Population Structure and Plant Performance of *Juniperus squamata* Buch.-Ham. ex D. Don along an Elevation Gradient in Manang, Nepal

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ABBREVIATION AND ACRONYMS

ANOVA	analysis of variance
asl	above sea level
ACAP	Annapurna Conservation Area Project
CBS	Central Bureau of Statistics
CCA	Canonical Correspondence Analysis
cm	centimeter
DNPWC	Department of National Park and Wildlife Conservation
DCA	Detrended Correspondence Analysis
GoN	Government of Nepal
GLORIA	Global Observation Research Initiative in Alpine Environments
GPS	geographical positioning system
ha.	Hectare
HLI	Heat Load Index
KATH	National Herbarium and Plant Laboratories, Godavari
m	meter
NPHC	National Population and Housing Census
NTFPs	Non-Timber Forest Products
NTNC	National Trust for Nature Conservation
PADIR	potential annual direct incident radiation
PCA	Principle Component Analysis
sq. m.	square meter
SD	standard deviation
SE	standard error
SE-	south east
SW-	south west
SPSS	statistical program for social science
TUCH	Tribhuvan University Central Herbarium
VDC	Village Development Committee

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Abstract

Manang is one of the most crucial landscape hosting a large number of plant species despite its difficult topography and unpredictable climate. This study aims to assess variation in population structure and plant performance of *J. squamata* Buch.-Ham. ex D. Don along elevation gradient in Manang, Central Nepal. The study area was divided into three elevation bands: low (3,400-3,700m), mid (3,700-4,000m) and high elevation band (4,000-4,300m). A total of 54 quadrats (18 quadrats per band) of 10 m×10 m size were sampled. In each plot, we recorded individual life stage (seedling, juvenile and adult), reproductive (number of fruits per plant) and vegetative (plant height, stem circumference and leaf biomass) traits of adult individuals of *J. squamata* separately. *J. squamata* preferred mostly dry and rocky habitats in SE- to SW- slopes, along Juniper- *Cotoneaster-Rosa-Berberis* woodland and Grassland vegetative assemblages. A total of 15 major vegetative assemblages and 94 species belonging to 37 families and 63 genera have been recorded in the study area. Elevation has a significant role on species richness. The total species richness was observed inclined linearly with increase in elevation gradient. Low and mid elevation bands mostly comprised woody shrub and tree species where as high elevation band hosted mainly herbaceous species. Highest density of all life stages: seedling, juvenile and adult, were observed in mid elevation of *J. squamata*. High contribution of seedling density was contributed in mid and high elevation band. Proportion of seedling was highest in mid and high elevation and least in low elevational band. The population structure showed a reverse J-shape indicating a good regeneration status in the study area. Most of the individuals were of small size. Mean leaf dry weight and stem circumference were 48.49±1.13gm per 0.0625m^2 canopy and 9.48±0.25 cm respectively. The mean value for the number of fruits onset was 84.88±6.2 per mature plant. The number of fruits per plant was observed linearly inclining towards higher elevations. Value for all traits studied except for leaf dry weight of *J. squamata* was observed increased along elevation band. Pearson correlation analysis revealed significant correlation among the number of fruits per plant with plant height and number of fruits per plant with stem circumference. Vegetative and reproductive traits along with population structure of *J. squamata* showed a remarkable variation with elevation gradient. Successful regeneration was observed in mid elevation band with moderate disturbance. Low elevation band was highly disturbed due to grazing, trampling, collection of fire wood and leaves, tourism and developmental activities.

CHAPTER I: INTRODUCTION

1.1 Background

High altitude regions in the world including Himalayas are biodiversity hotspots that exhibit substantial number of plant species and endemism. Estimates suggested that almost one third of the plant species assessed so far are reported from these regions (Körner 2007). Most of these species have evolutionary significances, and are the foundations of local livelihoods, culture and tradition.

The typical adaptive feature of high altitude plant species is their tolerance to harsh environmental conditions including freezing cold climates (Klotzli 1997; Körner 2003; Weppler 2006). Elevation being the principal factor many other environmental factors including climate, topography, edaphic factors plays crucial roles in shaping diversity and distribution of vascular plants in alpine regions (Rahbek 1995; Austin *et al.* 1996; Grytnes *et al.* 1999; Lomolino 2001; Bhattarai and Vetaas 2003). Out of these environmental factors, temperature and climate have predominant roles for describing the vegetation pattern and species richness along an elevation gradient (Körner 1995; Walker 1995). The climatic factors that vary with change in elevation include temperature, potential evapo-transpiration, growing period, humidity, nutrient availability, ultraviolet radiation and rainfall (Funnell and Parish 2001). Mountain slopes with significant bioclimatic amplitude generally have more species at the bottom than the top (Vetaas and Grytnes 2002). Studies revealed that the total species diversity at subalpine-nival gradients in Himalayas declines monotonically with subsequent rise in elevation (Lomolino 2001; Grytnes and Vetaas 2002; Bhattarai *et al.* 2004). In contrast, the endemic diversity is greatest in the alpine regions.

A population consists of individuals of the same species that survive, interact and migratethrough the same niche and habitat (Waples and Gaggiotti 2006). The word population (*L. populus*: people) refer to a group of organisms of a species occupying particular space at a particular time. Individuals in local populations share a common gene pool. The basic characteristic of a population is its size and density which is affected by population attributes like natality (birth), mortality and migration (immigration and emigration). The schedule of survival and fertility (birth) constituent the life history of a living organism (Ray and Choudhury 1989). The limits of a population depends upon the number, size and life form of a species, its mode of reproduction, mode of seed or juvenile dispersal, its habitat specificity and pattern of distribution within its geographical range. A population has a density and age structure, birth and death rate and a unique growth form (Verma and Agrawal 1996). Population biology is concerned with spatio-temporal variation in numbers and sizes of individuals in a population (Harper 1977).

There is always a continuous turnover in an existing population of living organisms due to four parameters, like birth, death, emigration and immigration. The process and stability of the population depends upon the organism's interactions with the environmental factors, such as food availability, predation, shelter and competition between the organisms for different resources which can be by changes in environment act as natural check on the performance of a population. The increase in size of population can bring both negative and positive effects to the individuals. The former types of effects may be intraspecific competition for nutrition, space, etc., and the later effects include protection from predators and influence on reproduction rate (Sharma 1999). Altitudinal gradients are complex gradients and involve many different co-varying factors, such as topography, soil, and climatic conditions (Austin *et al.* 1996). Out of these complex variables, temperature and climatic variables seem to be the most important than soil parameters for describing the vegetation along an altitude gradient (Woodward 1987; Körner 1995). Vegetation within a forest is significantly affected by variations in the microclimate due to altered aspect, altitude and slopes (Chaudhary 1999; Pande *et al.* 2002). Study on the variation in population density of seedlings, juveniles and adults along elevation gradient would be helpful in understanding the influences of environmental factors on population performance (Wang *et al.* 2004). Other traits like plant height stem diameter, leaf biomass and reproductive output can also be used to assess the growth status of a population.

A disturbance is a temporary change in average environmental conditions that causes a pronounced change in an ecosystem. White and Pickett (1985) define disturbance as "any relatively discrete event in time that disrupts ecosystem, community or population structure and changes resources, substrates availability, or the physical environment". According to Petraitis *et al.* (1989), disturbance is "the process that alters the birth and death rates of individuals present in a patch" by killing the individuals or by affecting the resource levels, natural enemies or competitors in ways that alter survival and fecundity. Humans not only affect ecosystems directly, but also are altering natural disturbance regimes. Ecological disturbances include fires, flooding, soil disturbance, wind/storm, insect outbreaks, nutrient inputs, as well as anthropogenic disturbances such as grazing forest clearing, trampling, land fragmentation and the introduction of exotic species (Connell 1978).

Vegetation biomass is a key ecological variable for understanding the evolution and potential future changes of the climate system. It can be defined as the dry weight of the given plant specimen or its part. Namgail *et al.* (2011) reported the hump-shaped relationship between aboveground phytomass and altitude in alpine community where as many other studies suggests sharp decline in biomass with increase in altitude in mountains (Garkoti and Singh 1995; Singh and Singh 1987).

1.2 Rationale and Objectives

High elevation ecosystems of Himalayas are the most productive as well as vulnerable geographic regions of the world (Glatzel 2009). These ecosystems are characterized by the most significant plant species having medicinal, religious and economic values, and most of such species like *Juniperus squamata* are under immense pressure due to anthropogenic disturbances.

In Himalaya, while most of the research interest has been on plant species whose economic potential is widely known (Ghimire *et al*. 2005; Ghimire *et al.* 2008; Shrestha and Jha 2009), but no detailed studies have been conducted for other species with less economic value but with immense socio-cultural values like that of *J. squamata*. Thus this study has been attempted to study the habitat characteristics, population size, reproductive trait and ecological parameters of *J. squamata*.

Population studies of Junipers conducted in Himalaya other than *J. squamata* signify a good regeneration pattern at local levels (Ghimire *et al.* 2008 and Chapagain 2014). However, there is no such information available in case of *J. squamata*, which has significant ethnobotanical, socio-cultural and economical value (Bhattarai *et al.* 2006). This plant is also collected heavily for commercial purposes, like juniper oil extraction (Adams 1996, 2014), and is thus under immense anthropogenic pressure. Thus, there is a need for baseline data regarding the important population attributes of *J. squamata* in order to know its present status and to make generalizations for future distributions, which may help better management of commercially valuable plant species in Himalaya.

The general objective of the study is to assess the population structure and plant performance of *J. squamata* along elevation gradient in Manang Valley of Manang district in Western Nepal.

Specifically we aim to

1. Assess habitat characteristics and frequency of associated species with *J. squamata* along elevation gradient
2. Study vegetation assemblages with *J. squamata* along elevation gradient
3. Assess relation between reproductive and vegetative traits of *J. squamata*.
4. Study variation in population density of seedlings, juveniles and adults of *J. squamata* along elevation gradient.

CHAPTER II: MATERIALS AND METHOD

2.1 Study Species

Gymnosperms (*gymnos*-nacked; *sperma*-seed) are one of the most ancient groups of plant including about 700-800 species belonging to 70-75 genera. Among gymnosperms, conifers are most dominant with about 550-600 species belonging to 60 genera forming most of the dominant forests. Conifer forests are also typical of the Himalayas (Dar and Dar 2006).

Juniperus (Family: Cupressaceae) is the second most diverse genus of conifer comprising approximately 75 species and 27 varieties growing on the Laurasian land mass (northern hemisphere), except *J. procera* Hochst. ex Endl., which grows along the rift mountains in east Africa into the southern hemisphere; and some of the Mediterranean junipers such as *J. oxycedrus*, *J. phoenicea*, and *J. thurifera* which grow in the mountains of the northernmost part of Africa (Adams 2014). The genus *Juniperus* is divided into three sections: Caryocedrus (1 species, *J. caryocedrus*), *Juniperus* (14 species, including *J. communis*) and Sabina (approximately 50 species, including *J. squamata*) (Adams 1995).

