Habitat characteristics, population structure, and vegetative and reproductive traits of *Juniperus indica* Bertol. along elevation gradient in Manang, Nepal

A dissertation submitted for the partial fulfilment of the requirements for M.Sc. in Botany

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CERTIFICATE

This is to certify that the M.Sc. dissertation work entitled **"Habitat characteristics, population structure, and vegetative and reproductive traits of *Juniperus indica* Bertol. along elevation gradient in Manang, Nepal"** has been carried out by Mr. Arjun Chapagain under our supervision. The entire work is based on the fieldwork and data analysis, and the results have not yet been published or submitted for any other degree. We recommend this dissertation work to be accepted as a partial fulfilment for M.Sc. degree in Botany (Plant Systematics and Biodiversity Unit).

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LETTER OF APPROVAL

The dissertation work submitted by Mr. Arjun Chapagain entitled "**Habitat characteristics, population structure, and vegetative and reproductive traits of *Juniperus indica* Bertol. along elevation gradient in Manang, Nepal**" has been accepted as a partial fulfilment of the requirements for M.Sc. degree in Botany (Plant Systematics and Biodiversity Unit).

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Arjun Chapagain
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ABBREVIATION AND ACRONYMS

AGM	above ground mass
ANOVA	analysis of variance
asl	above sea level
cm	centimetre
DBH	diameter at breast height
DPR	Department of Plant Resources
GBH	girth at breast height
GLM	generalized linear model
GoN	Government of Nepal
GLORIA	Global Observation Research Initiative in Alpine Environments
GPS	geographical positioning system
ha.	hectare
IUCN	International Union for Nature Conservation
KATH	National Herbarium and Plant Laboratories, Godavari
LMM	linear mixed model
m	meter
NTNC	National Trust for Nature Conservation
PADIR	potential annual direct incident radiation
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

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ABSTRACT

Elevation gradients are complex involving different co-varying factors that influence plant population structure and in traits related to life history. Studies pertaining to variation in such traits along the gradient provide opportunities to examine performance of plant populations under a range of environmental conditions. This study aims to assess variations in population structure and vegetative and reproductive traits of *Juniperus indica* Bertol. along elevation gradient in Manang, north-central Nepal. The whole of its distribution range was divided into lower- (3350-3580 m), mid- (3650-3880 m) and higher- (3950-4250 m) elevation bands, where populations were sampled in a total of 54 plots (18 plots per band) of 10 m x 10 m size. In each plot, we recorded habitat characteristics; number of individuals of *J. indica* classified into seedling, juvenile and adult; and vegetative (plant height, trunk diameter, canopy radius and leaf biomass) and reproductive (number of fruits per plant) traits of its adult individuals.

J. indica preferred dry, rocky habitats in SE- to SW-facing slopes, along with *Rosa-Berberis-Juniper* shrubland, subalpine and alpine grasslands, and open forests. Altogether, 88 plant species, associated with *J. indica*, were identified. Plots in the lower- and mid-elevation bands mostly comprised woody shrubs, whereas herbaceous species dominated the higher-elevation band. Mid-elevation band tended to show highest density of seedlings and juveniles, but adult density was high in the plots at lower-elevation band. *J. indica* exhibited almost similar population structure in three bands, with high contribution of juveniles than seedlings and adults. However, proportion of adult was high in lower-elevation, whereas proportions of seedling and juvenile tended to be high in mid- and higher-elevations. Density-diameter (d-d) curve for adult *J. indica* was reverse *J*-shaped, indicating continuous regeneration. Most of the individuals were of moderate to small size. Mean leaf dry biomass was 28.98 kg per ha. The number of fruits per plant ranged 10-1040 (mean 202.9). *J. indica* showed higher values of all studied traits in lower-elevation than in mid- and higher-elevation bands. Trunk diameter, leaf dry weight and fruits set parameters spatially varied within the same elevation band. Regression analysis showed that the canopy area was the strongest allometric variable for predicting total leaf biomass.

Population density of *J. Indica* and its vegetative and reproductive traits are influenced differently by the variations in elevation. It is concluded that *J. indica* in Manang exhibits successful regeneration despite harsh ecological conditions. Higher regeneration at mid- and higher-elevation bands indicates plants ability to tolerate adverse environmental conditions as well as a tendency for expansion of its distribution niche towards cooler habitat of high elevation. The use of outer canopy area is the best option for non-destructively estimating above ground biomass of *J. indica*.

***Key words**: biomass, population density, regeneration, reproductive trait, vegetative trait.*

CHAPTER ONE: INTRODUCTION

1.1 Background

Elevation gradients are complex involving many different co-varying factors like topography, soil, and climate (Austin *et al.* 1996). Elevation strongly influences length of growing season and the availability of soil moisture and nutrients (Namgail *et al.* 2010). Plant species growing along the elevation gradient show considerable variations in structure of their populations and in traits related to their life history and growth. Studies pertaining to variation in such traits along the elevation gradient provide opportunities to examine performance of plant populations under a range of environmental conditions (Kim and Donohue 2011). This study focuses on the variation in population structure and vegetative and reproductive traits of *Juniperus indica* Bertol. (Family: Cupressaceae) along elevation gradient in Manang, north-central Nepal.

Population structure has a direct impact on community structure and in turn demonstrates the development trend of community (Zhang *et al.* 2007). Population structure reflects biological and ecological characteristics of plants which are used to determine regeneration profile (Teketay 1996). A population with the presence of adequate number of seedlings or young individuals may represents reverse 'J'-shaped size-class distribution, indicating satisfactory regeneration. Inadequate numbers of seedlings or young individuals may represent a bell shaped size class distribution, indicating poor regeneration (Vetaas 2000, Tripathi and Khan 2007). Continuous regeneration is necessary for the long-term persistence of a species population (Thakuri 2010). Population density of seedlings and juveniles are considered as regeneration potential of a species (Bharali *et al.* 2012). Abundance of established seedlings and juveniles affects the future composition of forests (Thakuri 2010). The inclusion of seedlings and juveniles in plant population structures would provide better information about the status of the species at early stage of regeneration. Germination of seeds and establishment of seedlings and juveniles are related to the availability of space and moisture conditions and to adaptation to particular light regimes (Ramakrishnan *et al.* 1982).

Population dynamics of plant species can be described by demographic properties such as recruitment, mortality and growth. The balance among these properties regulates the dynamics and the structure of a population (Bharali *et al.* 2012). Plants generally grow and survive in a limited range of the environmental conditions, for example, temperature and light availability, and variation in these factors play important roles in shaping the age/size structure and regeneration at different elevations (Block and Treter 2001; Duan *et al.* 2009). Study on the variation in population density of seedlings, juveniles and adults along elevation gradients would be helpful in understanding the influences of environmental factors on forest regeneration (Wang *et al.* 2004).

Besides population structure, vegetative and reproductive traits characteristic to adult individuals, such as plant height, trunk diameter, canopy area, leaf biomass and reproductive output can be used to determine the present status and trend of growth of a population. Nondestructive measurements of plant allometric attributes (e.g., height, canopy dimensions, stem diameter) have long been used to estimate plant biomass (Mason and Hutchings 1967; Peek 1970; Ludwig *et al*. 1975) and reproductive output (Haymes and Fox 2012; Otárola *et al*. 2013). Recent interest in quantifying ecosystem carbon stocks and/or potential bioenergy uses has shown the need for using nondestructive methods to estimate total above ground biomass (AGM, Ansley *et al*. 2012). Studies indicate that canopy area and/or stem diameter can provide the best regression fits for AGM prediction in several tree species including *Juniperus* (Mason and Hutchings 1967; Ansley *et al*. 2012).

Plants growing along an elevation gradient exhibit differences in their height which generally become expressed by a shortening of their stems at higher elevations (Körner *et al.* 1983). Decreasing in diameter growth with increasing elevation must be dominantly caused by the corresponding decline in temperature. At cooler temperatures, there is a tendency for photosynthate to be transformed to sugars and starch rather than cellulose and this can limit diameter growth (Tranquillini 1979). The ecological significance of aspect is important because it influences diameter growth of tree, forest productivity, and species distribution (Hutchins *et al.* 1976). Canopy area typically requires at least two perpendicular measurements of canopy diameter, usually accomplished by inserting a measuring pole through the canopy. It is supportive to field measurement of outer canopy dimensions like plant height and trunk diameter to estimate above ground biomass (Ansley *et al.* 2012). In a considerable number of species, upland plants have been found to have smaller leaves (Kofidis and Bosabalidis 2008), which can be linked with leaf biomass at different elevations. There is a general belief that phytomass changes inversely with elevation in the tropical and temperate regions, perhaps due to the fact that trees are larger at the lower slopes of a mountain, but whether this applies to treeless, alpine environments with minimal precipitation is not clear (Namgail *et al*. 2010).

High altitude Himalaya is the most vulnerable geographic region of the world outside of the polar region to climate change (Cavaliere 2009; Salick *et al*. 2009). Climate change due to global warming has been considered as the most serious threat to mountain forests (Beniston 2003; Glatzel 2009). Subalpine forest represents the uppermost forest ecosystems along the elevation gradient. They are highly vulnerable to natural variation in climate (Sano *et al*. 2005) and are also under high anthropogenic pressure (Sharma *et al.* 2009). Ecological study of subalpine forests in the Nepal Himalaya is very scanty, though some initiatives have been taken in recent time (e.g., Shrestha *et al.* 2007; Ghimire and Lekhak 2007; Ghimire B.K. *et al.* 2008; Suwal 2010; Gaire *et al*. 2013). Lack of sufficient regeneration is a major problem of mountain forests. Studies on subalpine forests have reported poor seedling recruitment in understory of undisturbed old-growth forests (Shrestha *et al.* 2007). The challenge increases for woody species in the Himalayas if they fail to show a high freezing tolerance (Shrestha *et al.* 2007).

Juniperus indica is dominant in subalpine forest of Manang district, north-central Nepal (Ghimire B.K. *et al.* 2008). *Juniperus* forest in the Nepal Himalayas is under threat due to high anthropogenic pressure as well as harsh climatic conditions. The examples of anthropogenic challenges for juniper are destructive practices, such as over harvesting of leaves for incense and burning of bush to harvest its wood. Studies carried out in other parts of the world have shown that the principal ecological problems in junipers are related to low production of viable seeds (Juan *et al.* 2003; Otto *et al*. 2010). Juan *et al.* (2003) assessed viability in *J. oxycedrus* which show difficulties in the process of seed germination. In some species of *Juniperus*, low reproductive success is due to low amount of pollen that reaches female individuals resulting in less number of fruits set (Juan *et al*. 2003).

