* **335**:139–146 DOI 10.1016/j.foreco.2014.09.036.

Roff DA. 1992. *The evolution of life histories: theory and analysis.* London: Chapman & Hall.

Salick J, Ghimire SK, Fang Z, Dema S, Konchar KM. 2014. Himalayan Alpine vegetation, climate change and mitigation. *Journal of Ethnobiology* **34**:276–293 DOI 10.2993/0278-0771-34.3.276.

Scrucca L. 2004. Qcc: an R package for quality control charting and statistical process control. *R News* **4**:11–17.

Semwal DP, Saradhi PP, Nautiyal BP, Bhatt AB. 2007. Current status, distribution and conservation of rare and endangered medicinal plants of Kedarnath Wildlife Sanctuary, Central Himalayas, India. *Current Science* **92**:1733–1738.

Sheldon JW, Balik MJ, Laird SA. 1997. Medicinal plants: can utilization and conservation co exist? *Advances in Economic Botany* **12**:1–104.

Shrestha BB, Jha PK. 2010. Life history and population status of endemic Himalayan *Aconitum naviulare*. *Mountain Research & Development* **30**:353–364 DOI 10.1659/MRD-JOURNAL-D-10-00003.1.

Srivastava N, Sharma V, Kamal B, Jadon VS. 2010. *Aconitum*: need for sustainable exploitation (with special reference to Uttarakhand). *International Journal of Green Pharmacy* **4**:220–228 DOI 10.4103/0973-8258.74129.

Stearns SC, Hoekstra RF. 2000. *Evolution: an introduction*. Oxford: Oxford University Press, 381 pp.

Stevens S. 2003. Tourism and deforestation in the Mt Everest region of Nepal. *The Geographical Journal* **169**:255–277 DOI 10.1111/1475-4959.00089.

Ticktin T. 2004. The ecological implications of harvesting non–timber forest products. *Journal of Applied Ecology* **41**:11–21 DOI 10.1111/j.1365-2664.2004.00859.x.

Tonnabel J, Schurr FM, Boucher F, Thuiller W, Renaud J, Douzery EJP, Ronce O. 2017. Life-history traits evolved jointly with climatic niche and disturbance regime in the genus *Leucadendron* (Proteaceae). *The American Naturalist* **191**:220–234.

Totland Ø, Nyléhn J. 1998. Assessment of the effects of environmental change on the performance and density of *Bistorta vivipara*: the use of multivariate analysis and experimental manipulation. *Journal of Ecology* **86**:989–998 DOI 10.1046/j.1365-2745.1998.00318.x.

Tsarong TJ. 1994. *Tibetan medicinal plants*. New Delhi: Tibetan Medical Publications.

Uys RG, Bond WJ, Everson TM. 2004. The effect of different fire regimes on plant diversity in Southern African grasslands. *Biological Conservation* **118**:489–499 DOI 10.1016/j.biocon.2003.09.024.

Vetaas OR. 1992. Gradients in field-layer vegetation on an arid misty mountain plateau in the Sudan. *Journal of Vegetation Science* **3**:527–534 DOI 10.2307/3235809.

Webber PJ, May DE. 1977. The magnitude and distribution of belowground plant structures in the alpine tundra of Niwot Ridge, Colorado. *Arctic & Alpine Research* **9**:157–174 DOI 10.2307/1550578.

Weiner J, Thomas SC. 1986. Size variability and competition in plant monocultures oikos. *Oikos* **47**:211–222.

Zhang A, Cornwell W, Li Z, Xiong G, Fan D, Xie D. 2019. Strong restrictions on the trait range of co-occurring species in the newly created riparian zone of the three gorges reservoir area, China. *Journal of Plant Ecology* rtz016 DOI 10.1093/jpe/rtz016.

Received: 28 September 2020 | Revised: 12 March 2021 | Accepted: 19 March 2021

DOI: 10.1002/ece3.7520

ORIGINAL RESEARCH

Ecology and Evolution Open Access WILEY

Illegal harvesting and livestock grazing threaten the endangered orchid *Dactylorhiza hatagirea* (D. Don) Soó in Nepalese Himalaya

Deep Jyoti Chapagain[1,2] | Henrik Meilby[2] | Chitra Bahadur Baniya[1] | Shanta Budha-Magar[3] | Suresh Kumar Ghimire[1]

[1]Central Department of Botany, Tribhuvan University, Kirtipur, Nepal

[2]Department of Food and Resource Economics (IFRO), University of Copenhagen, Copenhagen, Denmark

[3]School of Science, Auckland University of Technology, Auckland, New Zealand

Correspondence
Deep Jyoti Chapagain, Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.
Email: chapagaindeep@gmail.com

Funding information
Danish Ministry of Foreign Affairs, Grant/Award Number: 13-07KU; Rufford Small Grants Foundation, Grant/Award Number: 19960-1

Abstract

Harvesting of orchids for medicine and salep production is a traditional practice, and increasing market demand is spurring illegal harvest. Ethno-ecological studies in combination with the effect of anthropogenic disturbance are lacking for orchids. We compared population density and structure, and tuber biomass of *Dactylorhiza hatagirea* (D. Don) Soó for three years in two sites: Manang, where harvesting of medicinal plants was locally regulated (protected), and Darchula, where harvesting was locally unregulated (unprotected). Six populations were studied along an elevation gradient by establishing 144 temporary plots (3×3 m^2) from 3,400 to 4,600 m elevations. Mean density of *D. hatagirea* was significantly higher in the locally protected (1.31 ± 0.17 plants/m^2) than in the unprotected (0.72 ± 0.06 plants/m^2) site. The protected site showed stable population density with high reproductive fitness and tuber biomass over the three-year period. A significant negative effect ($p < .1$) of relative radiation index (RRI) on the density of the adult vegetative stage and a positive effect of herb cover on juvenile and adult vegetative stages were found using mixed zero-inflated Poisson (mixed ZIP) models. The densities of different life stages were highly sensitive to harvesting and livestock grazing. Significant interactions between site and harvesting and grazing indicated particularly strong negative effects of these disturbances on densities of juvenile and adult reproductive stages in the unprotected site. Semi-structured interviews were conducted with informants ($n = 186$) in the villages and at the ecological survey sites. Our interview results showed that at the protected site people are aware of the conservation status and maintain sustainable populations, whereas the opposite was the case at the unprotected site where the populations are threatened. Sustainability of *D. hatagirea* populations, therefore, largely depends on controlling illegal and premature harvesting and unregulated livestock grazing, thus indicating the need for permanent monitoring of the species.

KEYWORDS

anthropogenic disturbances, interview survey, orchid, population density, population monitoring, salep, sustainability

1 | INTRODUCTION

Globally, 100 to 1,000 species per million become extinct every year, mostly due to anthropogenic habitat deterioration and fragmentation, land use change, urbanization, atmospheric nitrogen deposition, and climate change. Consequently, a large number of species, including many plants, are categorized as vulnerable or threatened (Di Marco et al., 2018; Jacquemyn et al., 2005; Kull & Hutchings, 2006; Pimm et al., 2014). The orchids typify the problem faced by many medicinal and aromatic plant species (MAPs). Orchids, with their complex biology (Rasmussen, 1995; Van der Cingel, 1995), tend to have small and isolated populations and exhibit high sensitivity to environmental changes (Vakhrameeva et al., 2008), and are at greater risk of extinction than most other plant groups (IUCN, 2020; Kull & Hutchings, 2006; Warghat et al., 2013). Human-mediated disturbances, mainly harvesting and habitat destruction, fragmentation, or loss of habitats are the most significant threats to the survival of orchid populations (Ehrlich, 1988; Laurance & Bierregaard, 1997; Saunder et al., 1991; Zhang et al., 2019). Also, increasing human activities may create novel environments (Zhang et al., 2019) which limit the persistence of orchid populations. Human disturbances may result in the breakdown of ecological connections between orchids and their pollinators and mycorrhiza, changes in edaphic and microclimatic conditions, and introduction of pests and diseases (Fay et al., 2015; Light et al., 2003). Disturbances may interrupt interspecific interactions leading to reduced reproductive output and eventually altering the plant demographic dynamics (Steffan-Dewenter et al., 2006). Studies have shown that, generally, the magnitude of disturbance impacts on plant populations depends on plant life stage and features of their reproductive system (Calvo, 1990). In the case of orchid populations, the severity of disturbance impacts also depends on the level of specificity of plant–animal interactions (e.g., interactions with pollinators) and the availability of sites suitable for seedling recruitment (Schulze et al., 2019).

The distribution and abundance of orchid populations depend on a suite of biological and ecological factors including seed production and dispersal, recruitment, availability of mycorrhizal fungi, and appropriate environmental conditions (McCormick & Jacquemyn, 2014). However, in case of the smallest orchid populations the seed output may be insufficient to ensure their long-term persistence (Faast et al., 2011). The life stage dynamics of orchid populations further depends on the elevation of their habitat. Environmental conditions and interactions associated with altitude play a significant role in the composition and distribution of orchid populations (Djordjević & Tsiftsis, 2020; Djordjević et al., 2016, 2020; Jacquemyn et al., 2005). Alpine and subalpine grasslands suffer from reduced nutrient availability and harsh environmental conditions, and plants growing in these habitats presumably develop adaptive coping strategies (Chapagain et al., 2019). Disturbance regimes, such as harvesting, grazing, trampling, and fire, also play influential positive (Chen et al., 2014; Dai et al., 2019) or negative (Chapagain et al., 2019; Kreziou et al., 2015) roles in determining the growth and persistence of plants and could be important elements of an optimal grassland management strategy for alpine meadows. Harvesting of whole plants or plant parts affects reproduction, survival, and growth and thereby also affects plant population dynamics (Gaoue et al., 2013; Ghimire et al., 2005, 2008; Huai et al., 2013; Ticktin, 2004). The extent of harvest impacts on plant populations, however, varies depending on habitat conditions, plant growth strategies, regeneration patterns, and microbial interaction, such as mycorrhizal association (Gaoue et al., 2013; Ticktin, 2015).

Inherently slow growth, high habitat specificity, dependency on pollinators, need of mycorrhiza for reproduction and germination, narrow range of ecological substitution options, unsustainable exploitation, and climate change are major challenges for the growth and development of orchids, such as *Dactylorhiza hatagirea* (Dhiman et al., 2019; Hinsley et al., 2017; Hutchings et al., 2018; Rasmussen & Rasmussen, 2018; Reiter et al., 2017; Shrestha et al., 2021; Yeung, 2017). Due to a marked decline in its natural populations, *D. hatagirea* has been listed as an endangered species in Nepal by Conservation Assessment and Management Plan (Bhattarai et al., 2001). According to the Forest Act of Nepal (2019), collection, use, sale, trade, and export of *D. hatagirea* are prohibited, and the species is strictly protected in list I of Government of Nepal (Go, 2011). It is also listed under appendix II in Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2020). Nevertheless, due to its high medicinal potency, *D. hatagirea* is still collected illegally at all life stages and traded to, especially, India and China (Olsen & Helles, 1997; Subedi et al., 2013), which has pushed local populations toward extinction (Manandhar, 2002).