Junipers, reproductive cones develop fleshy parenchyma surrounding seeds, dispersed by frugivore vertebrates (Garcia 1998). Evergreen species of the genus *Juniperus* can be separated from rest of the genera of Cupressaceae in that the female cone of the former species has seed-scale complexes completely fused and becoming fleshy to form a berry like fruit enclosing the seeds (Dar and Christensen 2003). The genus *Juniperus* is the source of numerous cultivars that are widely used for landscaping around the world (Adams 2008). Species of the genus *Juniperus* are of exceptional value, furnishing the greater proportion of timber and resin requirement, and in addition they are also the dominant forest makers of the world and play a significant role in the preservation of the environment (Tewari *et al.* 2010). In Africa, Asia, Europe, and the Caribbean countries, many species of the *Juniperus* are said to be threatened or endangered because of habitat loss, degradation and timber extraction (Adams 2014; Willson *et al.* 2008). However, in the South-Western hemisphere, many of the *Juniperus* species of section-*Sabina* are expanding their distributions on relatively dry, lower elevation habitats to the point of being “weedy” (Adams and Demeke 1993; Jackson *et al*. 2002; Miller *et al.* 1994; Willson *et al.* 2008).

In South Asia, the Himalayan Mountains provide habitat to about 10% of the world *Juniperus* species (Dar and Christensen 2003). The junipers of the Himalaya consist of *J. communis* L. var. *saxatilis* Pall., *J. indica* Bertol. var. *caespitosa* Farjon, *J. indica* var. *indica* Bertol., *J. rushforthiana* (R.P. Adams) R.P. Adams [syn. *J. indica* var. *rushforthiana* R.P. Adams], *J. recurva* Buch.-Ham. ex D. Don, and *J. squamata* Buch.-Ham. ex D. Don (Adams *et al*. 2009; Adams 2014). Four species of *Juniperus* are found in Nepal, of which the bushy and shrubby forms are *Juniperus communis* L., *J. squamata* Buch.-Ham. ex D. Don and the tall shrub or tree form are *J. recurva* and *Juniperus indica* Bertol. (Shrestha 1982; Ghimire *et al.* 2008). Manang hosts all these four species of junipers.

Juniper is extended all over Nepal above the altitude of 2,800 m asl in association with other vegetations. Juniper dominant forests are found widespread above 3,200m in several parts of Nepal (Chaudhary 1998). It is extended from east to west Nepal above the altitude of 2,800 m asl in association with other vegetations like *Populus ciliata*, *Rosa sericea, Berberis aristata*, *Hippophae salicifolia*, *Populus ciliata*, and *Salix babylonica* (Chaudhary 1998; Ghimire *et al.* 2001). Junipers also occur as a component of the montane scrub species community and grow up to the tree line. Junipers are the most important fuel-wood species of the Nepal Himalayas. They generally grow in poor, rocky and sandy soils (Adams 2014). Junipers are used as medicine (to treat cough, blood pressure, headache, dizziness, skin diseases, lung problems, etc.), fuel, incense, fence, manure and for many other purposes in Nepal (Bhattarai *et al.* 2006).

2.1.1 Taxonomy

J. squamata is an erect or procumbent, polymorphic shrub or small tree, which is up to 12 m tall with compact growth; branches ascending or horizontally spreading; branchlets densely arranged, straight or curved, usually short, not angled. Leaves are found in whorls of three, spreading or ascending, sometimes nearly appressed, needlelike, straight or slightly curved, (2.5-) 5-10 × 1-1.5 mm, green, slightly concave, with white stomatal bands adaxially, obtusely ridged with longitudinal, thin groove on ridge or at base abaxially, base decurrent, apex acute or acuminate. Pollen cones are terminal, ovoid, 3-4 mm; microsporophylls 9-12, each with 3 pollen sacs. Seed cones black or bluish black when ripe, ovoid or subglobose, 4-8 × 4-6 mm. Single seeded female cone. Seeds ovoid, 3.5-6 × 2-5 mm, ridged, with resin pits.

<u>Phenology</u>: Flowering in June-July. Female cones ripen in July-October.

2.1.2 Use

The genus *Juniperus* consists of all economically and medicinally important species. According to Bhattarai *et al.* (2006), junipers are used as medicine (to treat cough, blood pressure, headache, dizziness, skin diseases, lungs problems, etc.), fuel-wood, incense, fence, manure and reduces soil erosion. According to Manandhar (1987 and 2002), plant powder mixed with water is applied to treat skin diseases. Barks, fruits and leaves are the most important plant parts used for medicine. The species *squamata* is also found to be used in animal shed to remove insects and is used to cure animal's scabies and wound. Leaves of juniper species are used to extract essential oil, known as "juniper oil" (Adams 1996, 2014). The leaves oil of *J. squamata* constitute of α-pinene and sabinene, limonene, cis- and trans-thujone, sis-thujopsene and 8-α-acetoxyelemol. It also contains monoterpene alcohol, sesquiterpene and sesquiterpene alcohol (Adams *et al.* 1996).

2.2 Study Area

2.2.1 Overview of study area

Manang district is located in the trans-Himalayan region and covers about 25% of the total area within Annapurna Conservation Area, in between 28^{O} 77'N to 28^{O}43'N latitude and 83^{O} 44'E to 84^{O} 34'E longitude and elevation ranging from 1,830m asl to 8,156m asl. Southern part of Manang consists of chains of Himalayas like Manaslu, Annapurna I, II, III and IV. In past, this area was famous as a trade route between Nepal and Tibet. Nowadays, the area has been established as a well known eco-tourism destination in Nepal. The district is also unique for richness of biological resources, spectacular sceneries, indigenous culture and tradition (NTNC 2008). Manang district is one of the most sparsely settled districts with a total population of 6,538 (CBS 2011) within 2,246 sq.km area settled in 1,772 households.

Within Manang, our study area is located in north-eastern part of Upper Manang valley, lies in the coordinates between 28^{0}40' N and 84^{0}01' E with an elevation gradient of 3,450m asl to 4,300 m asl. The Upper Manang valley is covered by snow during winter for about 5 months (November to March). Analysis of temperature and precipitation data of over 30 years of the nearest meteorological station at Chame (lat: 28^{0}33', long: 84^{0}14', elevation: 2,680 m asl) revealed the annual mean maximum temperature of 16.73^{0}C, annual mean minimum temperature of 4.8^{0}C, and average annual precipitation of 948.73 mm. Highest precipitation (1,053.8 mm) was recorded in monsoon season.

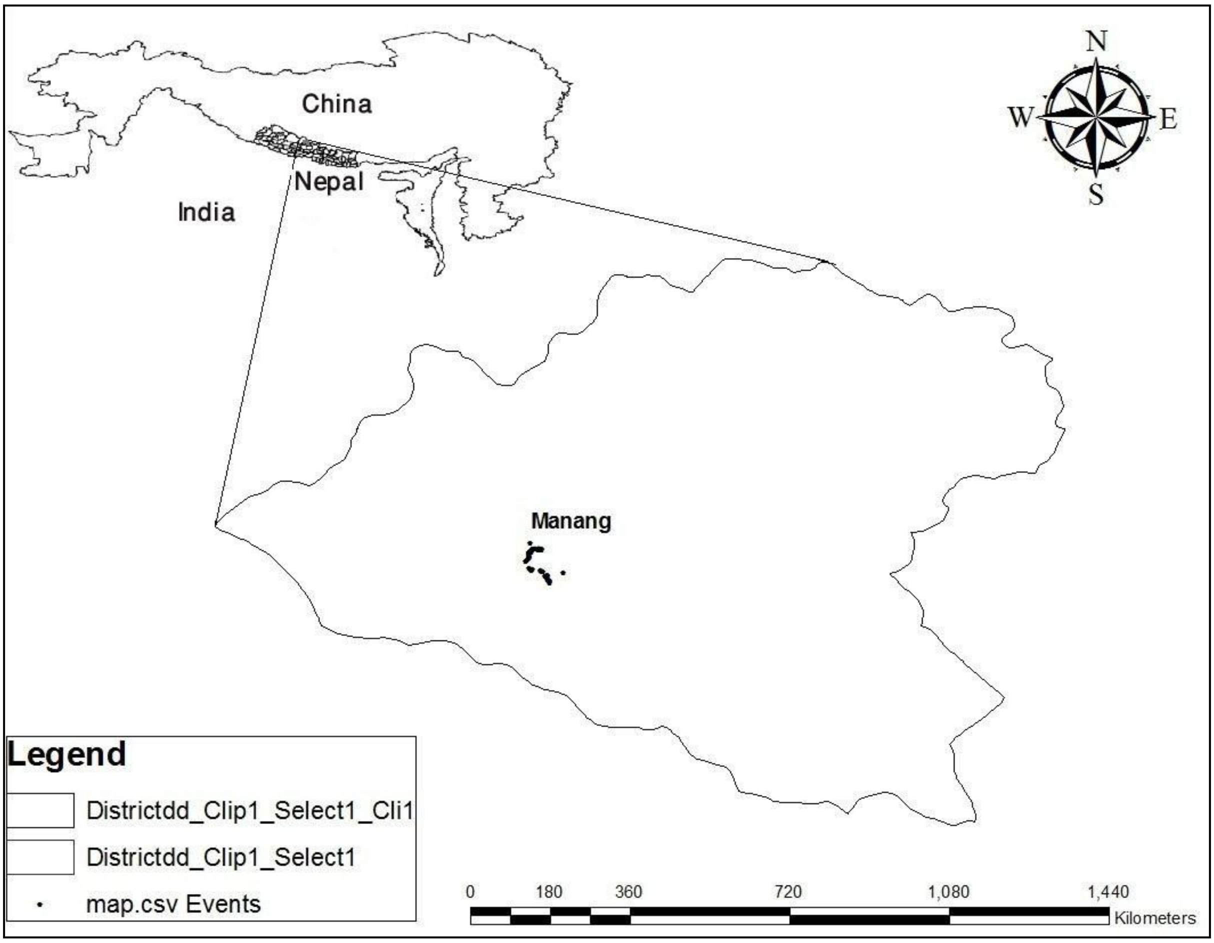


Figure 1: Map of study area

2.2.2 Vegetation

The vegetation of the study area is quite similar to that of Tibetan Plateau. In this valley, forest vegetation is not well developed. The dominant tree species are *Abies spectabilies* and *Pinus wallichiana* with scattered trees of *Betula utilis* in north facing slopes, whereas south facing slopes are characterized by *Juniperus indica* and *J. recurva* forming scattered thickets of bushy vegetation at high elevations (>4,000 m asl), but remaining arboreal in habit at lower elevations (<4,000 m asl) (Joshi 2013). The other components of woody thickets at subalpine level (3,000-4,000 m asl) are *J. squamata*, *Lonicera obovata*, *Rhododendron lepidotum* and *Caragana jubata* (Chaudhary, 1998). The alpine meadows above tree line are dominated by herbs and few scrubs like *Anaphalis royleana*, *Bistorta macrophylla*, *Gentiana depressa, Gentinella pedunculata, Kobresia nepalensis, Polygonatum cirrhifolium, Potentilla argyrophylla*, *Veronica ciliate*, *Rhododendron anthopogon, Salix calyculata, Primula primulina*, *Pedicularis pectinata, Artimesia falconeri* etc. Upper Manang valley harbors rich ethnobotanical knowledge that still persists widely among the indigenous people (Chaudhary *et al.* 2007; Pohle 1990; Rajbhandari 2001). Major medicinal herbs in trade from Manang are: *Ophiocordyceps sinensis*, *Dactylorhiza hatagirea*, *Nardostachys jatmansi*, *Allium hypsistum*, *Hippophae salicifolia*, *Picrorhiza kurroa*, *Paris polyphylla*, *Acorus calamus*, *Rheum australe*, *Eephedra girardiana*, *Swertia chirayita*, etc.