Ghimire B.K. *et al.*(2008) studied vegetation along an elevation gradient of *J. indica* forest in southern Manang Valley, but variation in the growth-related traits of *J. indica* along elevation gradient are not studied. Most of the works on Himalayan junipers are mainly confined to leaf essential oil variation (e.g., Adams and Chaudhary 1996; Adams *et al.* 1998), taxonomic determination (e.g., Adams *et al*. 2009), and ethnobotany (e.g., Bhattarai *et al.* 2006). Ethnobotanical study of junipers (*Juniperus indica* Bertol., *J. squamata* Buch.-Ham. ex D. Don and *J. communis* L.) in Manang by Bhattarai *et al.* (2006) revealed that the local community and traditional Tibetan doctors have been using different plant parts for different purposes. Its fruits, leaves, stem and barks are used in traditional medicine and leaves are burnt for incense. Plant parts of junipers are also used as fuel-wood, for fencing, and for making different kinds of household articles (Bhattarai *et al.* 2006). *J. indica* is one of the highly traded species (Ghimire S.K. *et al.* 2008). Its leaves are sold locally for incense and the essential oil obtained from steam distillation of leaves and fruits are exported outside the country.

In this research work, I sampled *J. indica* along an elevation gradient in Manang, and studied variation in its population ecology and traits associated with vegetative and reproductive performances. First, the habitat characteristics conditioning differences in plant composition along the elevation gradient were analyzed. Second, variation in population size and structure and in traits related to vegetative and reproductive performance (including trunk diameter, canopy area, number of fruits set and leaf biomass) were assessed among populations distributed along the elevation gradient. Finally, the relationships between performance traits of *J. indica* were analyzed.

1.2 Objectives

The general objective of the present study is to assess variation in population structure and in traits associated with vegetative and reproductive performances of *J. indica* along elevation gradient in Manang, north-central Nepal. The specific objectives are (i) to study habitat characteristics of *J. indica* in the study area, (ii) to predict its regeneration status along elevation gradient based on population structure (iii) to assess variation in vegetative and reproductive traits along elevation gradient, and (iv) to determine the relationship of allomatric traits with leaf biomass and reproductive outputs.

CHAPTER TWO: MATERIALS AND METHODS

2.1 Study Species

Juniperus is the second most diverse genus of gymnosperm having approximately 67 species and 28 varieties in the world (Adams *et al.* 2009). Most of the taxa are found on the Laurasian land mass, besides the rift mountain in east Africa into the southern hemisphere and the mountains of the northernmost part of Africa (Adams *et al.* 2009). Four species of *Juniperus* are found in Nepal and all these grow in subalpine and alpine zone. These four species are *J. communis* L., *J. squamata* D. Don., *J. recurva* Buch.Ham ex D.Don. and *J. indica* Bertol. (Shrestha 1982; Ghimire S.K. *et al.* 2008).

J. indica (Kalo Dhupi in Nepali, Ghimire S.K. *et al.* 2008) is native to high altitude Himalaya, occurring from the northern Indus Valley in Kashmir east to western Yunnan in China through North Eastern Tibet. It occurs throughout Nepal at elevations ranging from 3300 to 4500 m asl (Ghimire S.K. *et al.* 2008). The plant is found on open and rocky alpine slopes in drier areas; sometimes forming forests at lower elevation.

It is a woody shrub or small tree growing to 0.5-2 m tall, with largely horizontal branching. The plant occurs as dwarf shrub at higher elevations exceeding 4200 m and as tree at lower elevations, 3300-4000 m (Ghimire S.K. *et al.* 2008). The leaves are dark grey-green, dimorphic, with adult plants having mostly scale-like leaves, which are decussate or sometimes in whorls of 3, closely appressed, 1-3 mm long; while young plants have mostly needle-like leaves, which are borne in whorls of 3, 5-8 mm long. Needle-like leaves can also be found on shaded shoots of adult plants. The plant is dioecious, with male (pollen) and female (seed) cones on separate plants. The pollen cones are sub-globose or ovoid 2-3 mm long; seed cones are ovoid, berry-like, 6-10 mm long, glossy black when ripe, and contain a single seed. The cones are seen in April to May and maturing in October to December. The seeds are mostly dispersed by birds which eat the cones.

J. indica has high ethnobotanical importance. Almost all parts of *J. indica*, but mostly its leaves, are burnt as incense by Buddhist Society. Leaves, stems, barks and fruits of *J. indica* are also used in traditional medicine (Bhattarai *et al.* 2006; Ghimire S.K. *et al.* 2008). Its leaves and fruits are used medicinally in kidney, skin and lymph disorders, fever, cough and cold, sores, wounds, and paralysis of limbs (Ghimire S.K. *et al.* 2008). The plant is also used as fencing material and for carving different household articles (Bhattarai *et al.* 2006). *J. indica* (called Kalo Dhupi in Nepali) is highly valued in trade. Dried leaves are sold locally for incense, and essential oil obtained from steam distillation of fresh leaves is traded internationally for its use in medicines and cosmetics (Ghimire S.K. *et al.* 2008; Gurung 2010). The leaves are harvested throughout the year and fruits during July to August (WWF Nepal, 2007).

2.2 Study Area

2.2.1 Location and geomorphology

The study area is located in Manang district in north-central part of Nepal (Fig. 1). Manang district, with elevation range of 2000–6000 m asl, lies on broad U-shaped valley of Marshyangdi river to the north of the Annapurna mountain range. The valley is surrounded by the Annapurna range (7000 m asl) on the south; Manasalu (8163 m asl)on the east; Peri (6767 m asl), Himlung (7126 m asl) and Choya (6820 m asl) on the north; and Damodar (6364 m asl) and Muktinath (3760 m asl) on the west.

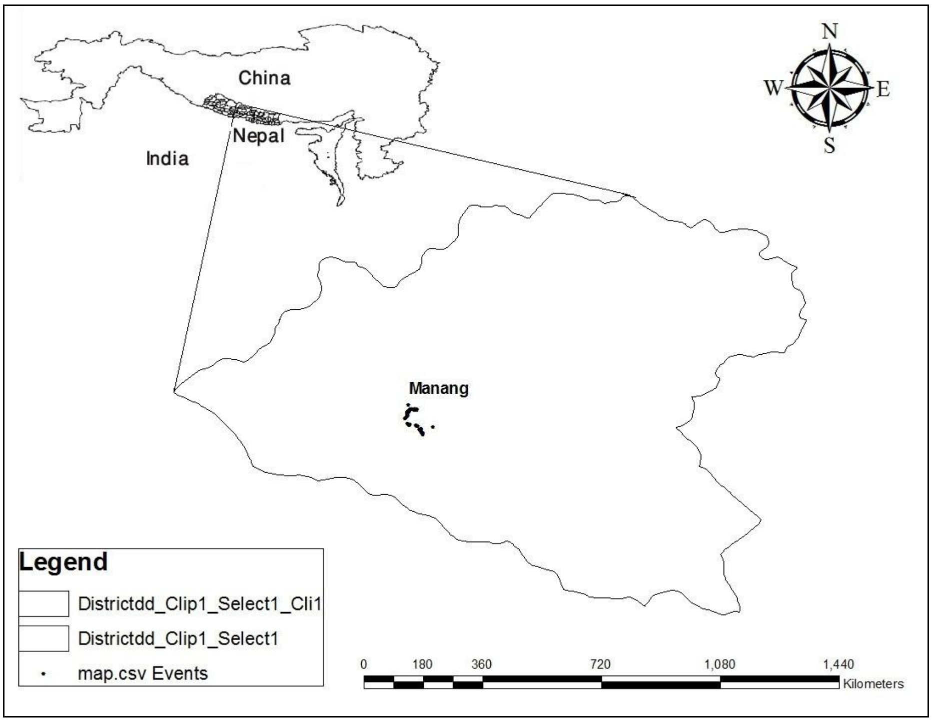

Fig.1. Map of the study area.

2.2.2 Climate

Analysis of climatic data (from 2006 to 2010) of the nearest meteorological station in the district headquarters Chame, which lies south of the main mountain ranges, showed mean annual precipitation of 1072.78 mm with highest precipitation recorded in July (263.04 mm) (Fig. 2). However, owing to the rain-shadow effect of surrounding mountain ranges (> 7000 m asl), the northern part of Manang Valley receives very low monsoonal precipitation (*ca.* 444 mm per annum at 3420 m asl) compared to southern hilly regions (Miehe *et al.* 2001; Baniya *et al.* 2009). Average minimum temperatures in Chame station remained negative from December (–2.78^{0}C) to February (–1.04^{0}C), and maximum temperature peaked in June (21.84^{0}C) (Fig 2), while the mean temperature being 11.03^0 C. The mean temperature at northern trans-Himalayan part, on the other hand, is comparatively low [for example 6.2°C of mean temperature was recorded in Jomsom station at 2750 m asl (Miehe *et al.* 2001)]. There is decreasing moisture from east to west in the upper Manang Valley, and the south-facing slopes are much drier than those facing north (Bhattarai *et al.*, 2004; Ghimire B.K. *et al.*, 2008). The area is covered by snow during winter for about five months (November to March). This snow melt water is the main source of soil moisture for forest.

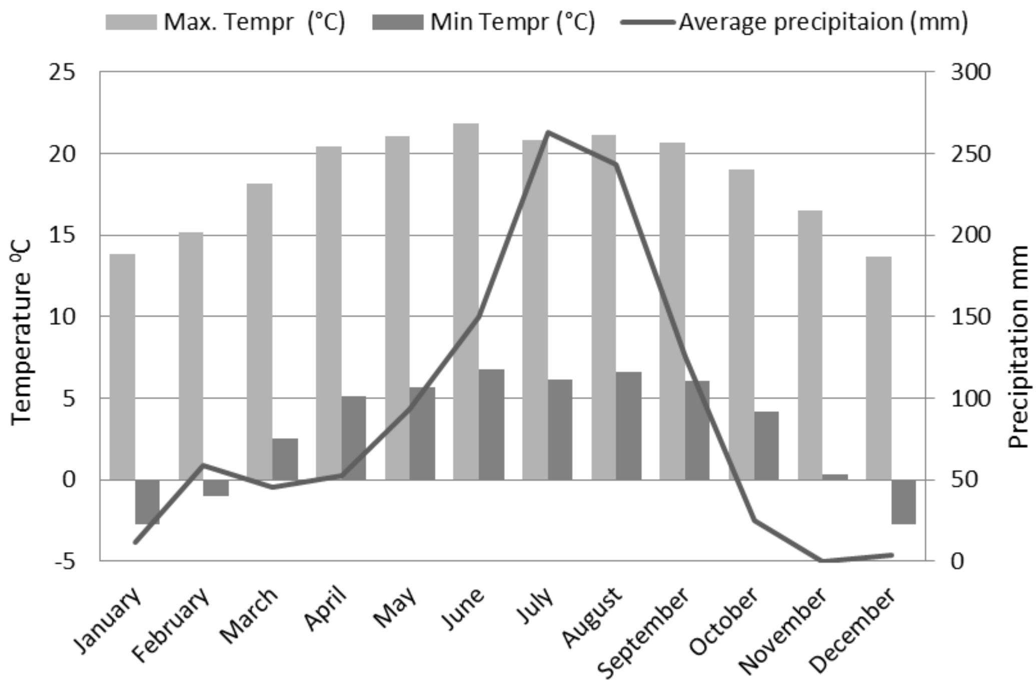

Fig. 2. Five year (2006-2010) average minimum and maximum temperatures and average precipitation recorded at Chame weather station (2650m asl), Manang (*Source*: Department of Hydrology and Meteorology, Government of Nepal).