Given that many orchids like *D. hatagirea* are currently threatened or endangered, a better understanding of the factors that influence orchid population ecology and dynamics may be critical to their long-term conservation (Shefferson et al., 2020). Formulation of strategies for conservation of a species requires a sound knowledge of environmental factors, population ecology, and demographic parameters (Margules & Pressey, 2000). Disentangling factors determining successful orchid establishment and its persistence under changing conditions is a major challenge. For the long-term conservation of endangered orchids, the development of concrete conservation plans based on indigenous knowledge, long-term monitoring, genetic analysis, and scientific inputs is crucial (Dobriyal et al., 2002; Jacquemyn et al., 2007). Endangered orchid species are in need of specific conservation actions (Charitonidou et al., 2019; Mincheva & Kozuharova, 2018; Tsiftsis et al., 2011), and more attention should be paid to the management of existing sites of orchids (Stipkova & Kindlmann, 2015).

Thus, the objectives of this paper are to (a) analyze the variation in population density, structure, and tuber production in *D. hatagirea* over three years in two sites subjected to different levels of anthropogenic disturbances, (b) study the impact of elevation and anthropogenic disturbances on the population density of *D. hatagirea*, (c) examine the interaction between site and environmental factors (harvesting, grazing, and herb cover) and its effect on population density of *D. hatagirea*, and (d) study the socio-cultural role of *D. hatagirea* and assess people's perception of its status.

To meet these objectives, we identified a locally unregulated site in the western part of Nepal and a well-managed, locally regulated site in the central part of Nepal. At these sites, we established permanent plots at different elevations and carried out a survey among local people.

2 | MATERIALS AND METHODS

2.1 | Study area

This study was carried out in two sites: (i) Lolu-Pilkanda (N29°60.095′ and E080°56.754′ to N29°57.719′ and E080°57.672′) within Api Nampa Conservation Area (ANCA) in Darchula District, northwest Nepal, and (ii) Bhimthang (N28°37.607′ and E084°28.343′ to N28°40.284′ and E084°29.166′), located within the strip of land separating Annapurna Conservation Area (ACA) and Manaslu Conservation Area (MCA) in Manang District, north-central Nepal (Figure 1).

The Lolu site, which lies in the upper Chamelia valley within ANCA, experiences high human pressure related mainly to livestock grazing and commercial harvesting of medicinal and aromatic plants (MAPs) (DNPWC, 2015). The site is managed by ANCA under the Department of National Parks and Wildlife Conservation (DNPWC) of the Government of Nepal, and due to poor implementation of regulatory mechanisms, the human exploitation of natural resources at this site is very heavy (Pyakurel et al., 2018). Further, the local community has not taken any particular initiatives to conserve the area. Commercial and illegal trade of MAPs from the area has increased drastically in the last few decades (Pyakurel et al., 2018). Hence, the Lolu site is hereafter referred as the locally "unprotected site."

ANCA is the youngest and the most remote conservation area of Nepal. The climate is temperate to nival with an annual average precipitation of 2,100 mm and annual mean minimum and maximum temperatures of 4.7°C and 27°C, respectively (DNPWC, 2015). Important livelihood activities are collection and trade of high-value MAPs, notably *Ophiocordyceps sinensis*, *Dactylorhiza hatagirea*, *Fritillaria cirrhosa*, *Neopicrorhiza scrophulariiflora* (Pouliot et al., 2018; Pyakurel et al., 2018), and traditional mountain farming systems tightly integrated with transhumance and other livestock systems (DNPWC, 2015). The area has legal permission for commercial harvest of MAPs, and over 25,000 collectors from different parts of western Nepal harvest valuable MAPs from rangelands within ANCA (DNPWC, 2015).

The Bhimthang site, which lies in Gyasumdo valley (lower Manang), experiences low human pressure in terms of livestock

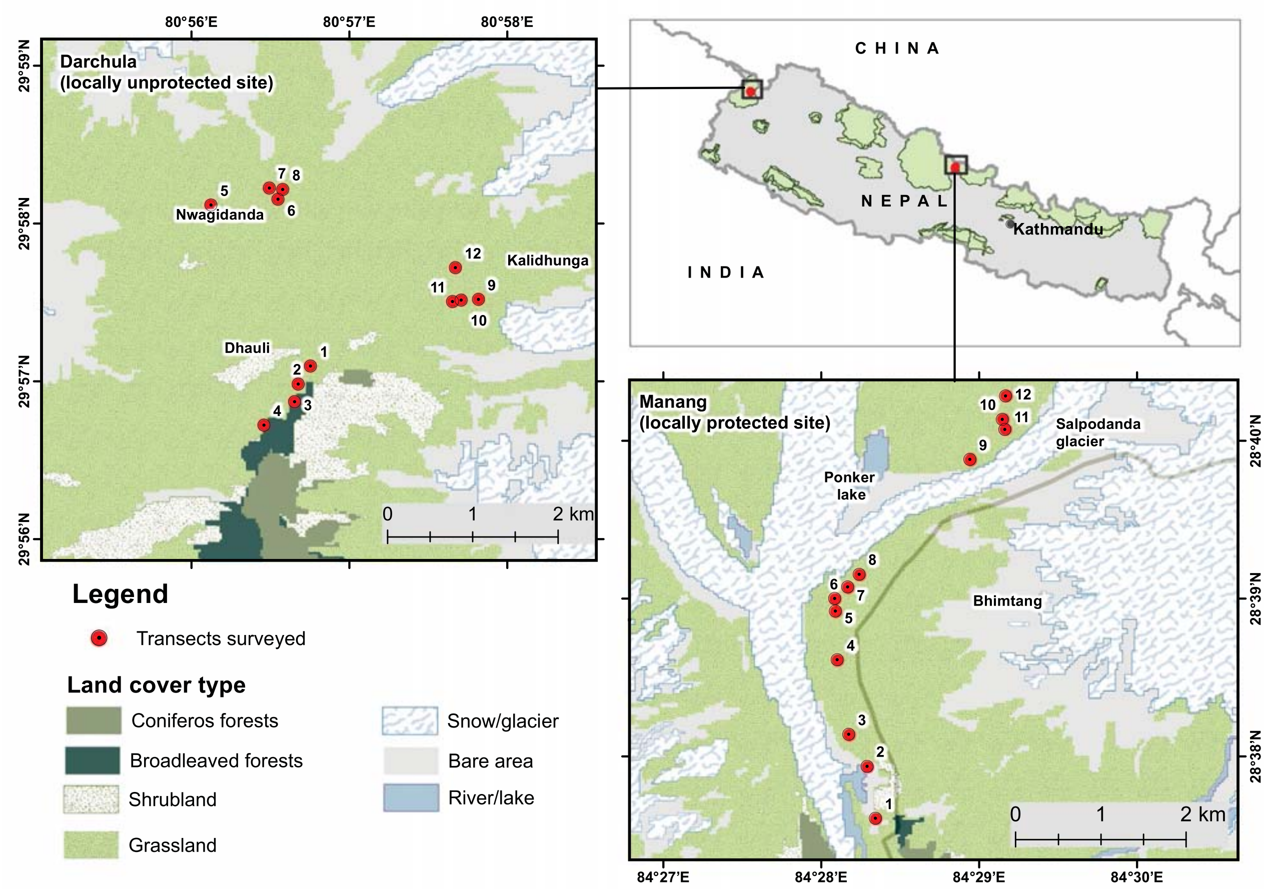


FIGURE 1 Map of the study area

FIGURE 2 Left: Blooming *Dactylorhiza hatagirea* (D. Don) Soo. Right: Tubers of *Dactylorhiza hatagirea* beaded for drying by the locals (PC: Chandra Kanta Subedi)

grazing and commercial and illegal harvesting of MAPs. This site is rich in biodiversity and natural beauty and is located in one of the major tourist areas of Nepal. Thus, tourism is the major source of income for the local community. The area is managed by the community in collaboration with Annapurna Conservation Area Project (ACAP), and the exploitation of valuable MAPs is very limited due to strict regulation by the community (NTNC, 2016). The locals are involved in patrolling to prevent illegal harvesting of MAPs during the maturation period. Further, the local people practice rotational harvest (one or two years depending on the availability of MAPs) of highly valued MAPs (NTNC, 2016). Therefore, the Bhimthang site is hereafter referred to as the locally "protected site."

At the protected site in Manang, the climate varies from temperate to nival and is influenced by the summer monsoon. The annual mean minimum and maximum temperatures are 4.7°C and 16.8°C, respectively, and the average annual precipitation is 972 mm (DNPWC, 2015). The basic livelihood activities include and combine traditional mountain farming systems, transhumance and animal husbandry, small-scale trade at lower altitude during winter, tourism, and the collection and trade of highly valued MAPs (Chhetri, 2014; Subedi & Chapagain, 2013).

The study sites differ with respect to edaphic, topographic, and substrate conditions. The unprotected site has a silty-loamy soil and is rich in herbs and grass and also has some bare ground cover, while the protected site has a sandy-loamy soil and is rich in moss, lichen, litter, rock, and scree cover. *D. hatagirea* is found on slopes ranging between 1 and 66° at the unprotected site and 4–65° at the protected site. The sites do not vary much in terms of relative radiation index (RRI) (Table S1: Appendix S1). The unprotected site is subjected to higher anthropogenic pressure as revealed by higher disturbance scores (harvesting, grazing, trampling, and animal droppings) than observed at the protected site.

2.2 | Study species

Dactylorhiza hatagirea (D. Don) Soó is locally known as Panchaunle ("five fingered hand"). It is distributed in Nepal, India, Bhutan, Pakistan, China, Afghanistan, and Mongolia (Roskov et al., 2020). *D. hatagirea* is an erect perennial herb (30–90 cm tall) that grows in moist alpine meadows and forest gaps between 2,800 and 4,600 m asl (Ghimire et al., 1999) favoring high soil Ca content (Thakur et al., 2020). It bears palmate tubers, 5–7 lanceolate or oblong leaves, which are progressively smaller toward the top, and has a robust stem (Figure 2, left). The inflorescence is up to 15 cm long, with a large number of densely packed flowers. Flowers are resupinate and purple to light pink, arranged around the rachis, resembling a hyacinth. Capsules bear thousands of dust-like seeds. Seeds are minute and have no endosperm and therefore lack storage reserves, and the orchid partly depends on the mycorrhizal fungi *Rhizoctonia* for nutrition (Giri & Tamta, 2012; Kalimuthu et al., 2007; Warghat et al., 2014). The rate of vegetative propagation is very slow and seed germination in nature is very poor, that is, 0.2%–0.3% (Vij, 2001).

The tubers of *D. hatagirea* (Figure 2, right) yield a high quality salep (a beverage made from the powder of the orchid tuber), which is used as an aphrodisiac or a nutritive and restorative tonic, and are also eaten raw as a farinaceous food (Baral & Kurmi, 2006; Sood et al., 2005; Thakur & Dixit, 2007; Vij, 1995; Watanabe et al., 2005). It is also used in the treatment of diabetes, chronic diarrhea, dysentery, coughs, hoarseness of voice, paralysis, fractures, during convalescence and to correct malnutrition (Das, 2004; Singh & Duggal, 2009). The whole plant possesses antibacterial properties and is used in curing various bacterial diseases (Ranpal, 2009). Tubers of *D. hatagirea* contain a wide range of chemical compounds including dactylorhins, dactyloses, glucosides, starch, and albumin (Kizu et al., 1999; Lama et al., 2001).