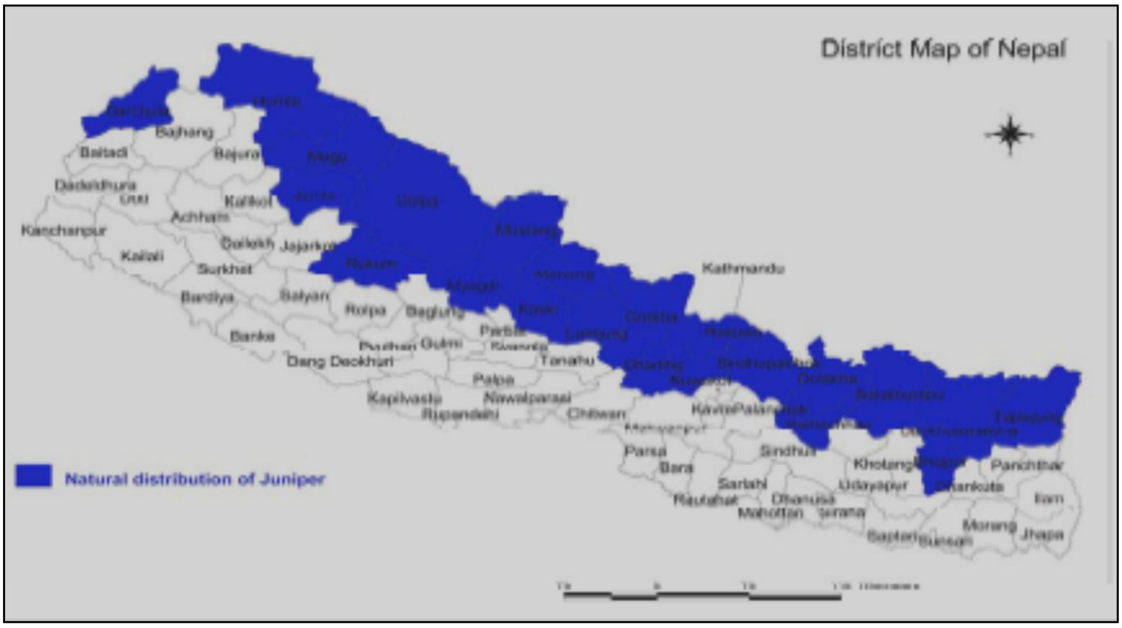


Figure 2: Junipers in Nepal Himalaya. Map Source: GTZ (2010).

2.3 Methods

2.3.1 Sampling design and data collection

Field study was conducted during September 2011 in north-eastern part of Manang Valley. Vegetation sampling was conducted along the elevation gradient from 3400m asl (in the river side of Brakha VDC) to 4,300 m asl (Ice Lake). The study area was divided into three elevation bands: high band (4,000m-4,300m asl), mid band (3700m-4000m asl) and low band (3,400m-3,700m asl). Population structure of *J. squamata* occurring in each study stands along the elevation gradient was studied using systematic random sampling method. Total nine transects were defined in the study area each containing six sample quadrats of 10m×10m size in about 100m (±36.71m) intervals. So there were altogether 54 quadrats.

For population study, individuals of *J. squamata* were divided based on plant height, stem circumference and reproductive maturity into three different growth stages (Schemske *et al.* 1994): seedlings (height <10 cm and/or stem circumference <4cm), juveniles (height 10-

40cm and/or stem circumference 4-6 cm) and adult (height usually >40 cm, stem circumference >6 cm and/or bearing reproductive structure). In each quadrat, the number of seedling, juvenile and adult individuals were counted separately, and fruit numbers were recorded from each mature/adult species.

In each quadrat, one mature individual was selected randomly and its leaves were collected for leaf biomass. A quadrat of size 25cm×25cm (0.0625m^2) was laid randomly over the crown of mature individual to collect the leaf sample. Thus collected leaves were kept in separate paper bags then labeled; and fresh weight was taken using spring balance. The sample leaves were shade-dried and kept in separate cloth bag and finally was transported to the university laboratory. Leaf samples were oven dried at 60℃ for 72 hours and dry weight was recorded using digital weighing balance. Adult individuals were not found in 6 quadrats so the collection of leaf sample was possible only from 48 quadrats.

2.3.2 Habitat parameters

Habitat parameters like latitude, longitude, altitude, slope, aspects, disturbance and vegetation assemblages were recorded for each sampling quadrat. Aspect, slope and altitude were recorded by using compass, clinometer and altimeter respectively. Similarly, latitude and longitude as well as altitude were recorded with the help of Global Positioning System (GPS) device. Altitude data was standardized using data both from GPS and altimeter to minimize error. In each quadrat, disturbance was recorded as a measure of anthropogenic disturbance. Indicators recorded for measuring disturbance included trampling, dung deposition and grazing or browsing. In addition, natural impacts were recorded by observing landslides, fallen rocks and river damage. Disturbance was recorded by assigning by categorical value as 0 (no disturbance), 1 (low disturbance), 2 (moderate disturbance) and 3 (high disturbance) based on visual estimation method. Each of 54 quadrats was further divided into 4 sub-quadrats of 5m×5m size. In each sub-quadrat, presence and absence (1 and 0 respectively) of plant species associated with *J. squamata* was recorded to study the frequency and abundance.

2.3.3 Association of *J. squamata* with different vegetation assemblages

Plant species dominant in the quadrats and sub-quadrats were considered for designing vegetation assemblages/ types. The vegetation assemblages were identified based on percentage coverage of dominant species. *J. squamata* growing in poor, rocky and sandy soils are found associated with major shrubs like *J. indica, Rosa sericea*. The other species found in the habitat include those of *Rhododendron, Cotoneaster, Berberis, Gentiana, Primula, Androsace, Potentilla, Bistorta* and several species of grasses (Panthi *et al.* 2007). *Pinus wallichiana* is found dominated in low-elevation whereas shrubs like *Cotoneaster, Berberis* and *Rosa* were found mostly dominated in mid-elevation. Similarly, herb and ground species were dominated in high-elevations.

2.3.4 Specimen collection and identification

Plant specimens present in sampling quadrats were collected for proper identification. Some specimens were identified in field based on standard taxonomic reference like Malla *et al.* (1976), Polunin and Stainton (1984) and Stainton (1988). The unidentified specimens were compared with relevant specimens deposited at National Herbarium and Plant Laboratories (KATH) and Tribhuwan University Central Herbarium (TUCH).A set of herbarium specimen were deposited in TUCH after proper identification. Botanical Nomenclature follows Press *et al.* (2000). Four species of *Carex* and one of each *Coelogyne, Conyza, Equisetum, Tanacetum* and *Trifolium* could not be identified up to specific level; but were incorporated for statistical analysis.

2.4 Statistical analysis

2.4.1 Habitat characteristics

The habitat characteristics of *J. squamata* were analyzed based on ecological traits and associated species diversity and composition. Associated species richness was calculated as the total number of species per quadrat (10m×10m). Abundance of each species was calculated by combining presence-absence data from all four sub-quadrats. The species data was used to calculate their frequencies in overall quadrats and in quadrats from three elevation bands (low, mid and high).

Aspect was folded about the northeast-southwest line (NE becoming zero degree and SW becoming 180^{O}) to find folded aspect (folded aspect = 180 – |Aspect – 180|) which was used to calculate heat load index (McCune and Keon 2002).

$\text{Heat Load Index}\ (\text{MJ cm}-2\ \text{yr}-1) = \frac{1-\text{Cos}(\theta-45°)}{2}$ [Here, Θ represents folded aspect.]

Approximation of heat load was conducted to make the scale symmetrical about the northeast-southwest line. The above equation rescales aspect to 0-1, with zero being the coolest slope (northeast) and one being the warmest slope (southwest).It gives a relative value of how much solar radiation a particular spot receives.

Bio-physical variables recorded in quadrats of three elevation bands were compared using one-way ANOVA when the data met assumptions of parametric test (i.e. normal distribution and homogeneity of variance). Bio-physical variables that did not meet the assumption of parametric test even after transformations were treated with non-parametric tests.

2.4.2 Density and size distribution of *J. squamata*

Density and population structure of *J. squamata* were analyzed for each quadrat and each elevation band (Bharali *et al.* 2012). Density is the number of individual per unit area. Density gives the numerical strength of species and was calculated by using following formula:

$$\text{Density (per hectare)} = \frac{\text{Total number of individuals in all plots}}{\text{Total number of plots in one transect} \times \text{size of plot}} \times 100 \times 100$$

The relative proportions of seedling, juvenile, adult and total density were calculated to identify population structure. Rejuvenation/regeneration potential of *J. squamata* was evaluated based on the density of seedlings and juveniles (Shankar, 2001).It was expressed by following formula:

$$\text{Rejuvenation} = \frac{\text{Seedling density} + \text{Juvenile density}}{\text{Adult density}}$$

2.4.3 Variation in vegetative and reproductive traits

Vegetative (plant height, circumference of stem and leaf dry weight) and reproductive (fruit production per plant) traits were computed for each adult individual in the respective elevation band. The total data set comprised of 362 adult individuals for which plant height and circumference of stem were recorded. Leaf dry weight was measured for a total of 48 individuals from each quadrat where adult individuals were present. Sample size for estimating fruit production comprised of 48 individuals that bore fruits during the study period, of which 15, 17 and 16 individuals were recorded in fruiting condition from low- mid- and high-elevation band, respectively. Vegetative and reproductive traits in three elevation bands were compared using one-way ANOVA.

Relationships between vegetative and reproductive traits were analyzed by calculating Pearson correlation coefficients. The data were first checked for normality and homogeneity of variance, when the data (plant height, circumference of stem and number of fruits) appeared not normal, they were log transformed for normality. Those traits that exhibit statistically significant correlations were further analyzed through linear regression analysis to evaluate the strength of relationships.

CHAPTER III: RESULTS

3.1 Habitat Characteristics

Juniperus squamata was found growing in drier regions with rocky habitats and sandy substrates facing SE- to SW- slopes of Manang valley ranging from 3,451m to 4,261m asl. The major dominant species in the study area was juniper (*J. squamata, J. indica* and *J. communis*) whereas woody species were dominant towards lower elevation and herbaceous species towards higher elevation. Most of the vegetations were characterized by subalpine-alpine shrubland and meadows.