2.2.3 Vegetation

The harsh climate of Manang Valley supported steppe vegetation similar to that of Tibetan Plateau. At elevations above 3000 m, there is forest of *Pinus wallichiana*, *Betula utilis* and *Abies spectabilis* on the north-facing slopes, and some forest of *P. wallichiana* on the dry south-facing slopes (Baniya *et al.* 2009). *Juniperus indica* and *Rosa sericea* with other shrubs are dominant on the dry south-facing slopes. In the lower elevation, the vegetation mainly comprised of *Juniperus squamata*, *Lonicera obovata* and *Caragana gerardiana*. The ground layer is composed of *Aster indamellus, Anaphalis royleana*, *Veronica ciliata*, *Gentiana depressa*, *G. robusta*, *Viola biflora* and scattered patches of thorny cushions such as *Astragalus cobresiiphilus* along with species of *Primula*, *Saxifraga* and *Androsace*. The riverbanks are occupied by *Salix calyculata*, *Populus ciliata* and *Hippophae tibetana* (Panthi *et al.* 2007). The higher elevation areas are dominated by species such as *Bistorta macrophylla*, *Cortia depressa*, *Rhododendron anthopogon*, *Potentilla saundersiana*, *P. fruticosa*, *Euphorbia stracheyi*, *Saxifraga hirculoides*, *Rhodiola humilis* and *Kobresia pygmaea*.

2.3 Methods

2.3.1 Sampling

Sampling of *Juniperus indica* population was made in September 2011 in north-eastern part of Manang Valley. A systematic random sampling approach was used. The study was started from Bhraka village (3350 m asl) of Manang almost at the valley bottom to Ice Lake (4250 m asl). The whole of the distribution range was divided into lower- (3350-3580 m), mid- (3650-3880 m) and higher- (3950-4250 m) elevation bands. In each elevation band three horizontal transects were laid at *ca*. 75-100 m elevation intervals. In each transect, 6 plots of size 10 m× 10 m were randomly sampled at *ca*. 50-100 m length intervals, totaling 54 plots.

In each sampling plot, individuals of *J. indica* of different size classes were recorded separately. Size classes were recognized related to the growth stage (Schemske *et al.* 1994). The size classes were broadly defined according to plant height or trunk diameter as seedlings (height <0.1 m, trunk diameter <1 cm), juveniles (height 0.1–1.0 m, trunk diameter <1 cm) and adult (height usually >1 m, trunk diameter >1 cm and also bearing reproductive structure). Adult individuals were recorded for their trunk diameter, canopy area and number of fruits set. Trunk diameter of adults >1-3 m tall was measured at 25 cm aboveground, whereas the trunk diameter of adults >3 m tall was measured at 137 cm aboveground. Crown cover was directly measured in terms of canopy area occupied by each adult individual using measuring tape. For leaf biomass, harvest method (Bhattarai *et al.* 2004) was followed. In each plot, an adult individual was randomly selected, and its leaves were collected within an area of 0.0625 m^2 by randomly placing a small quadrat (0.25 m × 0.25 m) on the crown surface. The leaves were packed in paper bags and fresh weight was taken in a spring balance (error of ±2.53 gm).In the field, all leaf samples collected from each plots were dried in shade by placing them in cotton bags. After returning to the laboratory, the leaf samples were oven dried at 60^0 C for 72 hours and dry weight was measured in digital weighing balance (error ±1.77 gm). Leaf samples were collected only from 46 plots due to the absence of adult individuals from the rest 8 plots.

Latitude, longitude and altitude of each plot were recorded with the help of GPS device. Aspect and slope of each plot were recorded using compass and clinometer respectively. Each 100 m^2 plot was further divided into 4 subplots of 5 m × 5 m size. In each subplot, presence/absence of plant species associated with *J. indica* was recorded. Vouchers of plant species encountered in sampling plots were collected. The vouchers were identified using taxonomic reference (e.g., Polunin and Stainton 1984; Stainton 1988) and comparing with specimens housed at TUCH and KATH. Herbarium specimens were deposited in TUCH. Nomenclature follows Press *et al.* (2010).

2.3.2 Data analysis

2.3.2.1 Habitat characteristics

Habitat characteristics of *J. indica* were evaluated in terms of variation in physical/topographic variables, and by analyzing patterns of associated species diversity and composition along the elevation gradient. The value of aspect, slope and latitude were combined to calculate potential annual direct incident radiation (PADIR, MJ cm^{-2} yr^{-1}) by using the formula given by McCune and Keon (2002). Aspect has been folded about the north-south line (rescaling 0-360^0 to 0-180^0, such that NE = NW, E = W, etc) before calculating PADIR using the following formula: folded aspect = 180 – |Aspect – 180| (McCune and Keon 2002). The formula for calculating PADIR is given by: PADIR = –1.467+1.582×COS(G3) × COS(H3)–1.5×COS(I3) ×SIN(H3) × SIN(G3) - 0.262×SIN(G3) ×SIN(H3) + 0.607×SIN(I3) × SIN(H3); where latitude, slope, and folded aspect are in columns G, H, and I, respectively, all in radians in Microsoft Excel. It gives a relative value (ranging from 0.03-1.11) of how much solar radiation a particular spot receives. Bio-physical variables recorded in *J. indica* growing sites in 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 transformation were treated with non-parametric Kruskal-Wallis tests.

Richness of associated species was calculated as the total number of such species present per plot. Presence-absence data from all the four subplots were combined to calculate abundance of each associated species per plot, in an ordinal scale from 0 (absent from all four subplots) to 4 (presence in all subplots). The abundance data of 88 associate species including *J. indica* from all 54 plots were used to calculate their frequency in lower-, mid- and higher-elevation bands, and overall frequency.

2.3.2.2 Density and size distribution

Density and population structure (the relative proportions of seedling, juvenile and adult to total density) of *J. indica* were analyzed for each plot and each elevation band (Bharali *et al.* 2012). Regeneration potential of *J. indica* was evaluated based on the density of seedlings and juveniles (Shankar 2001).It was expressed as the sum of seedling and juvenile density divided by the density of adults. In addition, density-diameter (d-d) curve was also developed for individuals with trunk diameter $\geq$1 cm to further assess regeneration patterns and population structure of adults. Variation in population density among elevation bands was compared using one-way ANOVA. Liner mixed model (LMM) (McCulloch and Searle 2000; Verbeke and Molenberghs 2000) was used to study the effects of elevation on population structure (proportion of life stages - seedlings, juveniles and adults) and regeneration potential. Elevation band and transect nested within elevation band were used as fixed factor and study plot was used as random variable in the model. PADIR and aspect (folded about the north-south line) were used separately in the model as cofactor to account for the effect of insolation and heat load (McCune and Keon 2002) respectively. LMM procedure fits models more general than those of the generalized linear model (GLM) procedure and it encompasses all models in the variance components procedure. The major capabilities that differentiate LMM from GLM are that LMM handles correlated and unbalanced data and unequal variances

(McCulloch and Searle 2000). LMM also handles more complex situations in which experimental units are nested in a hierarchy.

2.3.2.3 Variation in vegetative and reproductive traits

Vegetative (plant height, trunk diameter, canopy area, and leaf dry weight) and reproductive (fruits production) traits were computed for each adult individual in the respective elevation band. The total data set comprised of 134 adult individuals (57, 25 and 52 individuals from low- mid- and high-elevation band respectively) for which plant height, trunk diameter and canopy area were recorded. Leaf dry weight was measured for a total of 56 individuals (24, 15 and 17 individuals from low- mid- and high-elevation band respectively). Leaf dry weight was computed at the level of 0.065 m^2 canopy area and on individual basis (by multiplying the value obtained for 0.0625 m^2 canopy by total canopy area of each individual). Values of leaf dry weight from all adult individuals were added to obtain total value for each plot. Sample size for estimating fruit production comprised of 90 individuals that bore fruits during the study period, of which 30, 22 and 38 individuals were recorded in fruiting from low- mid- and high-elevation band, respectively.

Same LMM as above was used to assess the effect of elevation on vegetative and reproductive traits. Elevation band and transect nested within elevation band were used as fixed factor and identity of individuals was used to account for correlated random effect. PADIR and aspect (folded about the north-south line) were used separately in the model as cofactor to account for the effect of insolation and heat load (McCune and Keon 2002), respectively.

2.3.2.4 Relationships among traits

Relationships between vegetative and reproductive traits were analyzed by calculating Pearson correlation coefficients. Traits exhibiting statistically significant correlations were further analyzed through linear regression analysis to evaluate the strength of relationships and derive allometric equations. Particularly we focused on significant allometric traits to predict leaf biomass and fruit production. To meet the assumptions of parametric statistics (normality and homogeneity of variance), trunk diameter, plant height, leaf dry weight and number of fruits were log transformed, whereas canopy area was square root transformed. SPSS 17.0 was used for all statistical analyses.

CHAPTER THREE: RESULTS

3.1 Habitat Characteristics of *J. indica*

Juniperus indica was recorded from open rocky habitats in SE- to SW-facing slopes in upper Manang valley, with main vegetation type being *Rosa-Berberis-Juniper* shrubland, subalpine and alpine grasslands, and open forests (mainly of *Pinus wallichiana* at lower elevation). It grows on dry, rocky and sandy substrates. The plots at three elevation bands differed in all biophysical variables studied (Table 1). *J. indica* occurred on gentle SW-facing slopes towards higher elevations receiving high solar radiation (Table 1). Altogether, 88 plant species (belonging to 64 genera and 37 families), associated with *J. indica*, were identified in the study area, 33 species in lower-elevation band, 49 in mid-elevation band and 72 in higher-elevation band (Appendix II). Richness and abundance of associate species were significantly high at higher-elevation plots, the values of which decreased linearly towards lower elevation (Table 1). Asteraceae was the dominant family in the study area comprising 16 species within 10 genera, followed by Rosaceae, Gentianaceae, Ranunculaceae, Fabaceae, Scrophulariaceae and Cyperaceae. *Anaphalis, Artemisia, Carex, Juniperus* and *Pedicularis* were the largest genera, each comprising 3 species (Appendix III).

Table 1. Bio-physical variables (mean ± SE) recorded in *J. indica* growing sites in three elevation bands (low, mid, high) in Ice Lake area, upper Manang. For elevation, range values are given in the parentheses.