Recent findings indicate that *D. hatagirea* also has anticancerous properties (Popli, 2017).

2.3 | Sampling design used in the vegetation survey

This research is part of a long-term study carried out between 2015 and 2017 to understand the population dynamics of *D. hatagirea* in ANCA and Manang (research permit issued by Department of National Park and Wildlife Conservation [DNPWC]).

We sampled the vegetation in two ways: (i) To meet the first objective regarding variation in the populations over time, we established three permanent plots (20 × 20 m^2) in each population at the two study sites. (ii) Similarly, to meet the second and third objectives regarding impacts of environmental variables on population density, we established a total of 144 temporary plots (3 × 3 m^2), 72 at each site, from the lowest to the highest elevation to cover the whole distributional range of *D. hatagirea* at the study sites.

In each site, we subjectively identified three fairly dense populations where all life stages were present so that the population could be monitored in the following years (as a part of PhD dissertation of the first author). In each population, we established three randomly located permanent 20 m × 20 m plots and divided each plot into four 10 m × 10 m subplots. In each subplot, all plants were tagged in 2015, categorized with regard to life stage, and monitored in 2016 and 2017.

We established the temporary plots using the methods described by Chapagain et al. (2019). At each site, we identified three populations of *D. hatagirea* along an elevation gradient ranging from 3,400 to 3,900 m along the Upper Chamelia valley of ANCA and 3,400–4,600 m along the Gyasumdo valley in Manang (Figure 1, Table S1: Appendix S1). In each population, we established four transects at a minimum vertical distance of approximately 100 m. In each transect, we established six (3 m × 3 m) plots at a minimum horizontal plot to plot distance of 10 m. Each plot was divided into nine 1 m × 1 m subplots, and the four corner subplots were systematically sampled and measured.

For each plot, the geographical location (latitude and longitude) and topographical characteristics (elevation, slope, and aspect) were recorded and used to calculate the relative radiation index (RRI) (Oke, 1987; Vetaas, 1992a, 1992b). In each subplot, the ground cover (%) for vascular plants (grasses, herbs, and shrubs), nonvascular plants (lichens and bryophytes), litter, bare ground, rock, and scree cover were estimated using standard methods (Pauli et al., 2015). Disturbance (harvesting, trampling, grazing, and animal droppings) scores ranging from 0 (none) to 4 (very high) were recorded for each subplot after careful observation of the evidence, for example, large holes caused by excavation, wilted or fresh uprooted aerial parts, tuber fragments, browsed plant parts, defoliated aerial parts, and animal droppings.

We classified the individual plants into four life stage classes based on the number and size of leaves and presence of reproductive structures. The four stages are as follows: seedlings (Sd; leaf breadth ≤1 cm, leaf number = 1–2), juveniles (Jv; leaf breadth ≥1 cm, ≤2 cm, leaf number = 2–3), vegetative adults (Adv; leaf breadth ≥2 cm, leaf number >2, nonflowering), and reproductive adults (Adr; flowering or fruiting individuals). Individuals at different stages were counted in each subplot to calculate the density. The population structure was described as the proportion of each life stage within the studied population.

To estimate reproductive traits, we selected fifteen mature individuals from each population at the two sites and recorded the reproductive traits (number of flowers and fruits). For the estimation of dry biomass of tubers, we recorded the weight of fifteen dried tubers of *D.hatagirea* from the local MAPs collectors.

2.4 | Interview survey

Semi-structured interviews were conducted during 2015–2017 among 117 persons in the unprotected site (Darchula) and 69 persons in the protected site (Manang). The informants were local MAPs users, leaders, teachers, and students in the villages, and hotel owners, local tourist guides, and cattle herders working in the sites where we carried out our ecological survey. We explained the goal of our research and obtained the informants' consent before starting the interview. The informants were also informed about their right to withdraw their consent at any stage of the interview. Informants' responses were documented by written notes and in possible cases were also supplemented by voice recording, with the permission of the informants.

We asked the informants to comment on the abundance of *D. hatagirea*, collectors (villagers or outsiders), reasons for collecting the plant (local use or commercial purpose), local uses, the collection (areas and time of collection), when they started collecting, indigenous knowledge transfer practices, special tools, prices, qualities, markets (to whom they sold), processing after collection, and their other sources of income. We also asked questions concerning nature conservation; changes observed in the biotope, perceived trends in populations of the species, causes of population change, threats, conservation status, protection measurements, and practices that could ensure survival and sustainable management.

2.5 | Data analysis

A relative radiation index (RRI), which is a relative measure of the exposure to solar radiation at noon at a specific location (Oke, 1987; Vetaas, 1992a, 1992b), was calculated for each plot as a function of aspect, latitude, and slope:

$$\mathrm{RRI} = \cos(180^\circ - \Omega) \times \sin(\beta) \times \sin(\Phi) + \cos(\beta) \times \cos(\Phi),$$

where Ω is aspect (slope azimuth in degrees), Φ is latitude (degrees), and β is slope inclination (degrees).

The densities of different stages (seedling, juvenile, vegetative, and reproductive adults) were compared by Kruskal–Wallis tests, and the reproductive traits were compared among the three populations in each of the two sites using one-way ANOVA.

Direct field observations confirmed the plant as rare in the study site, and we therefore expected that the data collected would exhibit a large number of zeros. We tried with different model alternatives but based on the Akaike information criterion (AIC) the best fit was obtained using mixed zero-inflated Poisson (ZIP) models. The mixed ZIP model allowed us to analyze relationships between density of *D. hatagirea* plants at different stages and a set of independent variables including population, cover of shrubs or herbs, relative radiation index (RRI), and anthropogenic disturbance indicators such as harvesting, trampling, grazing, and animal droppings.

We prepared ten sets of candidate models using the glmmTMB package (See Appendix S2) and finally prepared an average model based on the set of five best candidate models (selected on the basis of delta AIC) using the MuMIn package (Barton, 2018). More specifically, the final average models were prepared using five models with delta AIC values ≤402 for the seedling stage, ≤748 for the juvenile stage, ≤645 for the adult vegetative stage, and ≤763 for the adult reproductive stage. The full models were in all cases expressed as:

$$\text{Density (of a particular stage of } D.\ hatagirea) = a + b\,(\text{population}) + c_1\text{RRI}_{ij} + c_2\text{Herb cover}_{ij} + c_3\text{Harvesting}_{ij} + c_4\text{Trampling}_{ij} + c_5\text{Grazing}_{ij} + c_6\text{Animal dropping}_{ij}$$

where a (intercept), b (population), and $c_1 \ldots c_6$ are fixed model parameters. $i = 1 \ldots 144$ is the plot (included as a random effect); $j = 1 \ldots 4$ is the subplot. The population variable had six categories, three at each site (see the section Study area). Confounded variables were excluded from the analysis. All the tests were conducted using the R version 3.5.3 (R Development Core team, 2017).

3 | RESULTS

3.1 | Variation in population density and structure

The population density of *D. hatagirea* at the unprotected site ranged from 0.60 to 0.79 individuals/m^2 while the density ranged from 0.70 to 2.16 individuals/m^2 at the protected site. Population densities were highest in mid-elevation populations at both sites (Table 1). At the unprotected site, all populations showed highest densities for the juvenile and adult vegetative stages, whereas at the protected site, the density of the adult reproductive stage was mostly higher than for the juvenile and adult vegetative stages (Table 1). At the protected site, the variation of density among the populations was significant (Kruskal–Wallis test, $p < .05$), overall, and for all stages except the adult vegetative stage, but at the unprotected site, there was no significant variation among populations ($p > .05$).

The population structure varied between the sites (Figure 3). For all populations, the proportion of seedlings was lower at the

TABLE 1 Population density (m^{-2}) for different life stages of *Dactylorhiza hatagirea* in populations in the locally unprotected (Darchula) and locally protected (Manang) sites

Population	Elevation (m asl.)	Life stage class[a] Sd	Jv	Adv	Adr	Total
Dhauli^{up}	3,605	0.05 ± 0.03	0.20 ± 0.03	0.20 ± 0.04	0.13 ± 0.02	0.60 ± 0.06
Nwagidanda^{up}	3,799	0.06 ± 0.02	0.39 ± 0.09	0.21 ± 0.04	0.12 ± 0.03	0.79 ± 0.10
Kalidhunga^{up}	3,976	0.08 ± 0.03	0.25 ± 0.04	0.28 ± 0.07	0.17 ± 0.03	0.78 ± 0.12
Mean^{up}		0.07 ± 0.01	0.29 ± 0.03	0.23 ± 0.03	0.14 ± 0.01	0.72 ± 0.06
χ^2 Value		0.70	2.56	0.98	1.39	2.57
p-Value		.70	.28	.61	.50	.28
Bhimthang^{p}	3,713	0.17 ± 0.05	0.46 ± 0.10	0.18 ± 0.07	0.27 ± 0.06	1.07 ± 0.17
Ponker Hill^{p}	4,046	0.44 ± 0.17	0.49 ± 0.14	0.40 ± 0.10	0.82 ± 0.17	2.16 ± 0.40
Salpodanda^{p}	4,437	0.03 ± 0.02	0.09 ± 0.03	0.19 ± 0.05	0.40 ± 0.04	0.70 ± 0.07
Mean^{p}		0.21 ± 0.07	0.34 ± 0.07	0.26 ± 0.04	0.50 ± 0.07	1.13 ± 0.17
χ^2 Value		13.62	8.86	3.09	10.63	14.24
p-Value		<.01	<.05	.21	<.01	<.01
Combined						
χ^2 value		18.64	14.82	3.92	42.99	21.77
p-Value		<.001	<.01	.41	<.0001	<.001

Note: Densities are stated as mean ± *SE*. χ^2 and *p*-values were based on Kruskal–Wallis test, $df = 5$, $n = 144$.

up = locally unprotected site, p = locally protected site.

[a] Life stage classes—Sd: seedling; Jv: juvenile; Adv: adult vegetative; Adr: adult reproductive.