Altogether, 94 species (belonging to 37 families and 63 genera) were found to be associated with *J. squamata*. Asteraceae was the largest family (10 genera and 17 species), followed by Rosaceae (5 genera and 8 species) and Rananculaceae (4 genera and 5 species. There were 26 monogeneric families: Cupressaceae consisting of 3 species, Caprifoliaceae, Convallariaceae, Ericaceae, Euphorbiaceae and Saxifragaceae each consisting of 2 species and rest of the monogeneric species were monospecific. *Carex, Gentiana* and *Pedicularis* were the largest genera each comprising 4 species; followed by *Anaphalis, Artemisia, Aster* and *Potentilla* each comprising 3 species; and *Thalictrum, Tanacetum, Swertia, Saxifraga, Rhododendron, Polygonatum, Lonicera, Juniperus, Gentianella, Euphorbia, Cotoneaster, Bistorta, Astragalus* and *Androsace* each comprising 2 species. Rest 42 genera associated were found monospecific (Appendix I). We reported only three species of trees, i.e. *Betula utilis, Pinus wallichiana* and *J. indica* in the study area.

Out of 94 species associated with *J. squamata*, highest number of species (n=75), were present in the high elevation band, followed by mid (n=64) and low (n=36) elevation bands (Fig. 3). High elevation band comprised of 29 families and 52 genera whereas mid elevation band comprised 24 families and 43 genera. Lowest number of families (19) and genera (29) was present in low elevation band. The study discovered linear inclination of all family, genera and species number from lower to higher elevation band.

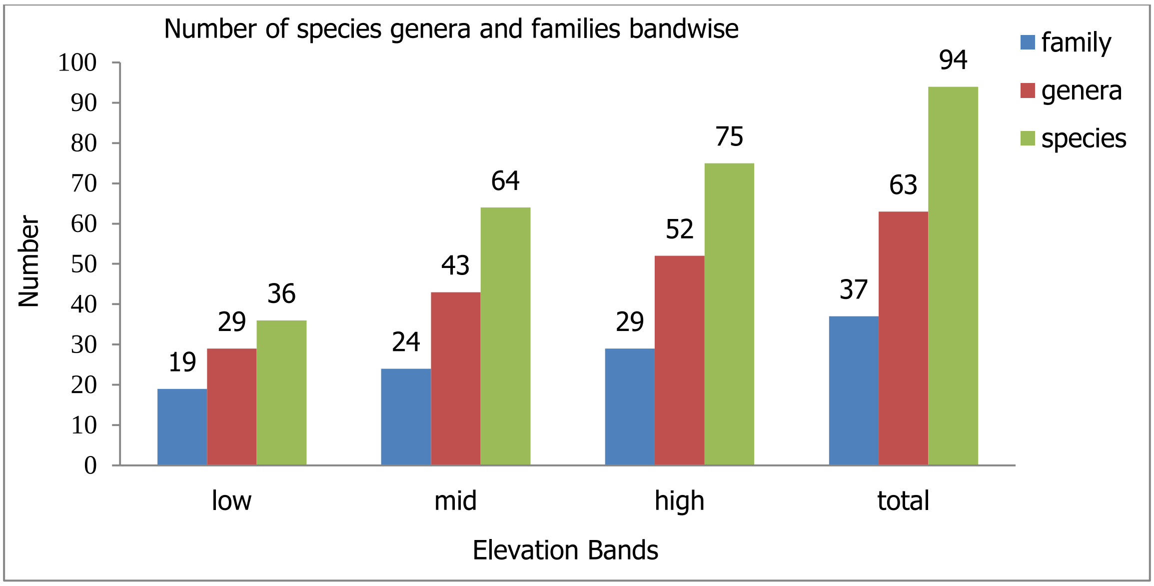


Figure 3: Number of associated species with *J. squamata* in three elevation bands along with number of genera and families.

15 major vegetation assemblages associated with the study species were identified in total 54 quadrats based on visual estimation method. Highest dominancy of Juniper woodland was observed in 17 quadrats. Juniper-*Cotoneaster* and Juniper-*Rosa* woodland vegetation assemblage were dominated in each five quadrats whereas *Berberis*, *Rosa* woodland and grassland vegetation assemblage were repeated in each four quadrats (Table 1). Similarly, four vegetation assemblage types, i.e. *Cotoneaster-Berberis*, Juniper-*Spiraea*, *Lonicera* and *Rosa-Berberis* woodland, were present in each single quadrat.

Five vegetation assemblage types (Juniper, Juniper-*Cotoneaster*, Juniper-*Rosa*, Grassland and *Rosa* woodland) were found dominantly distributed in all elevation bands (low, mid and high) whereas Juniper-*Berberis* dominated woodland was present in low and mid elevation bands and Juniper-*Lonicera* woodland was dominated in mid and high elevation bands. Similarly, juniper dominated grassland; Juniper-Pine and *Cotoneaster-Berberis* woodland were dominated only in low elevation band. Only two vegetation assemblage, i.e. *Cotoneaster* and *Lonicera* woodland were dominated in mid elevation bands; and Juniper-*Spiraea*, *Berberis* and *Rosa-Berberis* woodland were present in high elevation band only.

Table 1: List of vegetative assemblage types observed during study.

S.N.	Vegetation assemblages type	repeated number in plots	Band	Major associated species
1	Juniper woodland	17	LMH	*J. squamata, J. indica, J. communis, Rhododendron anthopogon, Tanacetum dolichophyllum, Berberis aristata Rosa sericea* and *Aster himalaicus.*
2	Juniper-*Cotoneaster* woodland	5	LMH	*J. squamata, J. indica, J. communis, Cotoneaster microphyllus, Rosa sericea, Berberis aristata,* and *Tanacetum* sp.
3	Juniper-*Rosa* woodland	5	LMH	*J. squamata, J. indica, J. communis, Rosa sericea, Berberis aristata, Lonicera hypoleuca* and *Potentilla* sp.
4	*Berberis* woodland	4	H	*Berberis aristata, J. squamata, Corydalis juncea, Tanacetum dolichophyllum, Delphinium brunonianum, Rhododendron lepidotum Tanacetum* sp. and *Potentilla* sp.
5	Grassland	4	LMH	*Carex* sp., *J. squamata, Aster* sp., *Rhododendron anthopogon* and *Tanacetum dolichophyllum.*
6	*Rosa* woodland	4	LMH	*Rosa sericea, J. squamata, J. indica, Carex* sp., *Berberis aristata, Rhododendron anthopogon* and *Tanacetum* sp.
7	Juniper-*Berberis* woodland	3	LM	*J. squamata, J. indica, J. communis, Berberis aristata, Rhododendron anthopogon* and *Lonicera hypoleuca.*
8	*Cotoneaster* woodland	2	M	*Cotoneaster microphyllus, J. squamata, Carex* sp., *Aster* sp.,*Corydalis juncea* and *Tanacetum* sp.
9	Juniper dominated Grassland	2	L	*J. squamata, J. indica, J. communis, Carex* sp., *Cotoneaster microphyllus, Tanacetum dolichophyllum, Berberis aristata* and *Corydalis juncea* and *Rhododendron anthopogon.*
10	Juniper-*Lonicera* woodland	2	MH	*J. squamata, J. indica, Lonicera hypoleuca, Rosa sericea, Corydalis juncea, Tanacetum* sp., *Aster* sp., *Rhododendron lepidotum* and *Potentilla* sp.
11	Juniper-Pine woodland	2	L	*J. squamata, J. indica, J. communis, Berberis aristata* and *Lonicera hypoleuca.*
12	*Cotoneaster-Berberis* woodland	1	L	*Cotoneaster microphyllus, Berberis aristata, Aster* sp., *J. squamata, J. indica, J.communis and Rhododendron anthopogon.*
13	Juniper-*Spiraea* woodland	1	H	*J. squamata, J. indica, Carex* sp., *Tanacetum* sp., *Rosa sericea, Rhododendron lepidotum* and *Potentilla peduncularis.*
14	*Lonicera* woodland	1	M	*Lonicera hypoleuca, J. squamata, Rosa sericea, Carex* sp., *Corydalis juncea* and *Tanacetum dolichophyllum.*
15	*Rosa-Berberis* woodland	1	H	*Rosa sericea, Berberis aristata, J. squamata, Carex* sp., *Tanacetum* sp., *Rhododendron lepidotum* and *Potentilla peduncularis.*

L= low, M= mid and H= high elevation band.

Bio-physical variations were observed in three elevation bands (Table 2). Higher disturbance was observed in low elevation band, followed by mid and high elevation bands (Table 2). Associated species abundance and richness were found to be significantly high in quadrats at high elevation band and the values were decreased gradually in quadrats towards lower elevation bands (Table 2).

Table 2: Bio-physical variables (mean ± SE) for all 54 quadrats recorded in *J. squamata* growing sites in three elevation bands (low, mid, high) in Manang.

Variables	Low	Mid	High	Overall	*F*	*p*
Elevation (m)	3546.56 ± 30.09	3857.17 ± 22.92	4153.17 ± 18.6	3852.3 ± 36.71	155.36	<0.001
Slope	40.28 ± 5.17	26.11 ± 3.2	18.61 ± 2.71	28.33 ± 2.5	8.2071	0.01
Disturbance	1.83 ± 0.19	1.61 ± 0.16	1.17 ± 0.09	1.54 ± 0.09	4.9713	0.011
HLI	1.08 ± 0.11	1.03 ± 0.11	1.15 ± 0.08	1.09 ± 0.06	0.3675	0.69
Species abundance*	31.72 ± 2.39	50.11 ± 4.67	63.89 ± 3.5	48.57 ± 2.74	19.663	<0.001
Species richness**	14.33 ± 0.59	20 ± 1.64	26.11 ± 1.37	20.15 ± 0.98	21.18	<0.001

*Associated species abundance is sum of vascular plant species repeated in subquadrats per quadrat.

** Associated species richness is number of vascular plant species associated with *J. squamata* per quadrat. *p* values based on one-way ANOVA.

Frequency of adult *J. squamata* was found to be 83.33%, 94.44%, and 88.88% in low, mid and high elevation bands respectively, with an overall frequency of 88.88%. Among the species associated with *J. squamata* from all sampling quadrats, the highest frequency was shown by *Juniperus indica* (88.89%), followed by *Tanacetum dolichophyllum* (87.04%), *Berberis aristata* (72.22%), *Rosa sericea* (61.11%) and *Cotoneaster microphyllus* (57.41%) (Appendix II).

In low elevation band, highest frequency was estimated for *Rhododendron anthopogon* and *Rosa sericea* (94.44%), followed by *Juniperus indica* (88.88%), *Juniperus communis* (83.33%), *Tanacetum dolichophyllum* (83.33%), *Berberis aristata* (77.77%) and *Aster himalaicus* (72.22%). Similarly, for mid elevation band, highest frequency of associated species was of *Juniperus indica* (88.89%) followed by *Cotoneaster microphyllus* and *Tanacetum dolichophyllum* (each 77.77%), *Berberis aristata*, *Carex* sp. 3 and *Lonicera hypoleuca* (each 61.11), and *Corydalis juncea* and *Rhododendron anthopogon* (each 55.6%). Finally, in high elevation band, highest frequency was calculated for *Delphinium brunonianum* and *Tanacetum dolichophyllum* (each 100%), *Rhododendron lepidotum* (94.44%), *Juniperus indica* (88.89), *Tanacetum* sp. (83.33%) and *Berberis aristata*, *Corydalis juncea* and *Potentilla peduncularis* (each 77.8%; Appendix II).