Variables	Low	Mid	High	*p*
Elevation (m)	3472.83 ± 20.11 (3351-3585)	3761.56 ± 18.70 (3655-3885)	4064.50 ± 20.72 (3947-4197)	<0.001
Aspect (^{0})	114.06 ± 10.34	136.39 ± 8.52	98.00 ± 8.89	0.021
Slope (^{0})	46.67 ± 3.96	24.72 ± 2.96	20.28 ± 3.17	<0.001
PADIR (MJ cm^{-2} yr^{-1})	0.84 ± 0.04	1.03 ± 0.03	1.00 ± 0.02	0.001
Associate species richness*	23.94 ± 1.54	33.00 ± 2.63	60.44 ± 2.88	<0.001
Associate species abundance	11.56 ± 0.48	15.78 ± 1.42	27.39 ± 1.32	<0.001

*Number of vascular plant species associated with *J. indica* per plot.*p* values based on Kruskal-Wallis tests or on one-way ANOVA.

Frequency of *J. indica* occurrence was estimated to be 68.06%, 61.11%, and 47.22% in lower-, mid- and higher-elevation bands, respectively with an overall frequency of 58.80%. Among the species associated with *J. indica* from all sampling plots in three elevation bands, *Tanacetum* *dolichophyllum* (54.17 %), *Juniperus squamata* (43.06 %), *Rosa sericea* (41.20 %), *Berberis aristata* (38.43 %), *Carex* sp. (37.50 %), *Tanacetum* sp. (33.80 %) and *Cotoneaster microphyllus* (33.33 %) exhibited overall high frequency of occurrence (Appendix II). The three elevation bands differed, to some extent, in composition of vascular plant species. Plots in the lower- and mid-elevation bands mostly comprised woody shrubs, whereas herbaceous species dominated the plots in the higher-elevation bands. *Carex* spp., *Rosa sericea*, *Tanacetum dolichophyllum*, *Berberis aristata* and *Rhododendron anthopogon* were the most frequent species (with frequency of occurrence >30%) in lower-elevation band. Similarly, *Rosa sericea*, *Cotoneaster microphyllus*, *Tanacetum dolichophyllum*, *Juniperus squamata*, *Berberis aristata*, *Potentilla fructicosa* and *Bistorta macrophylla* were the most frequent species (with frequency >30%) in mid-elevation band, and *Tanacetum dolichophyllum*, *Juniperus squamata*, *Tanacetum* sp., *Lonicera hypoleuca*, *Potentilla peduncularis*, *Rhododendron lepidotum*, *Conyza* sp., *Spiraea canescens*, *Berberis aristata*, *Carex* spp., *Delphinium brunonianum*, *Ajuga lupulina* and *Kobresia gammiei* were the most frequent species (with frequency >30%) in higher-elevation band (Appendix II).

3.2 Density and Size Distribution of *J. indica*

Density of seedling, juvenile and adult of *J. indica* in the entire study area were found to be 4.89 ± 0.67, 6.59 ± 0.95 and 2.26 ± 0.28 (mean±SE) individuals per 100 m^2 plot. The overall density, combining all three size classes was 13.74±1.47 individuals per 100 m^2 plot. Mid-elevation band tended to show high density of seedlings and juveniles, but the results were statistically insignificant (Table 2). On contrary, adult density was high in plots at lower-elevation band.

Table 2. Density (number of individuals per 10 × 10 m plot) of *J. indica* recorded in three elevation bands in Ice Lake area, upper Manang valley. Data shown are mean ± SE.

	Low	Mid	High	$F_{2,53}$	p
Seedling	5.39 ± 1.35	6.22 ± 1.39	3.06 ± 0.39	1.484	0.236
Juvenile	7.06 ± 1.63	8.39 ± 2.20	4.33 ± 0.64	0.582	0.563
Adult	3.17 ± 0.52	1.50 ± 0.27	2.11 ± 0.57	2.536	0.089
Total	15.61 ± 2.32	16.11 ± 3.45	9.50 ± 1.15	1.894	0.161

F and *p* values based on one-way ANOVA.

The proportions of seedling, juvenile and adult of *J. indica* in all 54 plots were 0.356, 0.480, and 0.164 respectively. In three elevation bands, *J. indica* exhibited almost similar population structure, with high contribution of juveniles than seedlings and adults (Fig. 3). However, proportion of adult was significantly high in lower-elevation band (LMM $F_{2,44}$ = 3.425, p = 0.041, Fig. 3, Table 3), whereas proportions of seedling and juvenile tended to be high in mid- and higher-elevation bands (but the results were statistically insignificant, Fig. 3 and Table 3). But significantly higher value of rejuvenation (expressed as the sum of seedling and juvenile density divided by the density of the adults) at mid- and higher-elevation bands compared to lower-elevation (LMM $F_{2,44}$ = 3.280, p = 0.047, Fig. 3, Table 3) signifies potentially higher regeneration of *J. indica* towards mid- and higher elevations.

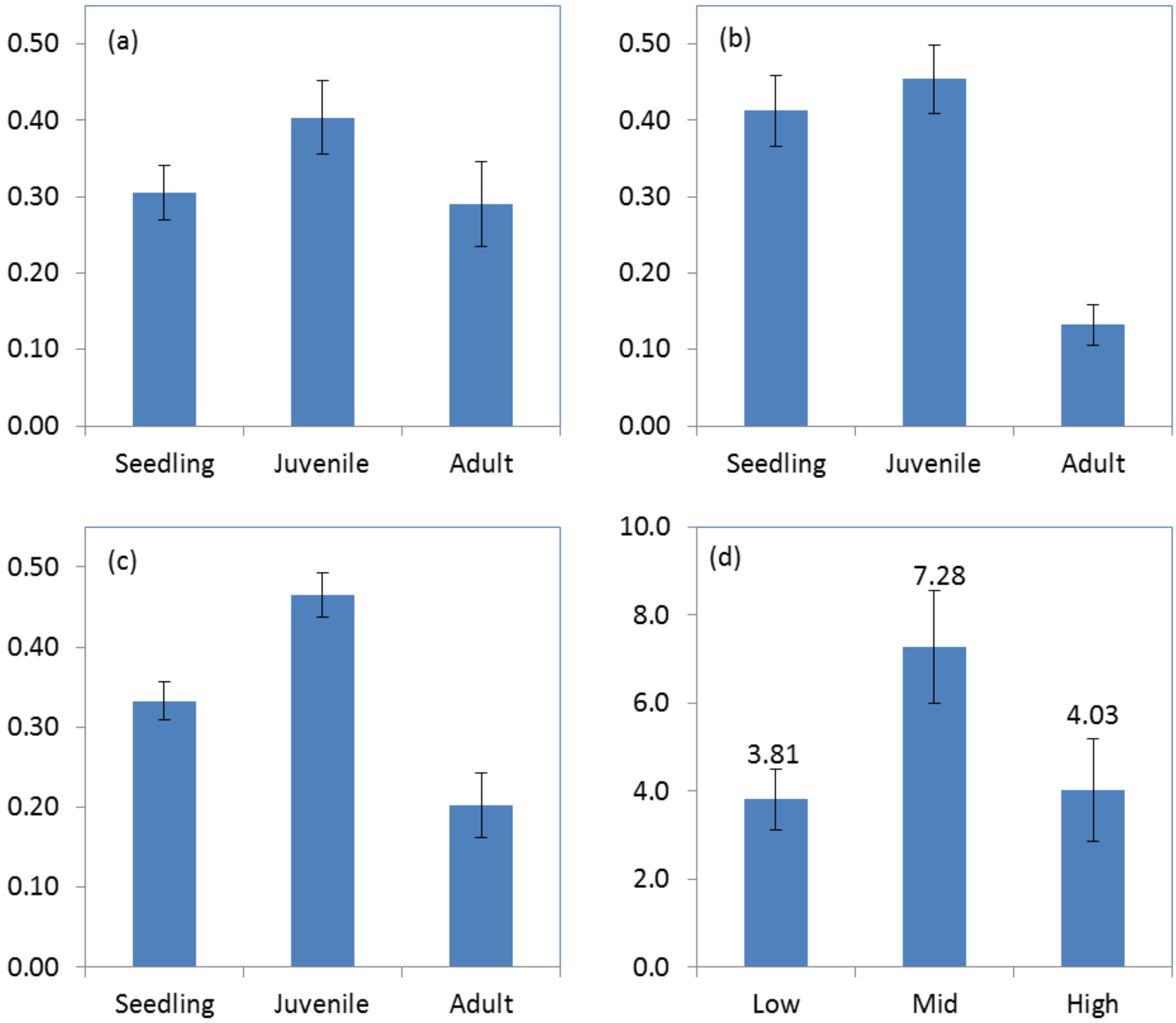

Fig. 3. Population structure [proportions (mean ± SE) of seedling, juvenile and adult] and regeneration pattern of *J. indica* at three elevation bands in Ice Lake area, upper Manang valley: (a-c) Population structure at three elevation bands (a – lower-elevation, b – mid-elevation, and c – higher-elevation), (d) rejuvenation expressed as the sum of seedling and juvenile density divided by the density of adults.

LMM analyses also gave significant results for adult proportion and rejuvenation when transects were nested within elevation band (Table 3), indicating that these parameters spatially varied within the same elevation band. However, in either of the case, the effect of aspect or PADIR (both used separately as covariate in the model) were not significant, indicating SE and SW gradient or incident radiation did not affect population structure. Among the remaining predictor variables considered in this study, richness and abundance of associate species also did not show significant relationship with either density of *J. indica* in three size classes or their proportions.

Table 3. Results of liner mixed model (LMM) showing the effects of elevation band and transect (nested within elevation band) as fixed factor and aspect (folded about the north-south line) as cofactor on size class proportions and rejuvenation of *J. indica*. Position of plots was used as random variable in the LMM.

	Seedling proportion				*Juvenile proportion*			
Source of variation	N_{df}	D_{df}	*F*	*P*	N_{df}	D_{df}	*F*	*P*
Elevation band	2	44	1.423	0.252	2	44	0.632	0.536
Transect (elevation band)	6	44	0.711	0.643	6	44	0.792	0.581
Aspect	1	44	1.526	0.223	1	44	0.297	0.589
	Adult proportion				*Rejuvination*			
Source of variation	N_{df}	D_{df}	*F*	*P*	N_{df}	D_{df}	*F*	*P*
Elevation band	2	44	3.425	0.041	2	44	3.280	0.047
Transect (elevation band)	6	44	2.678	0.027	6	44	5.037	0.001
Aspect	1	44	3.290	0.077	1	44	.471	0.496

N_{df} = numerator df; D_{df} = denominator df

Density-diameter (d-d) curve

The density-diameter (d-d) curve for adult *J. indica* from all study plots was nearly reverse *J*-shaped (Fig. 4a), where the density of *J. indica* was successively reduced with the increase of trunk diameter. In the lower-elevation band, smaller- and large-sized adult individuals were more or less equally present except for size class >4-6, which had highest density value (Fig. 4b);whereas in the mid- and higher-elevation bands, the large-sized adults(>8 cm in mid- and >6 cm in higher-elevation band) were completely absent (Fig. 4c,d).