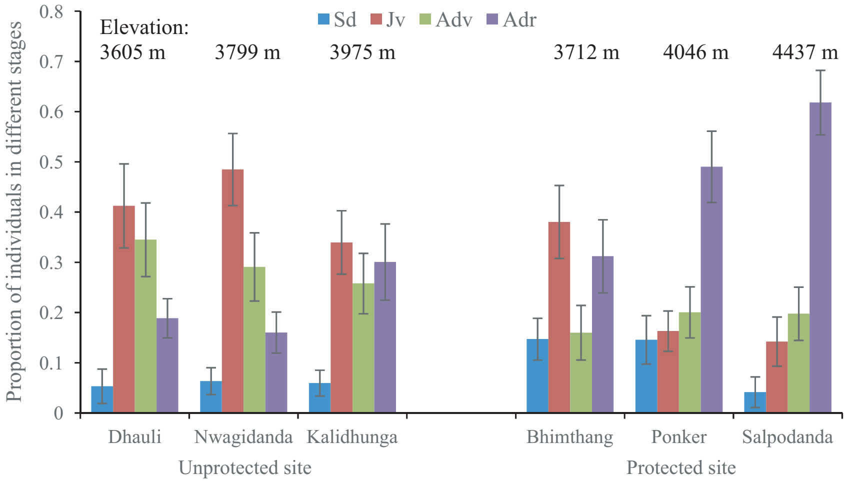


FIGURE 3 Population structure of *Dactylorhiza hatagirea* in (left) locally unprotected and (right) locally protected sites. Life stage classes: Sd = Seedling, Jv = juvenile, Adv = adult vegetative, and Adr = adult reproductive

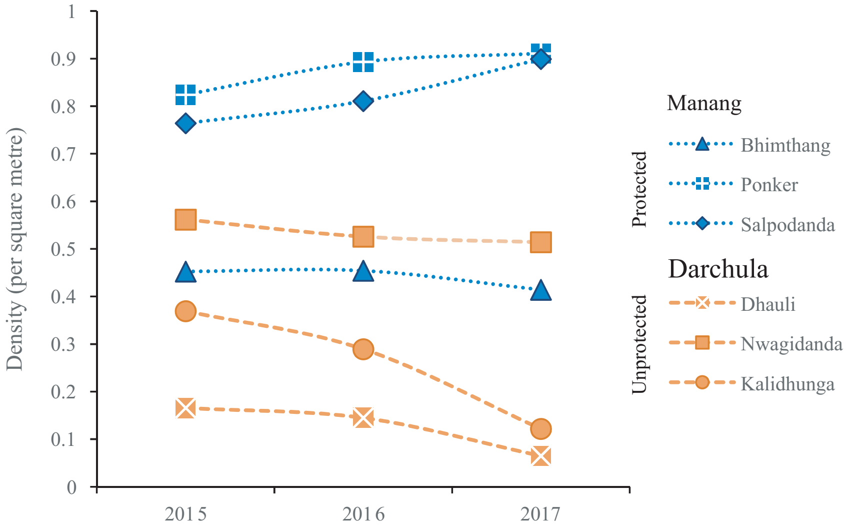


FIGURE 4 Density of *Dactylorhiza hatagirea* at the protected (Manang) and unprotected (Darchula) sites in 2015, 2016 and 2017

unprotected site than at the protected site. The proportion of juvenile and adult vegetative plants was the highest at the unprotected site, while the proportion of the adult reproductive stage was highest at the protected site. Comparing mean densities across three consecutive years (2015–2017), a drastic decrease was observed at the unprotected site (approximately by one third), while almost no change was seen at the protected site (Figure 4).

3.2 | Variation in reproductive traits and tuber production

At the protected site, the reproductive output and tuber biomass were about three times higher than at the unprotected site. In comparisons between populations, the reproductive traits (number of flowers, number of fruits, and total reproductive output per individual) were found to decrease from the lowest to the highest elevation at both sites (Table 2). The dry biomass of daughter tubers showed similar trends at both study sites. A comparison of tuber production across three consecutive years (2015, 2016, and 2017) showed reduced tuber production in 2017 at the unprotected site and increasing tuber production at the protected site (Figure 5).

3.3 | Effect of environmental variables on densities of different life stages and their interaction among the sites

Mixed zero-inflated Poisson (mixed ZIP) models showed significant negative effects of harvesting on the densities of juvenile and adult stages (both vegetative and reproductive). Grazing had a significant negative effect on juvenile ($p < .001$) and adult vegetative ($p < .05$) stages. Herb cover showed significant positive effects ($p < .05$) on the juvenile and adult vegetative stages. The relative radiation index had a weakly significant negative effect ($p < .1$) on the density of the adult vegetative stage. The zero-inflation model component was significant only for the adult reproductive stage (Table 3).

TABLE 2 Variation in reproductive output of *Dactylorhiza hatagirea* in populations in the locally unprotected (Darchula) and locally protected (Manang) sites

Population	Number of flower per individual	Number of fruit per individual	Total reproductive output per individual	Dry weight of daughter tuber (g)
Dhauli^{up}	15.27 ± 3.06	27.87 ± 1.94	39.07 ± 4.30	0.73 ± 0.08
Nwagidanda^{up}	14.89 ± 1.20	26.33 ± 1.92	34.93 ± 2.67	0.63 ± 0.13
Kalidhunga^{up}	13.11 ± 3.65	24.5 ± 2.07	27.40 ± 2.96	0.61 ± 0.09
Total				
F-value	0.49	2.22	9.47	3.06
p-Value	.49	.14	.15	.09
Bhimthang^{p}	29.5 ± 2.39	40.7 ± 3.05	71.2 ± 2.93	1.74 ± 0.13
Ponker Hill^{p}	27.9 ± 3.27	36.9 ± 2.18	64.8 ± 4.40	1.42 ± 0.16
Salpodanda^{p}	21.9 ± 1.61	31.4 ± 1.84	53.3 ± 2.32	1.17 ± 0.14
Total				
F-value	9.78	5.11	12.89	8.82
p-Value	.01	.02	.00	.00
Combined				
F-value	0.26	29.8	29.8	92.3
p-Value	.61	.00	.00	.00

Note: Values are stated as mean ± *SE*. *F* and *p*-values were based on one-way ANOVA, *df* = 5, *n* = 144.
up = locally unprotected site, p = locally protected site.

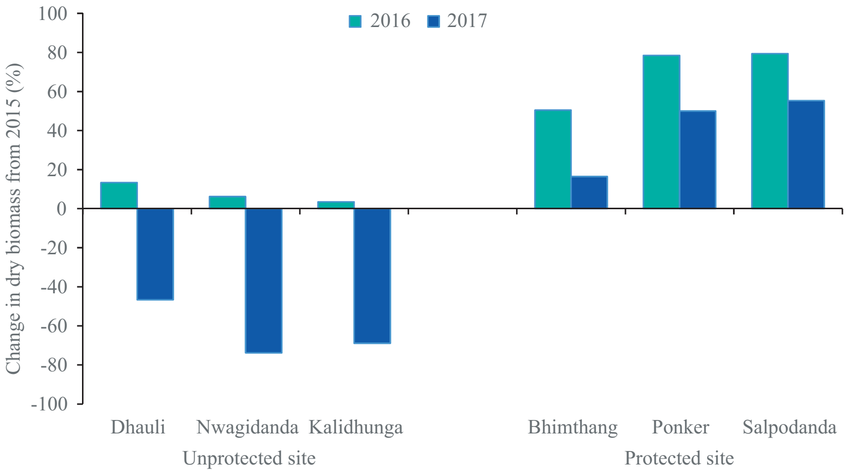


FIGURE 5 Variation in tuber production of *D. hatagirea* during 2015 to 2017 in populations in the locally unprotected (first three in the graph) and locally protected sites (last three in the graph)

The interaction effects of harvesting and grazing within population among the sites indicate that the unprotected populations showed significant negative effects of harvesting and grazing, while these effects were not distinct for the protected populations. The herb cover did not show any significant effects within populations among the sites (Table 4).

3.4 | Interview survey

About forty-three percent of the MAP users interviewed at the unprotected site (*n* = 117) were aware that *D. hatagirea* is strictly protected, seventy-one percent were aware of its use value, five percent were aware of its population ecology, and forty-two percent harvested *D. hatagirea* for local uses to treat cuts and wounds, boils, fractures, and to use it as a tonic. Ninety-two percent of the informants claimed that illegal harvesting of *D. hatagirea* was a common practice and that the population had decreased drastically over the last few decades. They further disclosed that there are illegal traders in the district headquarters of Darchula who motivate MAP users and cattle herders to harvest *D. hatagirea* by promising to buy the dried tubers. Further, perceived difficulties to collect *Ophicordyceps sinensis* and *Fritillaria cirrhosa* increased the temptation to carry out illegal and premature harvest of *D. hatagirea*. The informants mentioned that for every 1 kg of dried tubers sold, approximately 500–1,000 mature plants are harvested. Eighty-one percent of the informants are of the view that the major source of their livelihood is MAP collection.

At the protected site, about ninety-three percent of the MAP users interviewed ($n = 69$) were aware of the harvesting ban, ninety-eight percent were aware of the species' use value, nineteen percent were aware of its population ecology, and twenty-one percent occasionally harvested a few (2–5) individuals of *D. hatagirea* for home use to treat cuts and wounds, burns, and boils and for religious purposes by Buddhist Lamas. About twenty-four percent were of the opinion that the population is decreasing, while seventy-three percent thought that the population has been almost constant in the last few decades as the local community is involved in patrolling the area to control illegal collection during the maturation period. Our interview results also revealed that none of the families totally rely on MAP collection for their livelihood as they had access to income from the flourishing tourism. We also observed that awareness programs were run at community level, emphasizing the sustainable use of available MAPs. Further, they also used a specific route to protect sensitive plants from damage caused by grazing and trampling.

4 | DISCUSSION

The study is the first of its kind in this region, but similar research has been done in Greece (Charitonidou et al., 2019). Our work provides results based on a long-term study from the Nepal Himalayas and should therefore be of significance to the conservation management of the endangered orchid *D. hatagirea*. Environmental variables and human-mediated disturbances such as harvesting and livestock grazing had significant effects on the population structure, density, reproductive traits, and tuber production of *D. hatagirea*.

4.1 | Variation in population density and structure

We recorded a maximum population-level mean density of 1.31 plants/m^2 at the protected and 0.72 plants/m^2 at the unprotected site. The population density at the protected site was thus roughly twice as large as at the unprotected site. Particularly low proportions of seedlings and adult reproductive plants observed at the unprotected site could be due to overharvesting and grazing, since harvesters presumably tend to target the adult reproductive stage and recently established seedlings are sensitive to grazing. Moreover, seedlings browsed by livestock may be hard to find and could therefore be overlooked more frequently than the larger plants characterizing later life stages.

At both sites, we recorded a low proportion of seedlings. This could partly be explained as a consequence of orchids being habitat specific and seedling establishment depending on a suite of environmental factors, which are rarely present at the same time and place (Shefferson et al., 2020). Our results compare well with previous research as small populations have substantially lower viability compared to larger populations, and seedling recruitment rates can be considerably lower in small populations, which results in significantly lower population growth rate and density (Hens et al., 2017; Pellegrino & Bellusci, 2014). The low proportion of reproductive plants observed at the unprotected site compares well with observations made by Pellegrino and Bellusci (2014) for the orchid species *Serapias cordigera*, where human disturbances were noted to have a negative effect on the population size. The traditional practices of transhumance, harvesting, and habitat fragmentation are likely the major anthropogenic factors responsible for reducing the flowering density as well as the population size of *D. hatagirea* in the unprotected site. Human-induced disturbances like harvesting, grazing, and fire have negative effects on population density and performances (Aguilar et al., 2006; Chapagain et al., 2019; McKinney, 2002); however, there are also examples of the opposite, and Chen et al. (2014) actually reported a positive effect of human disturbance on some orchid species. Our results further suggest that at both sites the density of *D. hatagirea* reached a peak in mid-elevation populations (3,799 m asl in the unprotected and 4,046 masl in the protected site). This might indicate that we have actually managed to cover the elevation range and that the best habitats are thus found approximately in the middle of the range. It is also consistent with the idea that when examining small populations across environmental gradients, the peak density usually occurs at intermediate levels, as also noted by Chen et al. (2014).