Pearson correlation among habitat variables showed that altitude had strongest relationship with associate species richness and associate species abundance (r=0.673 and r=0.658 respectively). On contrary, altitude was negatively correlated with slope and disturbance. Slope was positively correlated with disturbance. Similarly, associate species richness was positively correlated with associate species abundance (Table 3).

Table 3: Pearson Correlation among habitat variables.

Variables	Attributes	Alt	Sl	Distb	HLI	AsSpRch	AsSpAbu
Altitude	Alt	1	-0.572**	-0.393**	0.088	0.673**	0.658**
Slope	Sl		1	0.383**	-0.078	-0.260*	-0.292*
Disturbance	Distb			1	-0.14	-0.183	-0.214
Heat Load Index	HLI				1	-0.071	-0.057
Associate species richness	AsSpRch					1	0.966**
Associate species abundance	AsSpAbu						1

** Correlation is significant at the 0.01 level (1-tailed).

* Correlation is significant at the 0.05 level (1-tailed).

3.2 Density of *J. squamata*

Population of *J. squamata* in three elevation band in study area was composed of 873 individuals (Fig. 4) with high number of individuals contributing in mid elevation band (436 individuals) followed by high and low elevation bands (311 and 126 individuals respectively). Number of individuals was high at moderately disturbed area and least in highly disturbed area.

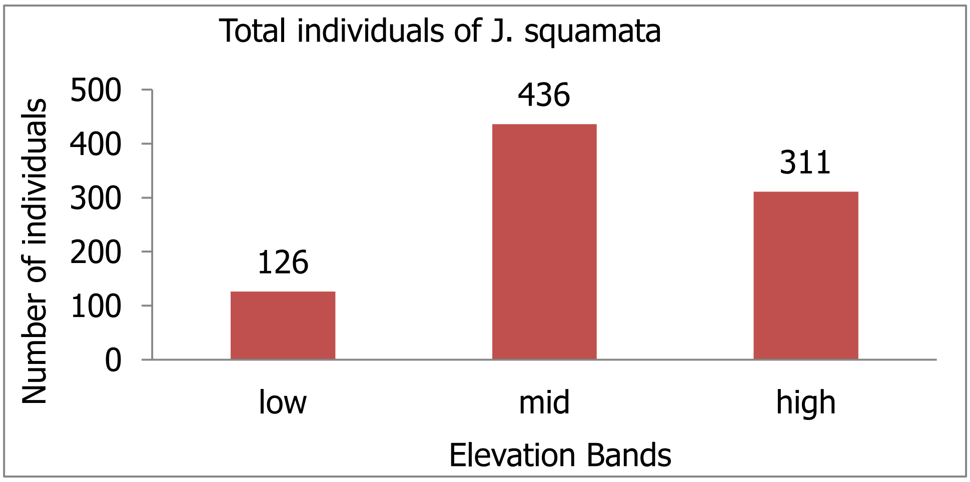


Figure 4: Number of individuals of *J. squamata* in three elevation bands in Manang valley.

Density of *J. squamata* combining all quadrats was 4850 per hectare (Fig. 5). The overall density of each seedling, juvenile and adult was found to be 2011.11, 1388.89 and 1450.00 per hectare respectively. In low elevation band, the density of seedling, juvenile and adult was 150, 305.56 and 244.44 per hectare respectively which were least value among three elevation bands. Seedling, juvenile and adult density were calculated to be maximum at mid elevation band (1083.33, 655.56 and 683.33 per hectare respectively). Likewise, moderate density of seedling (777.78 per hectare), juvenile (427.78 per hectare) and adult (522.22 per hectare) was found in high elevation band.

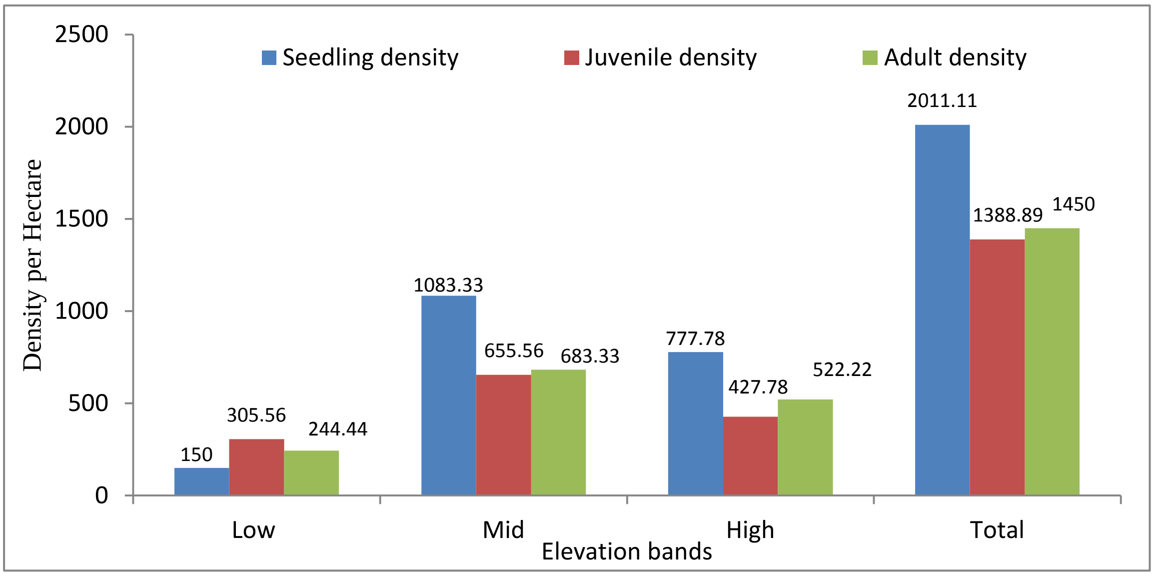
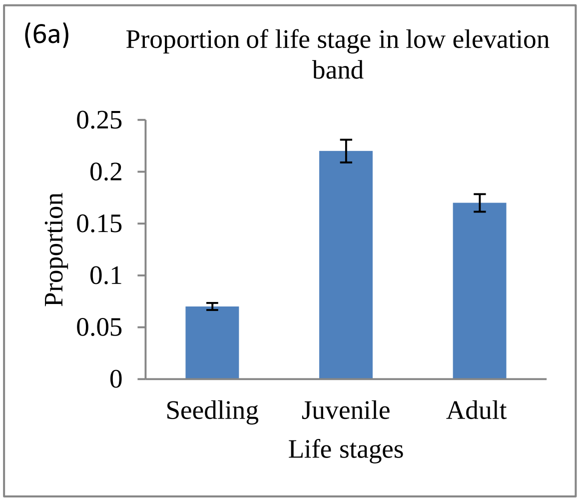
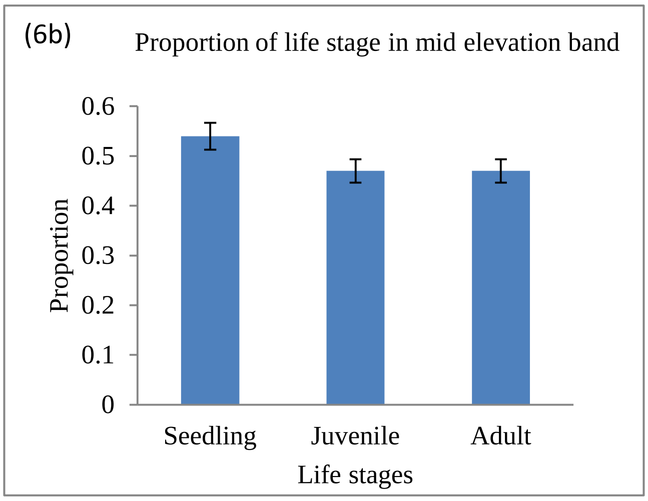


Figure 5: Density (number of individuals per hectare) of *J. squamata* recorded in three elevation bands in Manang valley.

The proportions of seedling, juvenile and adult of *J. squamata* in all 54 quadrats were evaluated to be 0.41, 0.286 and 0.298 respectively. In overall data, juvenile and adult proportional was almost similar. In mid elevation band, the proportion of all growth stages was found to have highest numerical value whereas low elevation band have least proportion value. Similarly, higher value of rejuvenation at mid elevation bands shows strong regeneration strength than that of high and low bands. Minimum rejuvenation was observed in low elevation band (Fig. 6).





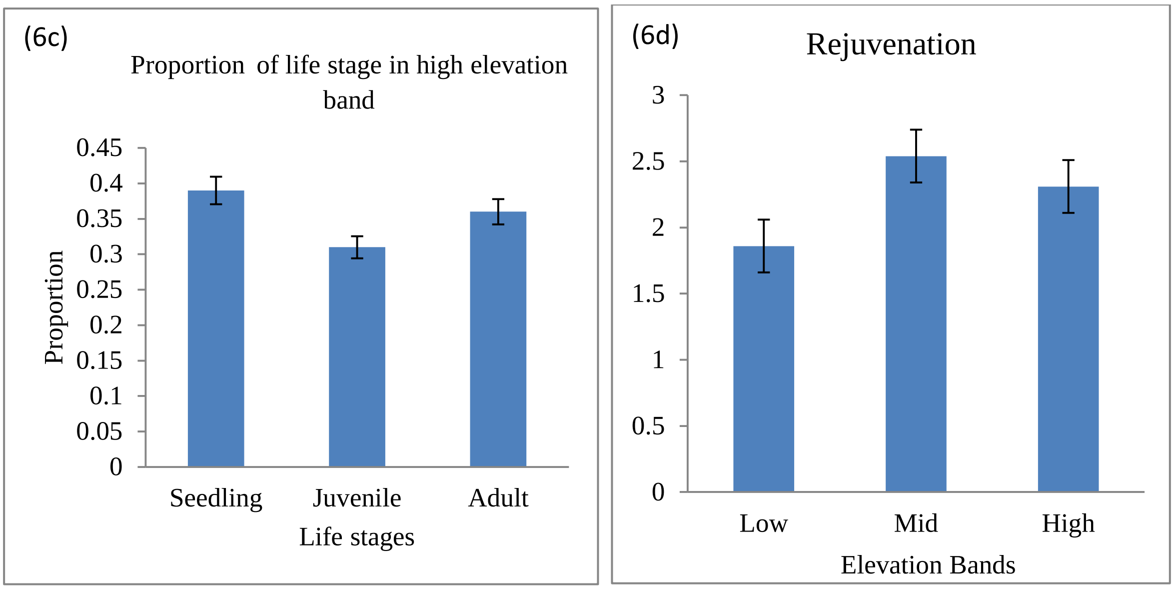


Figure 6: Population structure/proportions of seedling, juvenile, adult and rejuvenation of *J. squamata* at three elevation bands in Manang valley. Rejuvenation expressed as sum of seedling and juvenile density divided by adult density.