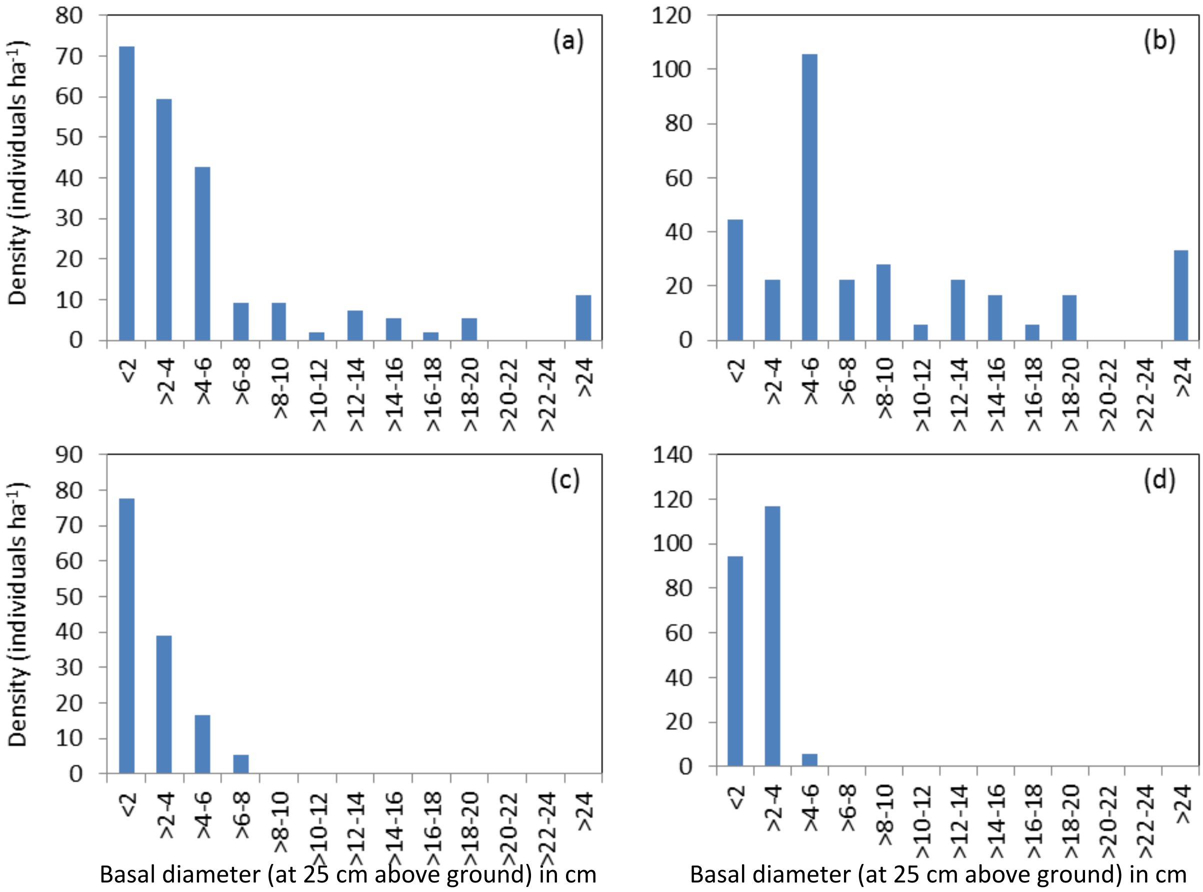

Fig.4. Density-diameter curve for adult *J. indica* in (a) the overall study plots, (b) lower-elevation band, (c) mid-elevation band, and (d) higher-elevation band.

3.3 Vegetative and Reproductive Traits of *J. indica*

3.3.1 Variation in vegetative and reproductive traits

Mean, standard error (SE) and range values of vegetative and reproductive traits of *J. indica* recorded in Ice Lake, upper Manang valley are given in Table 4. Most of the individuals of *J. indica* were of moderate to small size which height, trunk diameter and canopy area ranged 0.50-25 m, 0.95-60.48 m and 0.01-2.01 m^{-2} respectively. Mean leaf dry weight per 0.0625 m^2 canopy was 0.031 kg (range 0.013-0.058 kg) and on per hectare basis the mean dry leaf biomass was 28.98 kg. The mean number of fruits per plant ranged 10-1040 (mean 202.9).

LMM analysis revealed significant effect of elevation as fixed factor on vegetative and reproductive traits of *J. indica* (Table 5). *J. indica* showed higher values of all its vegetative and reproductive traits in lower-elevation than in mid- and higher-elevation bands (Fig. 5). Individuals of *J. indica* at lower-elevation were larger in vegetative size, with larger trunk, height, canopy area, and produced higher leaf biomass and greater number of fruits than did by individuals from mid- and higher-elevation (Fig. 5). However, LMM followed by pair-wise comparison of the main effects (elevation band) based on Bonferroni test revealed insignificant differences in almost all vegetative and reproductive traits of *J. indica* between mid- and higher-elevation bands (Fig. 5). There was also significant effect of nested factor (transects nested within elevation band) on trunk diameter, dry weight and fruits set, indicating that these parameters spatially varied within the same elevation band. Effect of aspect or PADIR (both used separately as covariate in the model) were not significant, indicating SE and SW gradient or incident radiation did not affect vegetative and reproductive traits.

Table 4. Mean, standard error (SE) and range values of vegetative and reproductive traits of *J. indica* recorded in Ice Lake, upper Manang valley.

Traits	*N*	Mean	SE	Minimum	Maximum
Plant height (m)	134	2.62	0.26	0.50	25.00
Trunk diameter (cm)	134	5.82	0.71	0.95	60.48
Canopy area (m^2)	134	0.16	0.03	0.01	2.01
Leaf dry weight (g) per 0.0625 m^2 canopy	56	31.12	1.65	12.50	58.10
Leaf dry weight (g) per plant	56	153.26	47.27	1.22	1801.51
Leaf dry biomass (kg ha^{-1})	46	28.98	8.37	0.18	202.20
Number of fruits per plant	90	202.90	21.64	10.00	1040.00

Table 5. Results of liner mixed model (LMM) showing the effects of elevation band and transect (nested within elevation band) as fixed factor and aspect (folded about the north-south line) as cofactor on vegetative and reproductive traits of *J. indica*.

	Trunk diameter				*Plant height*			
Source of variation	N_{df}	D_{df}	*F*	*P*	N_{df}	D_{df}	*F*	*P*
Elevation band	2	120	52.744	<0.001	2	118	36.159	<0.001
Transect (elevation band)	7	120	14.524	<0.001	7	118	9.735	<0.001
Aspect	1	120	2.387	0.125	1	118	.311	0.578
	Canopy area				*Leaf biomass per 0.0625 m^2canopy*			
Source of variation	N_{df}	D_{df}	*F*	*P*	N_{df}	D_{df}	F	*P*
Elevation band	2	116	53.745	<0.001	2	45	35.535	<0.001
Transect (elevation band)	7	116	24.468	<0.001	7	45	3.620	0.004
Aspect	1	116	2.530	0.114	1	45	.223	0.639
	Leaf biomass ha^{-1}				*Fruits set per plant*			
Source of variation	N_{df}	D_{df}	*F*	*P*	N_{df}	D_{df}	F	*P*
Elevation band	2	35	12.592	<0.001	2	77	15.784	<0.001
Transect (elevation band)	7	35	1.374	0.247	7	77	8.070	<0.001
Aspect	1	35	1.042	0.314	1	77	1.566	0.215

N_{df} = numerator df; D_{df} = denominator df

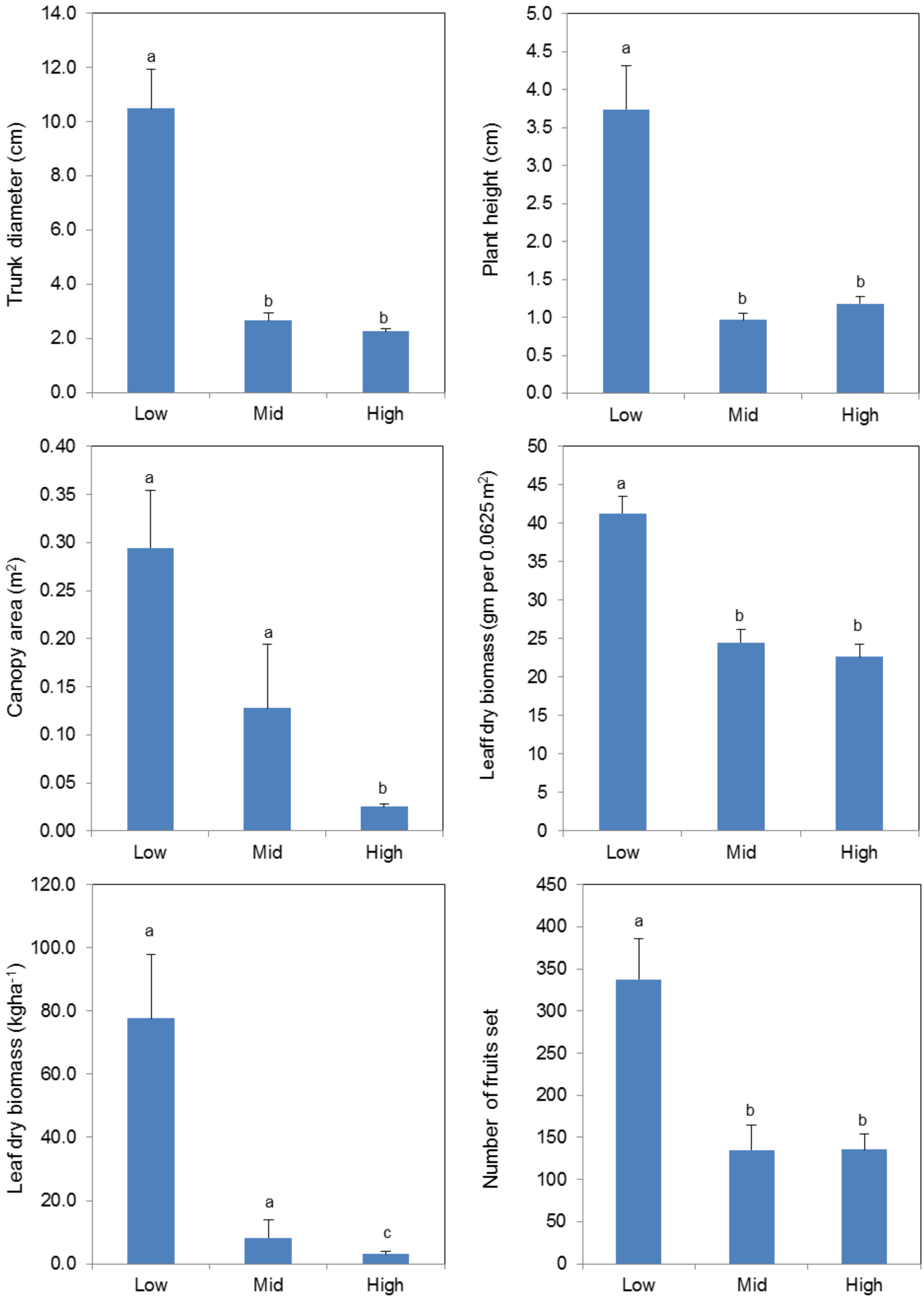

Fig.5. Variation in vegetative and reproductive traits of *J. indica* at three elevation bands (low, mid and high) in Ice Lake, upper Manang valley.