4.2 | Variation in the reproductive traits

We found reduced reproductive fitness in *D. hatagirea* at the unprotected site. Alterations due to anthropogenic disturbances in natural habitats often reduce the size and density of populations (Aguilar et al., 2006; McKinney, 2002). Anthropogenic disturbances increase the spatial distance between the plant populations as well as between individuals within a population, thereby disrupting insect movement between plants (Öckinger et al., 2009), decreasing pollinator abundance (Liu & Koptur, 2003), and altering their behavior and the frequency of flower visits (Aguilar et al., 2006). This process ultimately decreases the reproductive fitness of the plants (Peterson et al., 2008). The level of inbreeding may be higher in small, isolated populations (Miao et al., 2014) because of the higher rate of selfing and more frequent mating between close relatives. The resulting inbreeding depression can reduce the fitness of the plants compared with those in larger populations. Consequently, decreased outcrossing in small, sparse populations may reduce population fitness, potentially increasing the probability of extinction (Gargano et al., 2009; Stachurska-Swakoń et al., 2011). The weaker performance in terms of reproductive output at the unprotected site could further be attributed to the interruption of plant development caused by breakage of inflorescences during heavy grazing and trampling. A long history of grazing and trampling at the unprotected site (DNPWC, 2015) might have resulted in the production of reduced or defective reproductive parts. Grazing is likely to affect

TABLE 3 Mixed zero-inflated Poisson regression models for the density (m^{-2}) of seedling, juvenile, adult vegetative, and adult reproductive stages of *Dactylorhiza hatagirea*

			Locally protected site (Manang)			Locally unprotected site (ANCA)	
		ZI component	Count component				
Life stages	Average model	Zi	Bhimthang: Intercept	Ponker Hill	Salpodanda	Dhauli	Nwagidanda
Seedling	Full	−0.702 (0.691)	−1.982*** (0.619)	0.537 (0.553)	−2.037** (0.818)	−0.677 (0.876)	−0.740 (0.720)
	Conditional	−0.702 (0.691)	−1.982*** (0.691)	0.537 (0.553)	−2.037** (0.818)	−0.677 (0.876)	−0.740 (0.720)
Juvenile	Full	−2.150 (1.401)	−0.919** (0.363)	−0.133 (0.346)	−1.854**** (0.470)	−0.070 (0.488)	0.002 (0.381)
	Conditional	−2.150 (1.401)	−0.919** (0.363)	−0.133 (0.346)	−1.854**** (0.470)	−0.070 (0.488)	0.002 (0.381)
Adult vegetative	Full	0.122 (0.307)	−1.080* (0.560)	0.953** (0.421)	−0.201 (0.461)	1.026* (0.590)	0.437 (0.456)
	Conditional	0.122 (0.307)	−1.080* (0.560)	0.953** (0.421)	−0.201 (0.461)	1.026* (0.590)	0.437 (0.456)
Adult reproductive	Full	−0.781** (0.370)	0.894** (0.426)	1.138**** (0.287)	0.394 (0.307)	0.353 (0.413)	−0.106 (0.389)
	Conditional	−0.781** (0.370)	−0.894** (0.426)	1.138**** (0.287)	0.394 (0.307)	0.353 (0.413)	−0.106 (0.389)

Note: Density of different stages was modeled as a function of herb cover (%), relative radiation index (RRI), and disturbance (harvesting, grazing, and animal droppings), which were assessed using an ordinal 0–4 integer scale. Zi is the intercept in the zero-inflated component: all other parameters refer to the count component of the model. Parameter estimates with standard errors in brackets for full average and conditional average models. Significance levels are stated as: $*p < .1$, $**p < .05$, $***p < .01$, $****p < .001$.

TABLE 4 Mixed zero-inflated Poisson regression models expressing the interaction effect between site and environmental factors (harvesting, grazing, and herb cover) on density (m^{-2}) of seedlings, juvenile, adult vegetative, and adult reproductive stages of *D. hatagirea*

	Independent variables	Harvesting				Grazing
Life stages	Model	Intercept: Locally unprotected site	Locally protected site	Harvest	Interaction locally protected * harvest	Intercept: locally unprotected
Seedling	Conditional	−2.561**** (0.540)	0.180 (0.461)	−0.860*** (0.307)	1.012 (1.207)	−2.338**** (0.553)
	Zero Inflation	0.774 (0.549)				−0.789 (0.557)
Juvenile	Conditional	−0.546*** (0.201)	−0.708*** (0.244)	−0.833**** (0.138)	2.250** (0.922)	−0.354* (0.212)
	Zero Inflation	−1.327**** (0.466)				−1.614**** (0.466)
Adult vegetative	Conditional	−0.200 (0.251)	−0.654** (0.284)	−0.963**** (0.180)	0.903 (0.842)	0.019 (0.263)
	Zero inflation	0.093 (0.308)				0.047 (0.308)
Adult reproductive	Conditional	−0.851**** (0.234)	0.378* (0.230)	−0.949**** (0.204)	1.039** (0.501)	−0.660*** (0.256)
	Zero inflation	−0.760* (0.389)				−0.666* (0.368)

Note: Parameter estimates with standard errors in brackets. Significance levels are stated as: $*p < .1$, $**p < .05$, $***p < .01$, $****p < .001$.

D. hatagirea in numerous ways, both directly by damaging aboveground parts and indirectly by changing habitat characteristics (light intensity, litter accumulation, temperature, etc.) and interaction with other individuals or species (intra- and interspecific competition, pollination/herbivory).

The unprotected populations had a lower population size and a lower fruit set than did protected populations, suggesting that the latter populations are better buffered. This could be a consequence of inadequate pollinator visitation in small populations, resulting in insufficient pollen transfer, poor pollination, and lower seed set (Smithson, 2006; Tremblay et al., 2005; Xia et al., 2013). By contrast, larger populations of plants are likely to be more attractive to pollinators, resulting in higher visitation rates and therefore higher pollination success (Mustajärvi et al., 2001). Habitat fragmentation and disturbance lead to the interruption of interspecific interactions, indirectly causing changes in plant demographic dynamics via reduced reproductive output (Shefferson et al., 2020; Steffan-Dewenter et al., 2006). Some studies have shown that plants in disturbed habitats undergo a reduction in pollination efficiency and reproductive success as well as recruitment and survival rates, all of which negatively affect plant demographic dynamics (Aguilar et al., 2006; Bruna et al., 2009).

Transhumance and MAPs collection are common practices in the alpine and subalpine pastures of the unprotected site and have existed for a very long time (DNPWC, 2015). The site is legally open for commercial harvest of highly valued MAPs, such as *Ophiocordyceps sinensis* and *Fritillaria cirrhosa*, and the locals are highly dependent on the collection and trade of MAPs for their livelihood. A large number of collectors (locals as well as from other parts of the country) enters the site to collect *O. sinensis* and *F. cirrhosa* during the late spring

Kalidhunga	Herb cover	RRI	Harvesting	Grazing	Animal droppings
−0.514 (0.750)	0.009 (0.010)		−0.606 (0.369)	−0.424 (0.394)	0.019 (0.116)
−0.514 (0.750)	0.013 (0.009)		−0.606 (0.369)	−0.606 (0.336)	0.147 (0.292)
−0.213 (0.424)	0.010 (0.007)		−0.367** (0.177)	−0.644**** (0.188)	−0.080 (0.149)
−0.213 (0.424)	0.011** (0.004)		−0.376** (0.169)	−0.644**** (0.188)	−0.197 (0.179)
0.720 (0.490)	0.009 (0.007)	−0.106 (0.459)	−0.657*** (0.230)	−0.530* (0.290)	−0.014 (0.010)
0.720 (0.490)	0.010** (0.006)	−0.594* (0.903)	−0.657*** (0.230)	−0.595** (0.238)	−0.071 (0.217)
0.224 (0.373)	0.000 (0.001)	−0.098 (0.411)	−0.801**** (0.202)	−0.057 (0.134)	−0.010 (0.061)
0.224 (0.373)	0.000 (0.004)	−0.583 (0.853)	−0.801**** (0.202)	−0.177 (0.188)	−0.103 (0.173)

			Herb Cover			
Locally protected	Grazing	Interaction locally protected * grazing	Intercept: locally unprotected	Locally protected	Herb cover	Interaction locally protected * Herb cover
0.178 (0.488)	−1.011*** (0.348)	0.317 (0.558)	−3.569*** (0.899) −0.667 (0.719)	1.209 (0.916)	0.002 (0.011)	−0.004 (0.014)
−0.833*** (0.262)	−1.081**** (0.168)	0.757** (0.329)	−1.230*** (0.417) −0.668 (0.377)	0.132 (0.472)	0.000 (0.006)	0.000 (0.009)
−0.787** (0.321)	−1.126**** (0.207)	0.650 (0.408)	−0.667 (0.476) 0.732*** (0.267)	0.034 (0.541)	0.001 (0.007)	0.006 (0.010)
0.299 (0.256)	−0.956**** (0.200)	0.732** (0.294)	−1.551**** (0.464) −0.474 (0.337)	1.279*** (0.478)	−0.000 (0.007)	−0.002 (0.008)

when *D. hatagirea* also emerges. The resulting trampling presumably has a huge negative impact on its growth and development by breaking the aerial parts before fruit maturation and seed dispersal. Besides, collectors who are unable to collect sufficient amounts of *O. sinensis* and *F. cirrhossa* are tempted to illegally harvest the tubers of *D. hatagirea* irrespective of its degree of maturity. Such practices are also common in other parts of the world (Ghorbani et al., 2014; Kreziou et al., 2015). Further, the harvesting of the orchids involves destructive uprooting of the daughter tubers, which kills the plants.

When ascending from lowland to alpine environments in the Himalayas, plant species experience a large variation in abiotic conditions over an extremely short distance (Korner, 2003). With increasing elevation, changes in pressure, temperature, wind speed, UV exposure, and soil properties have been shown to affect different phenological and morphological properties of plants (Djordjević & Tsiftsis, 2020; Hodkinson, 2005), thus also influencing growth and reproductive performance. The decreasing number of reproductive parts observed along the altitudinal gradient could be further attributed to the time of flowering, which is influenced by the ambient temperature and the timing of the snow melt (Kudo & Hirao, 2006).

4.3 | Effects of different environmental variables on the density of different stages

The occurrence and distribution of orchid species are influenced by environmental and topographical factors such as latitude, altitude, slope, and aspect (Bulafu et al., 2007; Djordjević et al., 2016, 2020). In this study, we observed a very weak negative effect of the

Relative Radiation Index (RRI) on the adult vegetative density ($p < .1$) of *D. hatagirea*. Although the effect is weak, it may indicate that *D. hatagirea* prefers growing in humid places. Mixed ZIP models revealed a significant positive effect of herb cover on the juvenile and adult vegetative density. It may be possible to explain this as a consequence of other herbs providing shade and shelter for the growth and development of *D. hatagirea* individuals at different life stages. The harvesting showed significant negative effects on the density of all stages except seedlings, as also observed in other parts of the world (Ghorbani et al., 2014; Kreziou et al., 2015), whereas grazing showed significant negative effects on juvenile and adult vegetative stages only. Contrasting results from other parts of the world also exist. For example, Charitonidou et al. (2019) found that the current level of collection of *Dactylorhiza sambucina* in Greece is not significantly affecting the abundance of this orchid, and Mincheva and Kozuharova (2018) reported that wild orchids are not threatened by harvesting in Bulgaria.