3.3 Vegetative and Reproductive Traits of *J. squamata*

3.3.1 Variation in vegetative and reproductive traits

We calculated mean and standard error value of vegetative and reproductive traits of *J. squamata* (Table 4). The mean height of all the adult individuals was 59.11±2.12cm. Similarly, mean circumference and leaf dry weight per 0.0625 m^2 canopy were 9.48±0.25cm and 48.49±1.13gm respectively. Number of fruits was calculated to be 84.88±6.2 per mature/adult plant. Plant height and circumference were tended to be high at high elevation band whereas leaf dry weight was found high at low elevation band. Number of fruits per plant was significantly high in adult individuals growing at high elevation band that linearly decreased towards lower elevation bands (Table 4).

Table 4: Mean and standard error (SE) values of vegetative and reproductive traits of adult/mature *J. squamata* recorded in Manang valley.

		Mean					
Traits	N	Low	Mid	High	Overall	*F*	*P*
Plant height (cm)	48	55.22±4.89	58.92±2.69	62.97±3.32	59.11±2.12	1.088	0.364
Circumference (cm)	48	8.92±0.63	9.62±0.26	9.85±0.38	9.48±0.25	1.19	0.312
Leaf dry weight*	48	50.18±1.75	46.98±2.19	48.50±1.90	48.49±1.13	0.656	0.524
Number of fruits per plant	48	42.72±5.61	82.85±6.27	126.57±7.85	84.88±6.2	38.258	<0.001

*Leaf dry weight (g) per 0.0625 m2 canopy. *F* and *p* value based on one way ANOVA.

3.3.2 Correlation among traits

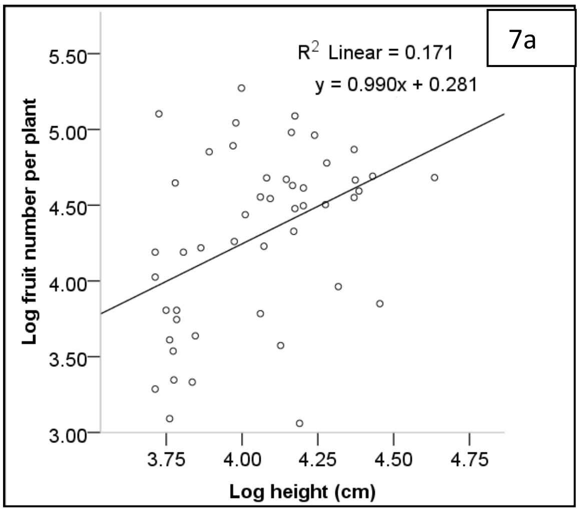
Pearson Correlation analysis revealed significant correlations between the plant height and number of fruits per plant (r= 0.373) and circumference of stem and number of fruits per plant (r= 0.308). The strength of relationship between plant height and number of fruits per plant (r^2= 0.171) and the circumference of stem and number of fruits per plant (r^2= 0.105) was observed linear (Fig 7; Table 6). On contrary, the relationship between circumference with leaf dry weight and leaf dry weight with number of fruits were negatively correlated to (Table 5).

Table 5*:* Pearson correlation coefficients among the vegetative and reproductive traits of *J. squamata*

Traits	Abbreviation	PlHt	Circum	DrWtPl	NoFr
Plant height+	PlHt	1	0.168	0.033	0.373**
Circumference +	Circum		1	-0.19	0.308*
Leaf dry weight+	DrWtPl			1	-0.019
Number of fruits+	NoFr				1

+ The values were log transformed before analysis.

Allomatric traits exhibiting statistically significant correlations with fruit output were further analyzed through linear regression analysis to evaluate the strength of relationships and derive allometric equations (Table 6, Fig. 7). Of the two allometric measurements (plant height and stem circumference), plant height was the strongest variable or predicting fruit output. The inclination of number of fruits per plant with increase in plant height was observed from analysis. Similarly, number of fruits was observed increased with increase in circumference thickness of stem also (Fig 7).



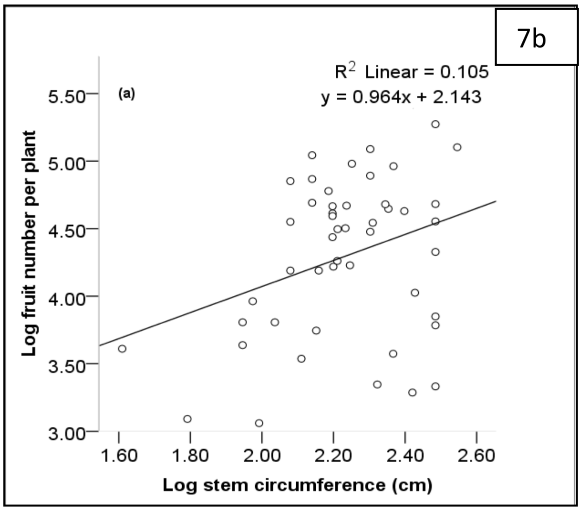


Figure 7: Relationships between (a) plant height and number of fruits per plant, and (b) Circumference and number of fruits. Fitted line based on linear regression model.

Table 6: Results of linear regression analysis predicting total fruit output per adult individual with significant tree allometric (predictor) variables in Manang. Measured variables were log transformed for regression analysis.

Response variable	Predictor variable	Intercept	Slope	R^2	SE_e	F	P
Fruit output (log)	Plant height (log)	0.281	0.990	0.171	0.532	9.489	0.003
	Circumference (log)	2.143	0.964	0.105	0.553	5.417	0.024

SE_e – standard error of the estimate.

CHAPTER IV: DISCUSSION

The present study of *J. squamata* was conducted along an elevational gradient from 3,400 m asl to 4,300 m asl in upper Manang valley of Manang district, Central Nepal. Conifers are the indicator of Himalayan forest providing a cool and soothing environment for livelihood, recreation and health (Dar and Dar 2006; Tewari *et al.* 2010). Nepal Himalaya exhibits some excellent conifer pure and mixed forests that are distributed throughout the country. Conifers like, junipers are mostly distributed from temperate to alpine ecological zone which has harsh climatic conditions in Nepal Himalaya. The distribution of the study species in Nepal ranges from east to west extending above 3,300 m asl to 4,400 m asl (Bhattarai *et al.* 2006). *J. squamata* nurture mostly in dry, rocky and sandy/gravel slopes in form of bushy patches mixed with other woody species at low and mid elevation while at high elevation it is associated with herbaceous species. Baseline studies of juniper have not been carried out in Nepal so far, this paper aims to add information regarding population structure and plant performance of *J. squamata*.

The present study showed 94 species (37 families and 63 genera) associated with population of *J. squamata* in the study area. Chapagain (2014) reported 88 associated species (37 families and 64 genera) with *J. indica* in the same study area. Similarly, Ghimire *et al.* (2008) reported 19 associated species in *J. indica* forest, representing 14 genera and 11 families. For a plant community, there are many factors (abiotic and biotic) which can affect the number of species present. Such variation in plant communities is due to variation in altitude, slope, latitude, aspect, rainfall, humidity, land use pattern and habitat heterogeneity which play vital role in formation of community structure (Bennie *et al*. 2006; Spies and Turner 1999).

In terrestrial plant community, several studies illustrate species richness declination with increasing elevations (Körner 1995; Woodward 1997). However, other studies show a hump shaped relationship in which species richness at first increases from low to mid elevations and then declines towards high elevation in Nepal Himalaya (Grytnes and Vetaas 2002). According to the previous critical literature review on species richness patterns in relation to altitude conducted in the Himalaya, the species richness decreases from mid altitude to high altitude (Bhattarai *et al.* 2004; Joshi 2013; Körner 2003; Rahbek 1997; Vetaas and Grytnes 2002) but the present result shows significant incline in species richness form lower to higher elevation. Chapagain (2014) also observed linear incline in species richness from 3,350m asl to 4,250m asl for associated species with *J. indica*. This contrary, in results regarding species richness along elevation, may be discussed under two circumstances. First, most of the previous studies represent true alpine areas, and our study area lie in transition to the subalpine and alpine zones. Second, the study area was subjected to significant disturbance like grazing, firewood collection, landslides, and tourism activities. The cumulative effect of all these disturbances may have posed lower elevation areas with decreasing species richness.

J. squamata was dominant along juniper, juniper-*Cotoneaster*, juniper-*Rosa* and *Berberis* woodland vegetation assemblage types. *J. squamata* was found to be mostly associated with *J. indica*, *Tanacetum dolichophyllum*, *Berberis aristata*, *Rosa sericea*, *Cotoneaster microphyllus*, *Carex* sp., *Rhododendron anthopogon*, and *Thalictrum cultratum*. The present overall value of associated species frequency was high for *J. indica*. Similarly, the second highest frequency was for *Tanacetum dolichophyllum* followed by *Berberis aristata*, *Rosa sericea* and *Cotoneaster microphyllus*.

The future community structure and regeneration status of the plant species could be predicted from the proportion of seedlings, juveniles and adults in the total populations of particular species (Bharali *et al.* 2012). The overall population of *J. squamata* in three elevation bands (low, mid and high) showed that contribution of seedling (362 individuals) was maximum to total population followed by adults (261 individuals) and juvenile (250 individuals). Seedling (41.47%) and juvenile (28.64%) population contributed about 70.11% of total populations which shows a reverse *J*-shaped structure, indicates a good regeneration pattern. At the same time, the result signifies the high mortality rate of seedling. Mortality might be due to different anthopogenic activities which disturb germination and growth of seedlings. The observed population structure signifies stable population of the studied species in the study area. Maximum number of total individuals in mid elevation band at 3,700-4,000m asl (436 individuals) were reported; followed by high elevation band at 4,000-4,300m asl and low elevation band at 3,400-3,700m asl (311 and 126 individuals respectively). In addition, the population of each life stages was found maximum at mid band where disturbance was observed moderate. It can be explained as: *J. squamata* can tolerate up to moderate disturbance but may be more vulnerable if disturbance impact increases continuously.

Total density of *J. squamata* in three elevation bands within the study area was 4,850 per hectare. Chapagain (2014) observed the density of *J. indica* (1,374 individuals/ ha) which is less than the present study result whereas the value (4,250 individuals/ ha) obtained by Chettri and Gupta (2007) in upper Mustang for *J. indica* is nearly similar to the result in the present study species. However, the density study conducted by Hussain *et al.* (2008) for *Abies pindrow* in Kumaon Himalaya and by Tiwari (2010) for *A. spectabilis* in Langtang national park was low, i.e. 151 trees/ha and 604trees/ha respectively. This result explains better population status of the study species in the study area than *J. indica* studied in same study area. The low, mid and high elevation bands contributed 700, 2,422 and 1,728 density per hectare respectively. Band wise highest density per hectare area for all age life stages in the present study was found in mid band. Chapagain (2014) also observed highest density for all life stages of *J. indica* in mid elevation band. Present study shows least density at the low elevation band and highest at mid elevation band. Lower value of density at low elevation band in the present study might be due to two reasons. Firstly, it might be due to different anthropogenic activities like tourism activities, grazing, trampling and cutting for daily uses such as collection of fire-wood and livelihood purposes. This can be an important finding from this study about how Himalayan biodiversity centers are being affected by anthropogenic disturbances. Second reason might be due to allelochemical influence of pine tree, quadrats of lower elevation band were associated with *Pinus wallichiana*. The needles of Pinus inhibit the growth of other species and even cause death of seedling in the surroundings (Junicost 2010; Reinoso and Carlos 2003).