3.3.2 Correlation among traits

Pearson correlation analyses revealed significant relationships among a numbers of traits of *J. indica* (Appendix IV). Significant correlations (p<0.01) were observed between trunk diameter and plant height (r = 0.580), trunk diameter and canopy area (r = 0.344), trunk diameter and leaf dry weight (r = 0.538), canopy area and leaf dry weight (r = 0.932), trunk diameter and number of fruits (r = 0.439), and plant height and number of fruits (r = 0.339). Allomatric traits exhibiting statistically significant correlations with leaf dry weight and fruit output (as in Appendix IV) were further analyzed through linear regression analysis to evaluate the strength of relationships and derive allometric equations (Table 6, Fig. 6). Of the three allometric measurements (trunk diameter, plant height and canopy area), only canopy area was the strongest variable or predicting total leaf biomass (Table 6).

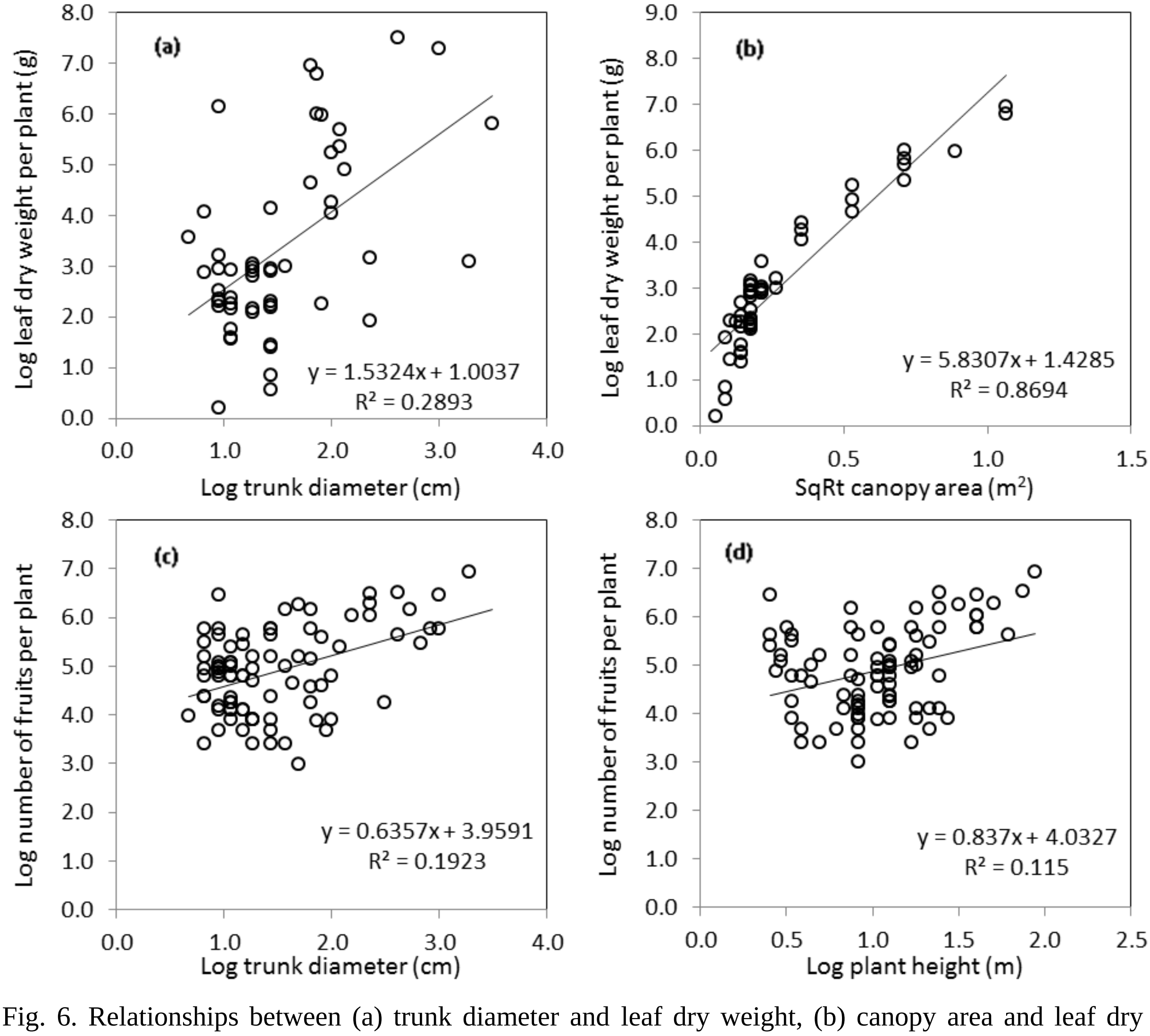

Fig. 6. Relationships between (a) trunk diameter and leaf dry weight, (b) canopy area and leaf dry weight, (c) trunk diameter and number of fruits, and (d) plant height and number of fruits. Fitted line based on linear regression model.

Table 6. Results of linear regression analysis predicting total leaf biomass (dry weight in g) and total fruit output per adult individual with significant tree allometric (predictor) variables at the Ice Lake area, upper Manang. Measured variables were log or square root transformed for regression analysis.

Response variable	Predictor variable	Intercept	Slope	R^2	SE_e	SE_i	SE_s	*F*
Leaf biomass (log)	Trunk diameter (log)	1.0037	1.5324	0.289	1.515	0.557	0.336	20.760
	Canopy area (sqrt)	1.4285	5.8307	**0.869**	0.570	0.123	0.323	326.310
Fruit output (log)	Trunk diameter (log)	3.9591	0.6357	0.192	0.807	0.227	0.141	20.235
	Plant height	4.0327	0.8370	0.115	0.844	0.277	0.252	11.042

SE_e – standard error of the estimate, SEi – standard error of the intercept, SEs – standard error of the slope.

CHAPTER FOUR: DISCUSSION

4.1 Habitat Characteristics of *J. indica*

Juniper is one of the diverse genera of the conifers comprising of about 75 species that are found from sea level to above timber line (Adams 2014). Most of the taxa of junipers are found on the Laurasian land mass, the rift mountain in east Africa into the southern hemisphere and the mountains of the northernmost part of Africa (Adams *et al.* 2009). They mostly prefer limestone, and found on dry rocky habitats (Junicost 2010; Ueckert 2013). In all the drier habitats of junipers throughout the world, the major factor limiting their abundance is soil water availability. *Juniperus indica* is the common species in the subalpine and alpine Himalayas. It prefers open rocky habitats in SE- to SW-facing slopes in upper Manang valley, with main vegetation type being *Rosa-Berberis-Juniper* shrubland, subalpine and alpine grasslands, and open forests (mainly of *Pinus wallichiana* at lower elevation). Generally, forests above 3800 m on the southern aspect in the trans-Himalayan dry valleys of central Nepal are bushy in nature and comprised mainly of *J. indica* (Ghimire B.K. *et al.* 2008; Ghimire *et al.* 2010). *J. indica* grows on dry, rocky and sandy substrates occurring on gentle SW-facing slopes towards higher elevations receiving high solar radiation (Bhattarai *et al* .2006; Rawat and Everson 2012). Being the root system highly developed and xerophytic nature, the plant has an advantage to establish in dry and rocky substrates. The species is therefore proposed to be particularly suited for afforestation program under xeric ecological conditions of trans-Himalaya (Rawat and Everson 2012).

In the present study, 88 vascular plant species (representing 64 genera and 37 families) associated with *J. indica* were identified within elevation range from 3350 to 4250 m asl. Asteraceae was the largest family. Studies on species composition in the subalpine forests of upper Manang valley were done by several workers (e.g., Baniya *et al.* 2009; Ghimire B.K. *et al.* 2008; Panthi *et al.* 2007), but none of the study relates abundance of *J. indica* with environmental attributes. Ghimire B.K. *et al.* (2008) reported 19 associated species in *J. indica* forest, representing 14 genera and 11 families within the similar elevation range from 3300 to 4000 m asl. Vegetation within a landscape is greatly affected by differences in the microclimate, aspect and altitude. Reinoso *et al.* (2003) reported the main source of geographic variation in the plant species composition of juniper communities along the south west coast of Spain was due to climate and soil texture. Aspect is one of the strong topographic factors affecting soils and microclimate thereby mediating species composition within a particular elevation level (Bennie *et al*. 2006). In upper Manang valley, dry south-facing slope is the most preferred habitat for the growth of *J. indica*. Compositional variation is also related to functional type interactions as different functional types are adapted to different environmental conditions in such a way that competitive interactions are minimized (Pausas *et al.* 2001).

4.2 Density and Size Distribution of *J. indica*

Total density of *J. indica* (1374 individuals per ha) recorded from three elevation bands in the present study is almost three times higher than the value (404 individuals per ha) obtained by Ghimire B.K. *et al.* (2008) in Manang, but almost three times less than the value obtained by Chhetri and Gupta (2007) in Mustang (4250 individuals per ha) and Rai (2013) in Langtang region (3500 individuals per ha). However, the elevation trend of population density was almost identical with that of previous reports. In the present study, total density and density of seedlings and juveniles of *J. indica* were found to be high at mid elevation (3650-3880 m asl), but the density of adult trees was high at lower elevation (3350-3580 m). Ghimire B.K. *et al.* (2008) also recorded the total density of *J. indica* to be highest at 3500-3800 m asl (516.66 individuals per ha), followed by 3800-4000 m asl (375 individuals per ha)and 3300-3500 m asl (320 individuals per ha).The variation in population size of seedlings, juveniles and adults at different elevations may be the results of climatic (especially rainfall and temperature) and edaphic factors (availability of soil water) that are critically important for the successful recruitment, establishment, survival and reproduction of plants (Bharali *et al.* 2012). There is sharp decline in temperature with the rise of elevation (temperature lapse rate for the western Himalayas is estimated to be 0.6-0.74°C per 100 m elevation raise for various months of the year, Jain *et al.* 2008). High elevation areas are also characterized by shorter growing season, resulting in reduced annual growth (Tranquillini 1979; Vetaas 2000). The pattern of decrease in density with elevation can vary with species as biotic interactions, mainly competition, also play a role in growth rate (McPherson and Wright 1989; Ghimire *et al.* 2010).