Grazing and trampling adversely affect aboveground parts and disturb the life cycle. In some cases, the underground parts are also exposed and eventually destroyed. The weak positive effect of harvest and grazing on plant density at the protected site for all the different stages could be linked to the narrow range of harvest and grazing intensities observed. By contrast, at the unprotected site, the negative impact of harvesting and grazing on density of different stages was highly pronounced. The prevailing disturbance practices (grazing, trampling, and overexploitation) and lack of awareness of the population ecology and the conservation status of the plant are the major challenges for sustainable management at the unprotected site (Pouliot et al., 2018). This is also in agreement with Poudeyal et al. (2019) who observed that intense human disturbances, especially harvest, played a crucial role as determinants of the density and structure of *Neopicrorhiza scrophulariiflora* populations.

4.4 | Interview survey

We observed a higher proportion of people at the protected site who knew about the harvest ban and the population ecology and use value of the species than at the unprotected site. The strict enforcement of MAPs harvesting rules by the community at the protected site is the key factor for the maintenance of sustainable populations of *D. hatagirea*. People at the protected site do not allow people from other districts to collect any types of MAPs in their territory (NTNC, 2016) and this helps to maintain a low harvesting pressure in populations of MAPs including *D. hatagirea*. Moreover, the Annapurna Conservation Area Project (ACAP), which has worked in this area for three decades, has also contributed to increase conservation awareness and promote sustainable use of natural resources (Baral & Heinen, 2007).

In contrast, in the unprotected site there is no strict local protection system and people from outside ANCA are also allowed to harvest MAPs. The local MAP users were also found with to have a low level of conservation awareness (in relation to *D. hatagirea*) as also reported for northwestern Greece by Kreziou et al., (2015). Moreover, due to the lack of protection measures and awareness, the MAP users were found to engage in intensive harvesting of *D. hatagirea*, and selling it to local traders (Pyakurel et al., 2018) despite the local legal acts of protection of *D. hatagirea*.

Additionally, a group of locals at the unprotected site has the view that the uncontrolled influx of unaware collectors from different parts of the country exacerbates the exploitation of the alpine vegetation. The prevailing unhealthy competition among MAP collectors in the unprotected site sometimes terminates in social conflicts which promotes the illegal harvest of *D. hatagirea* challenging its persistence. Thus, unregulated harvesting of *D. hatagirea* could be one of the major reasons for decline in the populations of the species.

5 | CONCLUSION AND CONSERVATION IMPLICATIONS FOR *D. HATAGIREA*

Harvesting and grazing and a low level of awareness about the population ecology and conservation among local people are the major challenges for sustainable development of *D.hatagirea* populations at the unprotected site. Harvesting and grazing showed significant negative effects on the density of different life stages of *D. hatagirea* and played a crucial role in deteriorating plant populations through reduction in reproductive outputs. Hence, this study indicated significantly reduced fruit production and lower productivity in terms of tuber biomass for plants at the unprotected site. Moreover, disturbances have the potential to cause a reduction in number of recruits and adult individuals (both vegetative and reproductive) as intensive destructive harvesting techniques are used at the unprotected site, irrespective of life stage and maturity. Coupled with other prevalent disturbances, the intensive and destructive harvesting may lead to local extinction of the species. Therefore, a good strategy for long-term conservation of the species would involve strengthening people's knowledge about the population ecology of *D. hatagirea*, increasing the enforcement of current regulations and introducing permanent monitoring of the populations. This study also recommends that governmental and nongovernmental organizations working in the field of conservation help in identifying alternative sources of income for the locals, so that their dependency on MAPs harvesting can be reduced, thus also reducing the pressure on populations of *D. hatagirea* and preventing local extinction of the species.

ACKNOWLEDGMENTS

The study was supported by the Research Committee for Development Research (FFU) of the Danish Ministry of Foreign Affairs, Grant No. 13-07KU, and a Rufford Small Grant (RSG-19960-1). We thank Department of National Parks and Wildlife Conservation for granting permission to carry out the fieldwork and the officials of Annapurna Conservation Area Project and Api Nampa Conservation Area for their support and cooperation during fieldwork. The local research assistants who helped us to establish the plots are highly acknowledged. We express our sincere thanks to the locals for their coordination and warm hospitality during the fieldwork and for sharing their knowledge. We thank Naresh Subedi for his constructive suggestions on an earlier version of the manuscript. We also thank Pratikshya Chalise and Yagya Raj Paneru for helping us during the fieldwork in Manang. The Central Department of Botany, Tribhuvan University, facilitated the fieldwork. Finally, we thank two anonymous reviewers and editors for critical comments which helped to improve the manuscript.

CONFLICT OF INTEREST

The authors declare no competing interests exist.

AUTHOR CONTRIBUTIONS

Deep Jyoti Chapagain: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Writing-original draft (equal); Writing-review & editing (equal). **Henrik Meilby:** Conceptualization (equal); Formal analysis (equal); Methodology (equal); Project administration (equal); Supervision (equal); Writing-original draft (equal); Writing-review & editing (equal). **Chitra Bahadur Baniya:** Supervision (equal); Writing-review & editing (supporting). **Shanta Budha-Magar:** Data curation (equal); Writing-review & editing (supporting). **Suresh Kumar Ghimire:** Conceptualization (equal); Formal analysis (equal); Methodology (equal); Project administration (equal); Supervision (equal); Writing-original draft (equal); Writing-review & editing (equal).

AUTHOR'S CONTRIBUTION

DJC and SKG designed the experiment; DJC and SBM collected the data; DJC, HM, CBB, and SKG analyzed the data and wrote the manuscript.

DATA AVAILABILITY STATEMENT

Data used for the ecological analysis are presented in the paper and the Supplementary material. Data that identify human participants in the study will not be made publicly available. However such data can be made available upon request to the authors.

ORCID

Deep Jyoti Chapagain https://orcid.org/0000-0003-2782-3974
Henrik Meilby https://orcid.org/0000-0002-3770-3880
Shanta Budha-Magar https://orcid.org/0000-0003-2599-063X

REFERENCES

Aguilar, R., Asworth, L., Galetto, L., & Aizen, M. A. (2006). Plant reproductive susceptibility to habitat fragmentation: Review and synthesis through a meta-analysis. *Ecology Letters*, *9*, 968–980. https://doi.org/10.1111/j.1461-0248.2006.00927.x

Baral, N., & Heinen, J. (2007). Resources use, conservation attitudes, management intervention and park-people relations in the Western Terai landscape of Nepal. *Environmental Conservation*, *34*(1), 64–72.

Baral, S. R., & Kurmi, P. P. (2006). *A compendium of medicinal plants in Nepal*. Kathmandu: Mass Printing Press.

Barton, K. (2018). *MuMIn: Multi-Model Inference*. R package version 1.42.1. Vienna: The R Foundation for Statistical Computing. Available at: https://CRAN.R-project.org/package=MuMIn

Bhattarai, N., Tandon, V., Ved, D. K. (2002). Highlights and outcomes of the Conservation Assessment and Management Planning (CAMP) Workshop, Pokhara, Nepal. In N. Bhattarai & M. Karki (Eds.), *Sharing local and national experience in conservation of medicinal and aromatic plants in South Asia* (pp. 46–53). Proceedings of the Regional Workshop held at Pokhara, Nepal, 21–23 January 2001. New Delhi, India: Medicinal and Aromatic Plants Program in Asia (MAPPA), International Development Research Centre (IDRC), South Asian Regional Office. https://idl-bnc-idrc.dspacedirect.org/bitstream/handle/10625/29240/120935.pdf?sequence=1

Bruna, E. M., Fiske, I. J., & Trager, M. D. (2009). Habitat fragmentation and plant populations: Is what we know demographically irrelevant? *Journal of Vegetation Science*, *20*(3), 569–576.

Bulafu, C. E., Mucunguzi, P., & Kakudidi, E. K. (2007). Diversity and distribution of wild terrestrial orchids of Mount Elgon Forest National, Park. *Eastern Uganda. African Journal of Ecology*, *45*(Suppl. 3), 21–28.

Calvo, R. (1990). Four-year growth and reproduction of *Cyclopogon cranichoides* Orchidaceae) in south Florida. *American Journal of Botany*, *77*, 736–741. https://doi.org/10.2307/2444365

Chapagain, D. J., Meilby, H., & Ghimire, S. K. (2019). Plant density and life history traits of *Aconitum spicatum* in North –central Nepal: Effects of elevation and anthropogenic disturbances. *PeerJ*, *7*, e7574. https://doi.org/10.7717/peerj.7574

Charitonidou, M., Stara, K., Kougioumoutzis, K., & Halley, J. M. (2019). Implications of salep collection for the conservation of the Elder-flowered orchid (Dactylorhiza sambucina) in Epirus, Greece. *Journal of Biological Research-Thessaloniki*, *26*, 1–13.(this paper is quite relevant to this work, since it refers to another Dactylorhiza species collected for salep, in Greece. Authors also refer to anthropogenic disturbances, and also used interviews in local communities.)

Chen, Y., Yang, X., Yang, Q., Li, D., Long, W. et al (2014). Factors affecting distribution patterns of wild plants with extremely small populations in Hainan Island China. *PLoS One*, *9*(5), e97751. https://doi.org/10.1371/journal.pone.0097751

Chhetri, P. (2014). *Livelihood Strategies of People in the Himalayan Region of Nepal: A Study in the Villages of Eastern Manang*. Independent Study Project. https://digitalcollections.sit.edu/isp_collection/1831

CITES (2020). *Convention on International Trade in Endangered Species of Wild Fauna and Flora: The CITES Appendices accessed on March 30, 2021 from*. https://cites.org/eng/app/appendices.php. Accessed on March 30, 2021.

Dai, L., Guo, X., Ke, X., Zhang, F., Li, Y., Peng, C., Shu, K., Li, Q., Lin, L., Cao, G., & Du, Y. (2019). Moderate grazing promotes the root biomass in Kobresia meadow on the northern Qinghai- Tibet Plateau. *Ecology and Evolution*, *9*, 9395–9406. https://doi.org/10.1002/ece3.5494

Das, S. P. (2004). *Indian orchids in indigenous medicine system. Orchids diversity and conservation*. St John's College, Tiruchirappalli: The Rapinat Herbarium.

Dhiman, N., Sharma, N. K., Thapa, P., Sharma, I., Swarnkar, M. K., Chawla, A., Shankar, R., & Bhattacharya, A. (2019). De novo transcriptome provides insights into the growth behaviour and resveratrol and trans-stilbenes biosynthesis in *Dactylorhiza hatagirea*—An endangered alpine terrestrial orchid of western Himalaya. *Scientific Reports*, *9*, 13133. https://doi.org/10.1038/s41598-019-49446-w

Di Marco, M., Venter, O., Possingham, H. P., & Watson, J. E. M. (2018). Changes in human footprint drive changes in species extinction risk. *Nature Communications*, *9*, 4621. https://doi.org/10.1038/s41467-018-07049-5

Djordjević, V., & Tsiftsis, S. (2020). The role of ecological factors in distribution and abundance of Terrestrial Orchids. In J.-M. Merillon, &

H. Kodja (Eds.), *Orchids phytochemistry, biology and horticulture, reference series in phytochemistry. Fundamentals and applications* (pp. 1–71). Springer Nature.