Analysis of present study discovered highest degree of disturbance in lower elevation band and least in high elevation band. This might be due to anthropogenic activities like over grazing, harvesting of leaves for incense and in animal shed, fuel wood, medicinal purposes, tourism and landslide. Ward (1981), Gilbert (1980) and Clifton *et al.* (1997) observed overgrazing and anthropogenic activities as a major reason for causing threat to England juniper. In addition to anthropogenic factors, several studies have observed low amount of pollen grains and complex reproductive phenomenon as major natural problem in junipers (Otto *et al*. 2010; Juan *et al*. 2003). Negative significant of disturbance with altitude and species richness was observed, i.e. more disturbance was observed in lower elevation bands favoring few species and gradually reduced in higher elevation bands hosting more species.

Vegetative and reproductive traits of *J. squamata* were calculated elevation band wise and in overall. Plant height, stem circumference and leaf dry weights were the vegetative traits and fruit number was reproductive trait incorporated in this study. Higher values of traits were observed in high elevation band except for leaf dry weight. In contrary, Chapagain (2014) observed all trait values higher at lower band in same study area for *J. indica*. Species with wider distribution differ from each other in mean value for vegetative traits. Klinka *et al*. (1996) correlated vegetative trait (plant height) with geographical attributes like latitude, longitude and altitude. Present study also correlated plant height with elevation which showed linear growth of plant height along elevation gradient. Reduction in radial growth at higher elevation plant is related to the shortened growing period largely as a result of the delay in start of seasonal growth (Tranquillini 1979), cold climate and short growing period (Körner *et al.* 1983). Similarly, short stem of high elevation plants help to avoid the damage due to strong wind and to improve photosynthetic conditions by keeping the leaves towards the warmer soil surface (Körner *et al.* 1983). On contrary, present study found circumference thickness and plant stem height boosting up towards higher elevation individuals.

Total leaf dry weight per $0.0625m^2$ in three elevation bands was found 48.49±1.13gm and was maximum at low elevation band followed by high and mid elevation bands. This might be due to presence of old individuals in lower elevation zone and small leaves in individuals towards higher elevation due to declining temperature and low nutrient ratio. Chapagain (2014) also reported the highest leaf biomass at low elevation band for *J. indica* in Manang. Rastetter *et al.* (2004) also reported negative relationship between biomass and elevation for several species due to low nutrient availability and temperature. Many plants growing along elevation gradients have smaller leaves in higher geographical regions (Kofids and Bosabalidis 2008) which might be a reason for low leaf biomass at higher elevation plants. Junipers are often low-growing plants covering ground vegetation. Conifers are effective to windbreaks; especially those that are evergreen and some gymnosperm invade into disturbed areas or vacant agriculture land. Pines and junipers are considered as notorious invaders, making the fertile land unusable (Tripathi *et al.* 2009).

Among allometric variables considered in this study, plant height (r^2= 0.171) and circumference of plant stem (r^2= 0.105) were more strong variables predicting total reproductive output. Measurement of basal trunk diameter and canopy height is difficult in bushy juniper like *J. squamata* because it has a very compact canopy with a high density of low growing stems (Ansley *et al.* 2012). None of the allometric traits considered in this study showed strong power for predicting leaf dry weight. Plants in lower elevation band are generally larger in size which favors production of greater number of fruits than those in higher elevation bands. Few numbers of fruits produced per plant in high elevation zones may also be due to limited pollination success rate and environmental factors (Juan *et al.* 2003). Conversely, this study reported large number of fruits tended in higher elevation individuals than in lower elevation. This may be due to greater disturbance in lower elevation.

CHAPTER V: CONCLUSION AND RECOMMENDATIONS

Juniperus squamata was the major dominant species along with *J. indica, Tanacetum dolichophyllum, Berberis aristata, Rosa sericea* and *Cotoneaster microphyllus* in alpine and subalpine zones. It was found to prefer dry and rocky habitats in South-east to south-west slopes in upper Manang valley. Altogether 94 species were found associated with study species in 15 different vegetation assemblages within the study area. *J. squamata* mostly preferred the habitat associated with Juniper, Juniper-*Rosa*, Juniper-*Cotoneaster*, *Berberis* woodland vegetation assemblage. Woody species were reported at lower elevation whereas herbaceous species were associated with *J. squamata* in upper regions. Species richness was observed increasing from lower elevation band to higher elevation band gradually, which might be due to anthropogenic factors such as cattle grazing, leaves and fire wood collection, tourism and developmental activities.

Population density of *J. squamata* was calculated elevation band wise and in overall. The results clearly showed stable population status with highest density of seedling followed by adult and juvenile densities. Good data of regeneration was found in the study area for *J. squamata*. Similar population structure was observed in overall, mid and high elevation bands where least number of seedlings was observed in low elevation band. This might be due to anthropogenic activities in lower regions.

Vegetative and reproductive structures were the most significant characteristics to study the population strength of *J. squamata*. Result observed a stable population structure of *J. squamata* in upper Manang valley. Plant height and stem circumference were found highly significant with number of fruits individually. Vegetative traits and associated species richness were found linearly increased towards higher elevations bands.

From the study, it can be concluded that various physical and biological factors interact differently in different sites to create habitat heterogeneity which determine the distribution pattern of various plant species and influence variations in species composition and diversity. Various ecological traits were seen important measures to study for carry out the research based on distribution of any species along elevation gradient.

Following recommendation have been suggested on the basis of the results of present study.

- Due to high anthropogenic activities in lower elevation band, population was not sustainable. Therefore, such activities should be maintained to minimum, if not possible to ban.
- Provide local people with training to harvest plant resources for use and trade in a sustainable way. Also the significance of pre- and post-harvesting should be informed.
- Assess sustainable use, sustainable levels of harvesting and the management practices for the conservation of medicinal plants: perception, utilization, conservation attitude, cultivation methods, etc.
- There is lack of data of *J. squamata* in many aspects such as distribution pattern, national population status, etc. So, I strongly recommend for the detail research of this species in future.

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APPENDIX

Appendix I: Number of families and genus associated with *J. squamata*

S.N.	Family	Genera number	Species number
1	Asteraceae	10	17
2	Rosaceae	5	8
3	Ranunculaceae	4	5
4	Fabaceae	3	4
5	Gentianaceae	3	8
6	Apiaceae	2	2
7	Cyperaceae	2	5
8	Lamiaceae	2	2
9	Polygonaceae	2	3
10	Primulaceae	2	3
11	Scrophulariaceae	2	5
12	Amaryllidaceae	1	1
13	Araceae	1	1
14	Asparagaceae	1	1
15	Berberidaceae	1	1
16	Betulaceae	1	1
17	Brassicaceae	1	1
18	Campanulaceae	1	1
19	Caprifoliaceae	1	2
20	Convallariaceae	1	2
21	Cupressaceae	1	3
22	Elaeagnaceae	1	1
23	Ephedraceae	1	1
24	Equisetaceae	1	1
25	Ericaceae	1	2
26	Euphorbiacea	1	2
27	Fumariaceae	1	1
28	Iridaceae	1	1
29	Morinaceae	1	1
30	Oleaceae	1	1
31	Orchidaceae	1	1
32	Pinaceae	1	1
33	Poaceae	1	1
34	Salicaceae	1	1
35	Saxifragaceae	1	2
36	Umbeliferae	1	1
37	Violaceae	1	1
Total		**63**	**95**

Appendix II: Frequency of occurrence (%) plant species associated with *J. squamata* in different elevation bands.

Associated plant species	**Low frequency**	**Mid frequency**	**High frequency**	**overall frequency**
Ajuga lupulina Maxim.	0	11.11	50	20.37
Allium sikkimense Baker	0	27.78	0	9.26
Anaphalis contorta (D. Don) Hook. F.	0	0	44.44	14.81
Anaphalis triplinervis (Sims) C. B. Clarke	0	0	5.56	1.85
Anaphalis xylorhiza Sch. Bip. ex Hook. f.	0	38.89	50	29.63
Androsace muscoidea Duby	0	27.78	0	9.26
Androsace tapete Maxima.	0	11.11	5.56	5.56
Anemone rupicola Cambess.	0	0	11.11	3.7
Arabidopsis himalaica (Edgew.) O. E. Schulz	0	16.67	33.33	16.67
Arisaema jacquemontii Blume	0	0	22.22	7.41
Artemisia falconeri C.B. Clarke	0	16.67	44.44	20.37
Artemisia roxburghiana Besser	27.78	0	5.56	11.11
Artemisia subdigitata Mattf.	0	38.89	16.67	18.52
Asparagus filicinus Buch.-Ham. ex D. Don	38.89	0	0	12.96
Aster albescens (DC.) Hand.-Mazz	22.22	16.67	33.33	24.07
Aster himalaicus C. B. Clarke	72.22	11.11	33.33	38.89
Aster indamellus Grierson	38.89	0	0	12.96
Astragalus candolleanus Royle ex Benth.	33.33	22.22	0	18.52
Astragalus multiceps Wall. ex Benth.	0	16.67	5.56	7.41
Berberis aristata DC.	77.78	61.11	77.78	72.22
Betula utilis D. Don	0	0	11.11	3.7
Bistorta affinis (D. Don) Greene	16.67	0	11.11	9.26
Bistorta macrophylla (D. Don) Sojak	0	83.33	5.56	29.63
Carex sp. 1	50	33.33	16.67	33.33
Carex sp. 2	61.11	38.89	38.89	46.3
Carex sp. 3	27.78	61.11	72.22	53.7
Carex sp. 4	5.56	44.44	27.78	25.93
Cicerbita macrorrhiza var. saxatilis (Edgew.) P. Brauv	0	27.78	11.11	12.96
Clematis buchananiana DC.	22.22	0	0	7.41
Conyza sp.	0	5.56	44.44	16.67
Cortia depressa (D. Don) C. Norman	0	0	22.22	7.41
Corydalis juncea Wall.	11.11	55.56	77.78	48.15
Cotoneaster affinis Lindl.	0	11.11	0	3.7
Cotoneaster microphyllus Wall. ex. Lindl.	61.11	77.78	33.33	57.41
Cremanthodium ellisii (Hook. f.) Kitam.ex	0	0	11.11	3.7
Cyananthus microphyllus Edgew.	0	0	11.11	3.7
Delphinium brunonianum Royle	0	38.89	100	46.3
Ephedra gerardiana Wall. ex. Stapf	0	0	61.11	20.37
Equisetum sp.	22.22	0	0	7.41
Euphorbia himalayensis Klotzsch	0	11.11	11.11	7.41
Euphorbia stracheyi Boiss.	0	0	16.67	5.56
Fragaria nubicola Lindl. ex. Lacaita	0	11.11	22.22	11.11