The future community structure and regeneration status of plant species can be predicted from the relative proportion of seedlings, juveniles and adults in the total population (Bharali *et al.*

2012). The overall population structure of *J. indica* in the three altitudinal bands of the present study area showed the highest contribution of juveniles to the total population size followed by seedlings and adults. The highest contribution of juveniles may be the result of maximum seedlings grown to juveniles. Seedlings and juveniles together constituted about 84.35% of the total population of *J. indica*. This shows good regeneration potential of *J. indica* in the study area. Density-diameter curve for *J. indica* further showed reverse J-shaped structure indicating continuous regeneration (Vetaas 2000). Similar reverse J-shaped patterns of population structure have been reported for different high-altitude tree species of Manang, central Nepal, such as *Abies spectabilis* (Ghimire and Lekhak 2007), *Betula utilis* (Shrestha *et al.* 2007), and *Pinus wallichiana* (Ghimire *et al.* 2010). .

Some of the previous studies have reported that regeneration potential of *Juniperus* spp. is generally low (Juan *et al.* 2003; Otto *et al*. 2010). It has been identified that the principal ecological problems related to *Juniperus* spp. is low production of viable seeds (Juan *et al.* 2003; Junicost 2010; Otto *et al*. 2010). The other factors considered for explaining low regeneration in *Juniperus* spp. are related to disturbance, increased competition, and absence of suitable dispersal vectors (McPherson and Wright 1989). Juan *et al.* (2003) assessed viability in *J. oxycedrus* which showed difficulties in the process of seed germination. In some dioecious species of *Juniperus*, low reproductive success is due to low amount of pollen that reaches female individuals resulting in less number of fruits set (Juan *et al*. 2003). In a study, inbreeding associated with fragmentation-led small population size in *Juniperus communis* has been attributed to low germination and establishment success (Oostermeijer *et al*. 2003; Gerard *et al*. 2004). In contrast to these findings, my study indicates successful regeneration of *J. indica* in Manang, central Nepal despite harsh ecological conditions. *J. indica* exhibits dioecious or monoecious habit (Adams 2014). Even in dioecious form, the distribution of male and female adults in most populations was random with no evidence of sex clustering resulting in high reproductive success by high amount of pollen reaching female individuals to produce high number of fruits set and this might be the reason why *J. indica* showed high regeneration potentiality in Manang.

However, regeneration of *J. indica* was comparatively low in lower elevation band, despite higher seed output (see below), than in mid and higher elevations. This might be due to several reasons. Firstly, this can be linked to disturbance, as the lower elevation band of the study area is close to settlement area where it received greater human pressure especially from livestock grazing. Secondly, *J. indica* in lower elevation band is associated with *Pinus wallichiana* where dominance of pine needles deposition of later species might have suppressed recruitment and successful establishment of *Juniper* seedlings. Reinoso *et al.* (2003), for example, observed that maritime juniper woodlands of Spain were affected by pine plantations, where deposition of pine needles reduced recruitment of juniper seedlings and increased their mortality. Junicost (2010) reported that as pines are growing much faster than junipers, they produce massive numbers of easily germinable seeds and modify the micro environment against other species by deposition of pine needles. Junipers have low germination capacity [e.g., <5% in maritime juniper (Reinoso *et al.* 2003)] are difficult to propagate from seed and are slow growing which, when coupled with human disturbance and competitive stresses, can make establishment difficult (Forestry commission 2003).

Increasing proportions of younger individuals of *J. indica* in mid and high elevation band show its capacity to tolerate harsh ecological conditions of higher elevations. Wide elevation amplitude (3300-4500 m within Nepal) together with its capacity to tolerate harsh conditions may enable *J. indica* to be quite successful species to adapt ecological changes. There is evidence that increasing carbon dioxide concentrations in the atmosphere during the last century may be benefitting junipers as they utilize the elevated carbon dioxide concentrations in C3 photosynthetic pathway (Mayeux *et al*. 1991). Conifers exhibit pronounced growth increase with increasing carbon dioxide concentrations in the atmosphere, suggesting that carbon dioxide enhancement may have played a role in the recent increases in the distribution and abundance of junipers throughout North America (Mayeux *et al*. 1991).

4.3 Vegetative and Reproductive Traits of *J. indica*

J. indica showed higher values of all its vegetative and reproductive traits in lower-elevation than in mid- and higher-elevation bands. In species with wider distribution, such as *J. indica* different populations differ from each other in the mean value of the vegetative characters. In such species, vegetative traits exhibit strong correlation with geography and are of direct adaptive value. Plant height, for example, has been observed to be correlated with latitude, longitude and altitude (Klinka *et al*. 1996). At the higher-elevation, on subalpine and alpine regions, the climate is cold whole over the year and growing season is short. Plants growing along an elevation gradient exhibit reduction in radial and vertical growth of their stem towards higher elevations mostly caused by corresponding decline in temperature (Körner *et al.* 1983). A reduction in radial growth at higher-elevation is related to the shortened growing period largely as a result of the delay in start of seasonal growth (Tranquillini 1979). At cooler temperatures, there is a tendency for photosynthate to be transformed to sugars and starch rather than cellulose and this can limit diameter growth (Tranquillini 1979). Shortening of stem allows plant to avoid the damaging effect of the strong winds at high elevations and to improve photosynthetic conditions by keeping the leaves closer to the warmer soil surface (Körner *et al.* 1983).

In the present study, the canopy area of *J. indica* was found to vary from 0.01 to 2.01 m^{-2}, which is far less than the findings of Ansley *et al.* (2012) who measured canopy area of redberry juniper of north Texas to be 27.9± 3.5 m^2(mean± SE, range: 1.4-73.9 m^2). Average canopy area of *J. indica* was found to be high in lower-elevation band which decreased gradually towards mid- and higher- elevations. Similar was the trend for leaf biomass of *J. indica*. A linear negative relationship between biomass and elevation has been reported for several plant speices (e.g., Rastetter *et al.* 2004) due to declining temperature and nutrient availability with increasing elevation. Kofidis *et al.* (2008) reported that many plants growing along altitudinal gradients have smaller leaves in their upland habitats and the leaves of the plant can undergo major alterations in relation to season. This may also result in reduction in leaf biomass with the increase in elevation. Low phytomass at high elevation has also been attributed to trampling and overgrazing by domestic livestock depleting plant resources on high altitude pastures (Zhou *et al.* 2006). As *Juniperus indica* is unpalatable to livestock, direct grazing impact on phytomass production of this species is therefore ruled out. However, trampling effect of livestock, and burning and harvesting of live plants for fuel and incense have imposed pressures on *J. indica* populations in the Himalaya. This study did not focus on the extent of human exploitation and its impact on *J. indica* populations, therefore further study is needed to quantify the anthropogenic impact on biomass production and regeneration status.

Of the three allometric measurements (trunk diameter, plant height and canopy area), canopy area was the strongest variable (r^2 = 0.869) for predicting total leaf biomass. This result agrees with the findings of Ansley *et al.* (2012) who found stronger linear relationship between canopy area and leaf biomass (r^2 = 0.940) on redberry juniper in Texas. Similarly, Sabin (2008) made similar measurements on western juniper in Oregon. The slope of our canopy area/leaf biomass curve (5.831) was considerably lower than the value (9.720) obtained by

Sabin (2008) over a similar range of juniper canopy sizes. Thus, for a given canopy area, Ansley *et al.* (2012) and Sabin (2008) have predicted a higher biomass than our results. Similarly, Miller *et al*. (1981) predicted greater leaf biomass of Utah juniper (*Juniperus osteosperma*) in Nevada for a similar range of canopy areas (which we calculated from their canopy diameter data only) than our predictions by combining diameter at breast height and average crown diameter to predict above ground biomass (r^2 = 0.963).

Among the other allometric variable, trunk diameter was less strong in predicting total leaf biomass (r^2 = 0.289). Plant height was even less effective predictor of leaf biomass, which is similar to the finding of Ansley *et al.* (2012). Measurement of basal trunk diameter and canopy height is difficult in bushy juniper because it has a very compact canopy with a high density of low growing stems that restricts access to central base stems. Thus, the use of outer canopy dimensions may be the best option for non destructively estimating above ground mass of *Juniperus indica* as reported in other species (Ansley *et al.* 2012).

None of the allometric traits considered in this study showed strong power for predicting reproductive output. Nevertheless, larger plants [with greater trunk diameter (r^2 = 0.192) and height (r^2 = 0.115)] produced more fruits than smaller plants (Table 6). As the plants in lower elevation band are generally larger in size they produced greater number of fruits than those in mid and higher elevation bands. Less number of fruits produced per plant in mid- and high elevation band may also be related to limited pollination success (Juan *et al.* 2003) as a result of wider spatial distance between male and female plants. However, further study is needed to support this hypothesis.

CHAPTER FIVE: CONCLUSIONS

Juniperus indica preferred dry and rocky habitats in SE- to SW-facing slopes, along with *Rosa-Berberis-Juniper* shrubland, subalpine and alpine grasslands, and open forests. Above 3800 m on the southern aspect, the forest was comprised of only bushy *J. indica.* Altogether, 88 plant species, associated with *J. indica*, were identified. Lower- and mid-elevation bands mostly comprised woody shrubs, whereas herbaceous species dominated higher-elevation band.

Population density of *J. indica* and its vegetative and reproductive traits are influenced differently by the variations in elevation. Mid-elevation band tended to show highest density of seedlings and juveniles, but adult density was high in the plots at lower-elevation band. *J. indica* exhibited almost similar population structure in three bands, with high contribution of juveniles than seedlings and adults. However, proportion of adult was high in lower-elevation, whereas proportions of seedling and juvenile tended to be high in mid- and higher-elevations. Density-diameter curve for adult *J. indica* was reverse *J*-shaped, indicating continuous regeneration.

Most of the individuals of *J. indica* were of moderate to small size. Individuals at lower-elevation were larger in vegetative size, with larger trunk, height, canopy area, and produced higher leaf biomass and greater number of fruits than did by individuals from mid- and higher-elevation. Trunk diameter, leaf dry weight and fruits set parameters spatially varied within the same elevation band.

Vegetative and reproductive traits are the most important characteristics to discriminate the populations of *J. indica*. It is concluded that *J. indica* in Manang exhibits successful regeneration despite harsh ecological conditions. Higher regeneration at mid- and higher-elevation bands indicates plants ability to tolerate adverse environmental conditions as well as a tendency for expansion of its distribution niche towards cooler habitat of high elevation.

The use of outer canopy area is the best option for non-destructively estimating above ground biomass of *J. indica*. This could be used for a variety of purposes, including estimation of biomass for bio-energy purposes or quantification of regional carbon stocks.

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Appendix I: Datasheet used in the field

Transect No: Altitude: Date:

Lat: Long: Slope: Aspect:

Ecology:

Vascular plant species associated with *Juniperus indica* in each sub plot:

S.N.	Name of the species	Presence in Sub- plots			
		A	B	C	D
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					
9.					
10.					

<u>For Size of each individual of *Juniperus indica*</u>

*Plot size: 10m * 10m with 4 sub-plots & sub-plot size: 5m * 5m*

S.N.	Size class Status		No. of individuals				Radius of the canopy (m)	Trunk Perimeter (cm)	Height from ground (cm)	No. of fruits
			A	B	C	D				
1	Seedling									
2	Juvenile									
3	Mature	I1								
		I2								
		I3								
		I4								
		I5								
		I6								
		I7								
		I8								
		I9								
Total										

Appendix II: Frequency of occurrence (%) ofvascular plant species associated with *Juniperus indica* in different elevation bands.