Djordjević, V., Tsiftsis, S., Lakušić, D., Jovanović, S., Jakovljević, K., & Stevanović, V. (2020). Patterns of distribution, abundance and composition of forest terrestrial orchids. *Biodiversity and Conservation*, *29*, 4111–4134. https://doi.org/10.1007/s10531-020-02067-6

Djordjević, V., Tsiftsis, S., Lakušić, D., Jovanović, S., & Stevanović, V. (2016). Factors affecting the distribution and abundance of orchids in grasslands and herbaceous wetlands. *Systematics and Biodiversity*, *14*(4), 355–370. https://doi.org/10.1080/14772000.2016.1151468

DNPWC (2015). *Management Plan for Api Nampa Conservation Area, Darchula (2015-2019)*. Kathmandu, Nepal: Government of Nepal Ministry of Forests and Soil Conservation, Department of National Parks and Wildlife Conservation. http://dnpwc.gov.np/media/publication/Api_Nampa_Conservation_Area_Management_Plan.pdf

Dobriyal, A. K., Vinteshwar, P. B., Neeraj, K., & Bisht, M. S. (2002). An appraisal of the fishery potential of river Nayar with a note on its improvement possibilities. In *Coldwater fish genetic resources and their conservation* (Vol. *7*, pp. 235–242). Nature Conservator Publication.

Ehrlich, P. R. (1988). The loss of diversity. In *Biodiversity* (pp. 21–27). Washington, D.C.: National Academy Press.

Faast, R., Facelli, J. M., & Austin, A. D. (2011). Seed viability in declining populations of *Caladenia rigida* (Orchidaceae): Are small populations doomed? *Plant Biology*, *13*, 86–95.

Fay, M. F., Pailler, T., & Dixon, K. W. (2015). Orchid Conservation: Making the links. *Annals of Botany*, *116*, 377–379. https://doi.org/10.1093/aob/mcv142

Gaoue, O. G., Horvitz, C. C., Ticktin, T., Steiner, U. K., & Tuljapurkar, S. (2013). Defoliation and bark harvesting affect life-history traits of a tropical tree. *Journal of Ecology*, *101*, 1563–1571. https://doi.org/10.1111/1365-2745.12140

Gargano, D., Gullo, T., & Bernardo, L. (2009). Do inefficient selfing and inbreeding depression challenge the persistence of the rare Dianthus guliae janka (Caryophyllaceae)? Influence of reproductive traits on a plant's proneness to extinction. *Plant Species Biology*, *24*(2), 69–76.

Ghimire, S. K., Gimenez, O., Pradel, R., McKey, D., & Aumeeruddy-Thomas, Y. (2008). Demographic variation and population viability in a threatened Himalayan medicinal and aromatic herb *Nardostachys grandiflora*: Matrix modelling of harvesting effects in two contrasting habitats. *Journal of Applied Ecology*, *45*, 41–51. https://doi.org/10.1111/j.1365-2664.2007.01375

Ghimire, S. K., McKey, D., & Aumeeruddy-Thomas, Y. (2005). Conservation of Himalayan medicinal plants: Harvesting patterns and ecology of two threatened species, *Nardostachys grandiflora* DC. and *Neopicrorhiza scrophulariiflora* (Pennell) Hong. *Biological Conservation*, *124*, 463–475.

Ghimire, S. K., Sah, J. P., Shrestha, K. K., & Bajracharya, D. (1999). Ecological study of some high altitude medicinal and aromatic plants in the Gyasumdo valley, Manang, Nepal. *Ecoprint*, *6*(1), 17–25.

Ghorbani, A., Gravendeel, B., Naghibi, F., & de Boer, H. (2014). Wild orchid tuber collection in Iran: A wake-up call for conservation. *Biodiversity and Conservation*, *23*, 2749–2760.

Giri, D., & Tamta, S. (2012). Propagation and conservation of *Dactylorhiza hatagirea* (D. Don) Soo, an endangered alpine orchid. *African Journal of Biotechnology*, *11*(62), 12586–12594. https://doi.org/10.5897/AJB11.3287

Go, N. (2011). Protected plants of Nepal: Its amendments. Kathmandu, Nepal: Ministry of Forests and Soil Conservation. *Nepal Gaz*, *60*, 38–45.

Hens, H., Pakanen, V. M., Jäkäläniemi, A., Tuomi, J., & Kvist, L. (2017). Low population viability in small endangered orchid populations: Genetic variation, seedling recruitment and stochasticity. *Biological Conservation*, *210*, 174–183.

Hinsley, A., de Boer, H. J., Fay, M. F., Gale, S. W., Gardiner, L. M., Gunasekara, R. S., Kumar, P., Masters, S., Metusala, D., Roberts, D., Veldman, S., Wong, S., & Phelps, J. (2017). A review of the trade in orchids and its implications for conservation. *Botanical Journal of the Linnean Society*, *186*(4), 435–455. https://doi.org/10.1093/botlinnean/box083

Hodkinson, I. D. (2005). Terrestrial insects along elevation gradients: Species and community responses to altitude. *Biological Reviews*, *80*, 489–551. https://doi.org/10.1017/S1464793105006767

Huai, H., Wen, G., Xu, W., & Bai, W. (2013). Effects of commercial harvesting on population characteristics and rhizome yield of *Anemone altaica*. *Economic Botany*, *67*(1), 41–50.

Hutchings, M. J., Robbirt, K. M., Roberts, D. L., & Davy, A. J. (2018). Vulnerability of a specialized pollination mechanism to climate change revealed by a 356-year analysis. *Botanical Journal of the Linnean Society*, *186*(4), 498–509. https://doi.org/10.1093/botlinnean/box086

IUCN (2020). *The IUCN Red List of Threatened Species*. Version 2020-3. *3*, Accessed on March 30, 2021 from: https://www.iucnredlist.org

Jacquemyn, H., Brys, B., Hermy, M., & Willems, J. H. (2005). Does nectar reward affect rarity and extinction probabilities of orchid species? An assessment using historical records from Belgium and Netherlands. *Biological Conservation*, *121*, 257–263. https://doi.org/10.1016/j.biocon.2004.05.002

Jacquemyn, H., Brys, R., Hermy, M., & Willems, J. H. (2007). Long-term dynamics and population viability in one of the last populations of the endangered *Spiranthes spiralis* (Orchidaceae) in the Netherlands. *Biological Conservation*, *134*, 14–21. https://doi.org/10.1016/j.biocon.2006.07.016

Kalimuthu, K., Senthilikumar, R., & Vijayakumar, S. (2007). In vitro micro propagation of orchid, *Oncidum* sp. *African Journal of Biotechnology*, *6*, 1171–1174.

Kizu, H., Kaneko, E., & Tomimori, T. (1999). Studies on Nepalese crude drugs - XXVI. Chemical constituent of Panchaunle, the root of *Dactylorhiza hatagirea* D. Don. *Chemical and Pharmaceutical Bulletin*, *47*(11), 1618–1625.

Korner, C. (2003). *Alpine plant life* (2nd ed.). Berlin: Springer-Verlag.

Kreziou, A., de Boer, H., & Gravendeel, B. (2015). Harvesting of salep orchids in north-western Greece continues to threaten natural populations. *Oryx*, *50*(3), 393–396.

Kudo, G., & Hirao, A. S. (2006). Habitat-specific responses in the flowering phenology and seed set of alpine plants to climate variation: Implications for global-change impacts. *Population Ecology*, *48*(1), 49–58. https://doi.org/10.1007/s10144-005-0242-z

Kull, T., & Hutchings, M. J. (2006). A comparative analysis of the decline in the distribution ranges of orchid species in Estonia and the United Kingdom. *Biological Conservation*, *129*, 31–39. https://doi.org/10.1016/j.biocon.2005.09.046

Lama, Y. C., Ghimire, S. K., & Aumeeruddy-Thomas, Y. (2001). *Medicinal plants of Dolpo*. Amchis' knowledge and conservation. Kathmandu: WWF Nepal.

Laurance, W. F., & Bierregaard, R. O. (1997). Tropical forest remnants: Ecology, management, and conservation of fragmented communities. *Journal of Mammalogy*, *79*(3), 1084–1088. https://doi.org/10.2307/1383118

Light, M. H., Kell, S. P., & Jackson, P. W. (2003). The role of education and training in orchid conservation: An overview and critique. In *Orchid conservation* (pp. 357–382). Kota Kinabalu, Sabah: Natural History Publications.

Liu, H., & Koptur, S. (2003). Breeding system and pollination of a narrowly endemic herb of the Lower Florida Keys: Impacts of the urban-wildland interface. *American Journal of Botany*, *90*(8), 1180–1187.

Manandhar, N. P. (2002). *Plants and people of Nepal*. Portland, Oregon: Timber Press Inc.

Margules, C. R., & Pressey, R. L. (2000). Systematic conservation planning. *Nature*, *405*(6783), 243–253.

McCormick, M. K., & Jacquemyn, H. (2014). What constraints the distribution of Orchid Populations? *New Phytology*, *202*, 392–400.

McKinney, M. L. (2002). Urbanization, biodiversity, and conservation. *BioScience*, *52*, 883–890.

Miao, Y. C., Lang, X. D., Zhang, Z. Z., & Su, J. R. (2014). Phylogeography and genetic effects of habitat fragmentation on endangered *Taxus yunnanensis* in southwest China as revealed by microsatellite data. *Plant Biology*, *16*(2), 365–374.

Mincheva, I., & Kozuharova, E. (2018). Is the traditional use of "salep" in the Bulgarian Rhodopes hazardous for the wild populations of terrestrial orchids? *Flora Mediterranea*, *28*, 399–418.

Mustajärvi, K., Siikamäki, P., Rytkönen, S., & Lammi, A. (2001). Consequences of plant population size and density for plant-pollinator interactions and plant performance. *Journal of Ecology*, *89*(1), 80–87. https://doi.org/10.1046/j.1365-2745.2001.00521.x

NTNC (2016). *Technical Report on biodiversity monitoring in Bhimthang valley*. National Trust for Nature Conservation (NTNC), Khumaltar, Lalitpur, Nepal. https://www.researchgate.net/publication/350485015_Biodiversity_Assessment_in_Bhimthang_valley_Manang_2016

Öckinger, E., Dannestam, Å., & Smith, H. G. (2009). The importance of fragmentation and habitat quality of urban grasslands for butterfly diversity. *Landscape and Urban Planning*, *93*(1), 31–37.

Oke, T. R. (1987). *Boundary layer climates*. New York: Mathuem & Co. Ltd.

Olsen, C. S., & Helles, F. (1997). Medicinal plants, markets, and margins in the Nepal Himalaya: Trouble in paradise. In *Mountain research and development* (Vol. *17*, pp. 363–374). International Mountain Society. https://doi.org/10.2307/3674025

Pauli, H., Gottfried, M., Lamprecht, A., Niessner, S., Rumpf, S., Winkler, M., Klaus, S., & Grabherr, G. (2015). *The GLORIA field manual–standard Multi-Summit approach, supplementary methods and extra approaches*. Vienna: GLORIA-Coordination: Austrian Academy of Sciences and University of Natural resources and Life Sciences.

Pellegrino, G., & Bellusci, F. (2014). Effects of human disturbance on reproductive success and population viability of *Serapias cordigera* (Orchidaceae). *Botanical Journal of the Linnean Society*, *176*(3), 408–420.