Genciana bryoides Burkill	0	33.33	11.11	14.81
Gentiana algida Pall.	0	27.78	0	9.26
Gentiana depressa D. Don	0	33.33	5.56	12.96
Gentiana robusta King ex. Hook	0	27.78	5.56	11.11
Gentianella paludosa (Hook.) H. Smith	0	5.56	0	1.85
Gentianella pedunculata (D. Don) H. Smith	0	11.11	11.11	7.41
Heracleum obtusifolium Wall. Ex. DC.	11.11	11.11	22.22	14.81
Hippophae tibetana Schlecht.	27.78	5.56	0	11.11
Iris kemaonensis Wallich. ex. Royle	0	11.11	11.11	7.41
Juniperus communis Buch-Ham ex. Andersson	83.33	33.33	5.56	40.74
Juniperus indica Bertol.	88.89	88.89	88.89	88.89
Kobresia gammiei C. B. Clarke	0	27.78	50	25.93
Leontopodium stracheyi (Hook. f.) C. B. Clarke ex. Hemsl.	27.78	33.33	44.44	35.19
Ligustrum confusum Decne.	0	0	33.33	11.11
Lonicera hypoleuca Decne.	0	61.11	55.56	38.89
Lonicera minutifolia Kitam.	0	11.11	11.11	7.41
Morina nepalensis D. Don	38.89	0	0	12.96
Orchidaceae	11.11	0	0	3.7
Oxytropis williamsii Vass.	11.11	0	0	3.7
Pedicularis flexuosa Hook. f.	0	16.67	33.33	16.67
Pedicularis pectinata Wall. ex. Benth.	0	0	33.33	11.11
Pedicularis pyramidata Royle. ex. Benth.	0	27.78	5.56	11.11
Pedicularis rhinanthoides Schrenk.	33.33	0	0	11.11
Pinus wallichiana A. B. Jackson	22.22	0	0	7.41
Pleurospermum apiolens C. B. Clarke	5.56	38.89	55.56	33.33
Poa annua L.	0	0	11.11	3.7
Polygonatum cirrhifolium (Wall.) Royle	0	11.11	22.22	11.11
Polygonatum hookeri Baker	0	16.67	44.44	20.37
Potentilla exigua Sojak	0	38.89	5.56	14.81
Potentilla fructicosa Lindl. ex.Lehm.	38.89	50	22.22	37.04
Potentilla peduncularis D. Don	0	11.11	77.78	29.63
Primula primulina (Spreng.) H. Hara	0	38.89	50	29.63
Rhododendron anthopogan D. Don	94.44	55.56	0	50
Rhododendron lepidotum Wall. ex. D. Don	0	22.22	94.44	38.89
Rosa sericeae Lindl.	94.44	27.78	61.11	61.11
Rumex nepalensis Spreng.	0	0	44.44	14.81
Salix calyculata Hook. f. ex. Andersson	5.56	0	0	1.85
Saussurea nepalensis Spreng.	0	22.22	55.56	25.93
Saxifraga andersonii Engl.	0	22.22	5.56	9.26
Saxifraga mucronulata Royle	0	0	11.11	3.7
Spiraea canescens D. Don	0	0	16.67	5.56
Swertia chirayita Karsten	0	0	50	16.67
Swertia cuneata D. Don	0	0	50	16.67
Tanacetum dolichophyllum Kitam.	83.33	77.78	100	87.04
Tanacetum sp.	22.22	38.89	83.33	48.15
Taraxacum eriopodum DC.	5.56	16.67	0	7.41

Thalictrum alpinum L.	0	33.33	11.11	14.81
Thalictrum cultratum Wall	33.33	44.44	72.22	50
Thymas linearis Benth.	0	16.67	11.11	9.26
Trifolium sp.	0	5.56	33.33	12.96
Verbascum thapsus L.	5.56	5.56	44.44	18.52
Viola biflora L.	0	22.22	5.56	9.26

Appendix III: Species present in the study quadrats and their respective families.

S. No.	Species present in the study area	Family
1	*Ajuga lupulina* Maxim.	Lamiaceae
2	*Allium sikkimense* Baker	Amaryllidaceae
3	*Anaphalis contorta* (D. Don) Hook. F.	Asteraceae
4	*Anaphalis triplinervis* (Sims) C. B. Clarke	Asteraceae
5	*Anaphalis xylorhiza* Sch. Bip. ex Hook. f.	Asteraceae
6	*Androsace muscoidea* Duby	Primulaceae
7	*Androsace tapete* Maxima.	Primulaceae
8	*Anemone rupicola* Cambess.	Ranunculaceae
9	*Arabidopsis himalaica* (Edgew.) O. E. Schulz	Brassicaceae
10	*Arisaema jacquemontii* Blume	Araceae
11	*Artemisia falconeri* C.B. Clarke	Asteraceae
12	*Artemisia roxburghiana* Besser	Asteraceae
13	*Artemisia subdigitata* Mattf.	Asteraceae
14	*Asparagus filicinus* Buch.-Ham. ex D. Don	Asparagaceae
15	*Aster albescens* (DC.) Hand.-Mazz	Asteraceae
16	*Aster himalaicus* C. B. Clarke	Asteraceae
17	*Aster indamellus* Grierson	Asteraceae
18	*Astragalus candolleanus* Royle ex Benth.	Fabaceae
19	*Astragalus multiceps* Wall. ex Benth.	Fabaceae
20	*Berberis aristata* DC.	Berberidaceae
21	*Betula utilis* D. Don	Betulaceae
22	*Bistorta affinis* (D. Don) Greene	Polygonaceae
23	*Bistorta macrophylla* (D. Don) Sojak	Polygonaceae
24	*Carex* sp. 1	Cyperaceae
25	*Carex* sp. 2	Cyperaceae
26	*Carex* sp. 3	Cyperaceae
27	*Carex* sp. 4	Cyperaceae
28	*Cicerbita macrorhiza* var. saxatilis (Edgew.) P. Brauv	Asteraceae
29	*Clematis buchananiana* DC.	Ranunculaceae
30	*Conyza* sp.	Asteraceae
31	*Cortia depressa* (D. Don) C. Norman	Apiaceae
32	*Corydalis juncea* Wall.	Fumariaceae

33	*Cotoneaster affinis* Lindl.	Rosaceae
34	*Cotoneaster microphyllus* Wall. ex. Lindl.	Rosaceae
35	*Cremanthodium ellisii* (Hook. f.) Kitam.ex	Asteraceae
36	*Cyananthus microphyllus* Edgew.	Campanulaceae
37	*Delphinium brunonianum* Royle	Ranunculaceae
38	*Ephedra gerardiana* Wall. ex. Stapf	Ephedraceae
39	*Equisetum* sp.	Equisetaceae
40	*Euphorbia himalayensis* Klotzsch	Euphorbiacea
41	*Euphorbia stracheyi* Boiss.	Euphorbiacea
42	*Fragaria nubicola* Lindl. ex. Lacaita	Rosaceae
43	*Gentiana algida* Pall.	Gentianaceae
44	*Gentiana bryoides* Burkill	Gentianaceae
45	*Gentiana depressa* D. Don	Gentianaceae
46	*Gentiana robusta* King ex. Hook	Gentianaceae
47	*Gentianella paludosa* (Hook.) H. Smith	Gentianaceae
48	*Gentianella pedunculata* (D. Don) H. Smith	Gentianaceae
49	*Heracleum obtusifolium* Wall. Ex. DC.	Umbeliferae
50	*Hippophae tibetana* Schlecht.	Elaeagnaceae
51	*Iris kemaonensis* Wallich. ex. Royle	Iridaceae
52	*Juniperus communis* Buch-Ham ex. Andersson	Cupressaceae
53	*Juniperus indica* Bertol.	Cupressaceae
54	*Juniperus squamata* Buch.-Ham ex. D. Don	Cupressaceae
55	*Kobresia gammiei* C. B. Clarke	Cyperaceae
56	*Leontopodium stracheyi* (Hook. f.) C. B. Clarke ex. Hemsl.	Asteraceae
57	*Ligustrum confusum* Decne.	Oleaceae
58	*Lonicera hypoleuca* Decne.	Caprifoliaceae
59	*Lonicera minutifolia* Kitam.	Caprifoliaceae
60	*Morina nepalensis* D. Don	Morinaceae
61	Orchidaceae	Orchidaceae
62	*Oxytropis williamsii* Vass.	Fabaceae
63	*Pedicularis flexuosa* Hook. f.	Scrophulariaceae
64	*Pedicularis pectinata* Wall. ex. Benth.	Scrophulariaceae
65	*Pedicularis pyramidata* Royle. ex. Benth.	Scrophulariaceae
66	*Pedicularis rhinanthoides* Schrenk.	Scrophulariaceae
67	*Pinus wallichiana* A. B. Jackson	Pinaceae
68	*Pleurospermum apiolens* C. B. Clarke	Apiaceae
69	*Poa annua L.*	Poaceae
70	*Polygonatum cirrhifolium* (Wall.) Royle	Convallariaceae
71	*Polygonatum hookeri* Baker	Convallariaceae
72	*Potentilla exigua* Sojak	Rosaceae
73	*Potentilla fructicosa* Lindl. ex.Lehm.	Rosaceae
74	*Potentilla peduncularis* D. Don	Rosaceae
75	*Primula primulina* (Spreng.) H. Hara	Primulaceae
76	*Rhododendron anthopogan* D. Don	Ericaceae

77	*Rhododendron lepidotum* Wall. ex. D. Don	Ericaceae
78	*Rosa sericeae* Lindl.	Rosaceae
79	*Rumex nepalensis* Spreng.	Polygonaceae
80	*Salix calyculata* Hook. f. ex. Andersson	Salicaceae
81	*Saussurea nepalensis* Spreng.	Asteraceae
82	*Saxifraga andersonii* Engl.	Saxifragaceae
83	*Saxifraga mucronulata* Royle	Saxifragaceae
84	*Spiraea canescens* D. Don	Rosaceae
85	*Swertia chirayita* Karsten	Gentianaceae
86	*Swertia cuneata* D. Don	Gentianaceae
87	*Tanacetum dolichophyllum* Kitam.	Asteraceae
88	*Tanacetum sp.*	Asteraceae
89	*Taraxacum eriopodum* DC.	Asteraceae
90	*Thalictrum alpinum* L.	Ranunculaceae
91	*Thalictrum cultratum* Wall	Ranunculaceae
92	*Thymas linearis* Benth.	Lamiaceae
93	*Trifolium* sp.	Fabaceae
94	*Verbascum thapsus* L.	Scrophulariaceae
95	*Viola biflora* L.	Violaceae

Appendix IV: Density (number of individuals per hectare) of *J. squamata* recorded in three elevation bands and overall in Manang valley.

Elevation Band	Seedling density	Juvenile density	Adult density	Total
Low	150	305.56	244.44	700
Mid	1083.33	655.56	683.33	2422.22
High	777.78	427.78	522.22	1727.78
Total	2011.11	1388.89	1450	4850

Appendix V: Proportion of *J. squamata* size classes and Rejuvenation/regeneration potential in three elevation band in Manang valley.

Bands	Seedling number	Juvenile number	Adult number	Total no. in band	Seedling Proportion	Juvenile Proportion	Adult proportion	Rejuvenation*
Low	27	55	44	126	0.07	0.22	0.17	1.86
Mid	195	118	123	436	0.54	0.47	0.47	2.54
High	140	77	94	311	0.39	0.31	0.36	2.31
Total	362	250	261	873	0.41	0.286	0.298	2.34

*Rejuvenation is calculated as sum of seedling density and juvenile density divided by adult density.