Peterson, A., Bartish, I. V., & Peterson, J. (2008). Effects of population size on genetic diversity, fitness and pollinator community composition in fragmented populations of *Anthericum liliago* L. *Plant Ecology*, *198*(1), 101–110.

Pimm, S. L., Jenkins, C. N., Abell, R., Brooks, T. M., Gittleman, J. L., Joppa, L. N., Raven, P. H., Roberts, C. M., & Sexton, J. O. (2014). The biodiversity of species and their rates of extinction, distribution, and protection. *Science*, *344*(6187), 1246752. https://doi.org/10.1126/science.1246752

Popli, D. (2017). *Elicitation of Dactylorhin-E and studying anti cancerous Potential of Dactylorhiza hatagirea(D.Don) Soo*. Master's Dissertation, Biotechnology, Jaypee University of Information and Technology, Waknaghat, India.

Poudeyal, M. R., Meilby, H., Shrestha, B. B., & Ghimire, S. K. (2019). Harvest effects on density and biomass of *Neopicrorhiza scrophulariiflora* vary along environmental gradients in the Nepalese Himalayas. *Ecology and Evolution*, *9*, 7726–7740. https://doi.org/10.1002/ece3.5355

Pouliot, M., Pyakurel, D., & Smith-Hall, C. (2018). High altitude organic gold: The production network for *Ophiocordyceps sinensis* from far-western Nepal. *Journal of Ethnopharmacology*, *218*, 59–68.

Pyakurel, D., Sharma, I. B., & Smith-Hall, C. (2018). Patterns of change: The dynamics of medicinal plant trade in far-western Nepal. *Journal of Ethnopharmacology*, *224*, 323–334.

R Development Core Team (2017). *A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available at http://www.Rproject.org/

Ranpal, S. (2009). *An Assessment of Status and Antibacterial Properties of Dactylorhiza hatagirea in Annapurna Conservation Area (A case study of Paplekharka, Lete VDC, Mustang)*. Master's Dissertation, Institute of Forestry, Tribhuvan University, Kathmandu.

Rasmussen, H. N. (1995). *Terrestrial orchids: from seed to mycotrophic plant*. Cambridge University Press.

Rasmussen, H. N., & Rasmussen, F. N. (2018). The epiphytic habitat on a living host: Reflections on the orchid–tree relationship. *Botanical Journal of the Linnean Society*, *186*(4), 456–472. https://doi.org/10.1093/botlinnean/box085

Reiter, N., Vicek, K., O'Brien, N., Gibson, M., Pitts, D., Brown, G. R., Bower, C. C., & Phillips, R. D. (2017). Pollinator rarity limits reintroduction sites in an endangered sexually deceptive orchid (*Caladenia hastata*): Implications for plants with specialized pollination systems. *Botanical Journal of the Linnean Society*, *184*(1), 122–136. https://doi.org/10.1093/botlinnean/box017

Roskov, Y., Ower, G., Orrell, T., Nicolson, D., Bailly, N., Kirk, P. M., Bourgoin, T., DeWalt, R. E., Decock, W., Nieukerken, E., Zarucchi, J., & Penev, L. (2020). *Species 2000 & ITIS Catalogue of Life, 2020-04-16 Beta*. Digital resource at www.catalogueoflife.org/col. Species 2000. http://www.catalogueoflife.org/col/details/reference/species/392654

Saunder, D. A., Hobbes, R. J., & Margules, C. R. (1991). Biological consequences of ecosystem fragmentation: A review. *Conservation Biology*, *5*, 18–32. https://doi.org/10.1111/j.1523-1739.1991.tb00384.x

Schulze, E.-D., Beck, E., Buchmann, N., Clemens, S., Müller-Hohenstein, K., & Scherer-Lorenzen, M. (2019). Interactions between plants, plant communities and the abiotic and biotic environment. In *Plant ecology* (pp. 689–741). Berlin, Heidelberg: Springer.

Shefferson, R. P., Jacquemyn, H., Kull, T., & Hutchings, M. J. (2020). The demography of terrestrial orchids: Life history, population dynamics and conservation. *Botanical Journal of the Linnean Society*, *192*, 315–332. https://doi.org/10.1093/botlinnean/boz084

Shrestha, B., Tsiftsis, S., Chapagain, D. J., Khadka, C., Bhattarai, P., Shrestha, N. K., Kolanoswska, M. A., & Kindlmann, P. (2021). Suitability of habitats in Nepal for Dactylorhiza hatagirea now and under predicted future changes in climate. *Plants*, *10*, 467. https://doi.org/10.3390/plants10030467

Singh, A., & Duggal, S. (2009). Medicinal orchids-an overview. *Ethnobot Leaflets*, *2009*(3), 3.

Smithson, A. (2006). Pollinator limitation and inbreeding depression in orchid species with and without nectar rewards. *New Phytologist*, *169*(2), 419–430.

Sood, S. K., Rana, S., & Lakhanpal, T. N. (2005). *Ethnic aphrodisiac plants*. Jodhpur, India: Scientific Publishers.

Stachurska-Swakoń, A., Cieślak, E., & Ronikier, M. (2011). Genetic variability of small isolated populations of *Cicerbita alpina* (L.) Wallr. (Asteraceae) in the Beskid Mały Mts (southern Poland). *Polish Journal of Ecology*, *59*(2), 279–288.

Steffan-Dewenter, I., Klein, A. M., Gaebele, V., Aebele, T., & Tscharnke, T. (2006). Bee diversity and plant–pollinator interactions in fragmented landscapes. In *Plant–pollinator interactions: From specialization to generalization* (vol. *31*, pp. 387–410). University of Chicago Press.

Stipkova, Z., & Kindlmann, P. (2015). Extent and Reasons for meadows in South Bohemia becoming unsuitable for Orchids. *European Journal of Environmental Science*, *5*, 142–147. https://doi.org/10.14712/23361964.2015.87

Subedi, A., Kunwar, B., Choi, Y., Dai, Y., van Andel, T., Chaudhary, R. P., de Boer, H. J., & Gravendeel, B. (2013). Collection and trade of wild-harvested orchids in Nepal. *Journal of Ethnobiology and Ethnomedicine*, *9*(1), 9–64.

Subedi, B., & Chapagain, P. (2013). Tourism Development in Upper Manang Valley of Annapurna Region. *Nepal Tourism and Development Review*, *1*(1), 56–68. https://doi.org/10.3126/ntdr.v1i1.7370

Thakur, D., Rathore, N., Sharma, M. K., Parkash, O., & Chawla, A. (2020). Identification of ecological factors affecting the occurrence and abundance of *Dactylorhiza hatagirea* (D. Don) Soo in the Himalaya.

Journal of Applied Research on Medicinal and Aromatic Plants, *20*, 100286. https://doi.org/10.1016/j.jarmap.2020.100286

Thakur, M., & Dixit, V. K. (2007). Aphrodisiac activity of *Dactylorhiza hatagirea* (D. Don) Soo in male albino rats. *Evidence-Based Complementary and Alternative Medicine*, *4*, 29–31.

Ticktin, T. (2004). The ecological implications of harvesting non-timber forest products. *Journal of Applied Ecology*, *41*, 11–21. https://doi.org/10.1111/j.1365-2664.2004.00859.x

Ticktin, T. (2015). The ecological sustainability for non-timber forest products harvest: Principles and methods. In C. M. Shackleton, A. K. Pandey, & T. Ticktin (Eds.), *Ecological sustainability for non timber forest products; Dynamics and case studies of harvesting* (pp. 31–52). Routledge.

Tremblay, R. L., Ackerman, J. D., Zimmerman, J. K., & Calvo, R. N. (2005). Variation in sexual reproduction in orchids and its evolutionary consequences: A spasmodic journey to diversification. *Biological Journal of the Linnean Society*, *84*(1), 1–54.

Tsiftsis, S., Tsiripidis, I., & Trigas, P. (2011). Identifying important areas for orchid conservation in Crete. *European Journal of Environmental Science*, *1*, 28–37. https://doi.org/10.14712/23361964.2015.44

Vakhrameeva, M. G., Tatarenko, I. V., Varlygina, T. I., Totosyan, G. K., & Zagulskii, M. N. (2008). *Orchids of Russia and adjacent countries*. ARG Gantner Verlag KG. http://www.nhbs.com/orchids_of_russia_and_adjacent_countries_tefno_159628.html

Van der Cingel, N. A. (1995). *An atlas of orchid pollination: European orchids*. Balkema.

Vetaas, O. R. (1992a). Gradients in field-layer vegetation on an arid misty mountain plateau in the Sudan. *Journal of Vegetation Science*, *3*(4), 527–534.

Vetaas, O. R. (1992b). Micro-site effects of trees and shrubs in dry savannas. *Journal of Vegetation Science*, *3*, 337–344. https://doi.org/10.2307/3235758

Vij, S. P. (1995). Orchid genetic diversity in India: Conservation and commercialization. In *Proc. 5th Asia Pacific Orchid Conference and Show, Fukuoka* (pp. 20–39).

Vij, S. P. (2001). Orchids and tissue culture: Current status. In S. K. Nandi, L. M. S. Palni, & A. Kumar (Eds.), *Role of plant tissue culture in biodiversity conservation and economic development* (pp. 491–502). Gyanodaya Prakashan.

Warghat, A. R., Bajpai, B. P., Srivastava, R. B., Chaurasis, H. S., & Sood, H. (2013). Population genetic structure and conservation of small fragmented locations of *Dactylorhiza hatagirea* in Ladakh region of India. *Scientia Horticulturae*, *164*, 448–454. https://doi.org/10.1016/j.scientia.2013.09.044

Warghat, A. R., Bajpai, P. K., Srivastav, R. B., Chaurasia, O. P., Chauhan, R. S., & Sood, H. (2014). In vitro protocorm development and mass multiplication of an endangered orchid, *Dactylorhiza hatagirea*. *Turkish Journal of Botany*, *38*, 737–746.

Watanabe, T., Rajbhandari, K. R., Malla, K. J., & Yahara, S. (2005). *A handbook of medicinal plants of Nepal*. Kobfai Publishing Project, Bangkok, Thailand: Kobfa Publishing Project, Bangkok.

Xia, J., Lu, J., Wang, Z. X., Hao, B. B., Wang, H. B., & Liu, G. H. (2013). Pollen limitation and Allee effect related to population size and sex ratio in the endangered *Ottelia acuminata* (Hydrocharitaceae): Implications for conservation and reintroduction. *Plant Biology*, *15*(2), 376–383.

Yeung, E. C. (2017). A perspective on orchid seed and protocorm development. *Botanical Studies*, *58*, 33. https://doi.org/10.1186/s40529-017-0188-4

Zhang, A., Cornwell, W., Li, Z., Xiong, G., Fan, D., & Xie, Z. (2019). Strong restrictions on the trait range of co-occurring species in the newly created riparian zone of the three gorges reservoir area, China. *Journal of Plant Ecology*, *12*(5), 825–833. https://doi.org/10.1093/jpe/rtz016

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Chapagain DJ, Meilby H, Baniya CB, Budha-Magar S, Ghimire SK. Illegal harvesting and livestock grazing threaten the endangered orchid *Dactylorhiza hatagirea* (D. Don) Soó in Nepalese Himalaya. *Ecol Evol*. 2021;00:1–16. https://doi.org/10.1002/ece3.7520