SN	Plant species	Family	Frequency of occurrence (%) in different elevation bands			
			Lower-elevation band	Mid-elevation band	Higher-elevation band	Overall
1.	*Ajuga lupulina* Maxim.	Lamiaceae	0.00	1.39	33.33	11.57
2.	*Allium sikkimense* Baker	Amaryllidaceae	0.00	0.00	2.78	0.93
3.	*Anaphalis triplinervis* (Sims) C. B. Clarke	Asteraceae	0.00	0.00	1.39	0.46
4.	*Anaphalis xylorhiza* Sch. Bip. ex Hook. f.	Asteraceae	0.00	26.39	27.78	18.06
5.	*Anaphalis contorta* (D. Don) Hook. f.	Asteraceae	0.00	0.00	13.89	4.63
6.	*Androsace muscoidea* Duby	Primulaceae	0.00	0.00	11.11	3.70
7.	*Androsace tapete* Maxima.	Primulaceae	0.00	0.00	1.39	0.46
8.	*Anemone rupicola* Cambess.	Ranunculaceae	0.00	0.00	12.50	4.17
9.	*Arabidopsis himalaica* (Edgew.) O.E. Schulz	Brassicaceae	0.00	1.39	13.89	5.09
10.	*Arisaema jacquemontii* Blume	Araceae	0.00	0.00	9.72	3.24
11.	*Artemisia* sp.1	Asteraceae	16.67	0.00	1.39	6.02
12.	*Artemisia* sp.2	Asteraceae	0.00	4.17	19.44	7.87
13.	*Artemisia subdigitata* Mattf.	Asteraceae	0.00	12.50	5.56	6.02
14.	*Asparagus filicinus*Buch.-Ham. ex D.Don	Asparagaceae	9.72	0.00	0.00	3.24
15.	*Aster himalaicus* C.B. Clarke	Asteraceae	11.11	25.00	1.39	12.50
16.	*Aster albescens* (DC.) Hand.- Mazz	Asteraceae	20.83	26.39	16.67	21.30
17.	*Astragalus multiceps* Wall. ex Benth.	Fabaceae	1.39	9.72	11.11	7.41
18.	*Astragalus candolleanus*Royle ex Benth.	Fabaceae	0.00	12.50	19.44	10.65
19.	*Berberis aristata* DC.	Berberidaceae	38.89	38.89	37.50	38.43
20.	*Betula utilis* D.Don	Betulaceae	0.00	0.00	4.17	1.39
21.	*Bistorta macrophylla* (D. Don) Sojak	Polygonaceae	0.00	33.33	1.39	11.57
22.	*Bistorta affinis* (D. Don) Greene	Polygonaceae	8.33	0.00	11.11	6.48
23.	*Carex* sp. 1	Cyperaceae	65.28	18.06	29.17	37.50
24.	*Carex* sp. 2	Cyperaceae	56.94	16.67	29.17	34.26
25.	*Carex* sp. 3	Cyperaceae	27.78	18.06	37.50	27.78
26.	*Chesneya nubigena* (D.Don) Ali	Fabaceae	0.00	0.00	4.17	1.39
27.	*Cremanthodium ellisii* (Hook.f.) Kitam.ex Kitam.& Gould	Asteraceae	0.00	0.00	5.56	1.85
28.	*Cicerbita macrorhiza* var. *saxatilis* (Edgew.) P.Brauv	Asteraceae	0.00	2.78	15.28	6.02
29.	*Clematis buchananiana* DC.	Ranunculaceae	6.94	0.00	0.00	2.31

SN	Plant species	Family	Frequency of occurrence (%) in different elevation bands			
			Lower-elevation band	Mid-elevation band	Higher-elevation band	Overall
30.	*Coelogyne* sp.	Orchidaceae	4.17	0.00	0.00	1.39
31.	*Conyza* sp.	Asteraceae	0.00	4.17	38.89	14.35
32.	*Cortia depressa* (D.Don) C.Norman	Apiaceae	0.00	0.00	20.83	6.94
33.	*Corydalis juncea* Wall.	Fumariaceae	5.56	16.67	22.22	14.81
34.	*Cotoneaster microphyllus* Wall. ex. Lindl.	Rosaceae	27.78	52.78	19.44	33.33
35.	*Cotoneaster affinis* Lindl.	Rosaceae	0.00	6.94	0.00	2.31
36.	*Cyananthus microphyllus* Edgew.	Campanulaceae	0.00	0.00	2.78	0.93
37.	*Delphinium brunonianum* Royle	Ranunculaceae	0.00	15.28	37.50	17.59
38.	*Ephedra gerardiana* Wall.ex Stapf	Ephedraceae	0.00	0.00	27.78	9.26
39.	*Equisetum* sp.	Equisetaceae	11.11	0.00	0.00	3.70
40.	*Euphorbia himalayensis* Klotzsch	Euphorbiacea	0.00	5.56	6.94	4.17
41.	*Euphorbia stracheyi* Boiss.	Euphorbiacea	0.00	0.00	11.11	3.70
42.	*Fragaria nubicola* Lindl. Ex Lacaita	Rosaceae	0.00	2.78	11.11	4.63
43.	*Galium aparine* L.	Rubiaceae	0.00	0.00	2.78	0.93
44.	*Gentiana robusta* King ex. Hook.	Gentianaceae	0.00	0.00	29.17	9.72
45.	*Gentiana depressa* D.Don	Gentianaceae	0.00	15.28	9.72	8.33
46.	*Gentianella pedunculata* (D.Don) H.Smith	Gentianaceae	0.00	2.78	27.78	10.19
47.	*Gentianella paludosa* (Hook.) H. Sm.	Gentianaceae	0.00	5.56	6.94	4.17
48.	*Kobresia gammiei* C.B. Clarke	Cyperaceae	0.00	13.89	31.94	15.28
49.	*Heracleum obtusifolium* Wall. ex. DC.	Umbeliferae	5.56	0.00	4.17	3.24
50.	*Hippophae tibetana* Schlecht.	Elaeagnaceae	0.00	5.56	0.00	1.85
51.	*Iris kemaonensis* Wallich ex. Royle	Iridaceae	0.00	0.00	12.50	4.17
52.	*Juniperus squamata* Buch-Ham ex. D.Don	Cupressaceae	22.22	40.28	66.67	43.06
53.	*Juniperus communis* L.	Cupressaceae	23.61	29.17	0.00	17.59
54.	*Salix calyculata* Hook.f. ex Andersson	Salicaceae	1.39	0.00	0.00	0.46
55.	*Leontopodium stracheyi* (Hook.f.) C.B. Clarke ex Hemsl.	Asteraceae	16.67	20.83	23.61	20.37
56.	*Ligustrum confusum* Decne.	Oleaceae	0.00	0.00	8.33	2.78
57.	*Lonicera hypoleuca* Decne.	Caprifoliaceae	0.00	5.56	58.33	21.30
58.	*Lonicera minutifolia* Kitam.	Caprifoliaceae	0.00	0.00	19.44	6.48
59.	*Morina nepalensis* D.Don	Morinaceae	20.83	0.00	0.00	6.94
60.	*Oxytropis williamsii* Vass.	Fabaceae	8.33	0.00	0.00	2.78
61.	*Pedicularis pectinata* Wall. Ex. Benth.	Scrophulariaceae	0.00	0.00	19.44	6.48

SN	Plant species	Family	Frequency of occurrence (%) in different elevation bands			
			Lower-elevation band	Mid-elevation band	Higher-elevation band	Overall
62.	*Pedicularis rhinanthoides* Schrenk.	Scrophulariaceae	11.11	0.00	0.00	3.70
63.	*Pedicularis flexuosa* Hook. f.	Scrophulariaceae	0.00	13.89	25.00	12.96
64.	*Pinus wallichiana* A.B. Jackson	Pinaceae	11.11	0.00	0.00	3.70
65.	*Pleurospermum apiolens* C.B. Clarke	Apiaceae	2.78	15.28	22.22	13.43
66.	*Polygonatum hookeri* Baker	Convallariaceae	0.00	8.33	23.61	10.65
67.	*Polygonatum cirrhifolium* (Wall.) Royle	Convallariaceae	0.00	5.56	13.89	6.48
68.	*Potentilla fructicosa* Lindl. ex Lehm.	Rosaceae	19.44	34.72	15.28	23.15
69.	*Potentilla peduncularis* D.Don	Rosaceae	0.00	6.94	41.67	16.20
70.	*Primula primulina* (Spreng.) H. Hara	Primulaceae	0.00	18.06	15.28	11.11
71.	*Rhododendron anthopogon* D.Don	Ericaeae	31.94	19.44	0.00	17.13
72.	*Rhododendron lepidotum* Wall. ex D. Don	Ericaeae	0.00	11.11	41.67	17.59
73.	*Rosa sericea* Lindl.	Rosaceae	41.67	63.89	18.06	41.20
74.	*Rumex nepalensis* Spreng.	Polygonaceae	0.00	0.00	22.22	7.41
75.	*Saussurea nepalensis* Spreng.	Asteraceae	0.00	5.56	15.28	6.94
76.	*Saxifraga andersonii* Engl.	Saxifragaceae	0.00	9.72	2.78	4.17
77.	*Spiraea canescens* D.Don	Rosaceae	0.00	0.00	38.89	12.96
78.	*Swertia cuneata* D.Don	Gentianaceae	0.00	0.00	27.78	9.26
79.	*Swertia chirayita* Karsten	Gentianaceae	0.00	0.00	23.61	7.87
80.	*Tanacetum dolichophyllum* Kitam.	Asteraceae	41.67	47.22	73.61	54.17
81.	*Taraxacum eriopodum* DC.	Asteraceae	1.39	4.17	0.00	1.85
82.	*Tanacetum* sp. (local name Khamsang)	Asteraceae	11.11	25.00	65.28	33.80
83.	*Thalictrum cultratum* Wall.	Ranunculaceae	0.00	1.39	27.78	9.72
84.	*Thalictrum alpinum* L.	Ranunculaceae	11.11	22.22	0.00	11.11
85.	*Thymas linearis* Benth.	Lamiaceae	0.00	0.00	12.50	4.17
86.	*Trifolium* sp.	Fabaceae	0.00	5.56	12.50	6.02
87.	*Verbascum thapsus* L.	Scrophulariaceae	4.17	12.50	8.33	8.33
88.	*Viola biflora* L.	Violaceae	0.00	0.00	6.94	2.31

Appendix III: Families with number of genera and species recorded from the study area.

S.N.	Families	No. of Genera	No. of species
1	Asteraceae	10	16
2	Rosaceae	5	7
3	Gentianaceae	3	6
4	Ranunculaceae	4	5
5	Fabaceae	4	5
6	Scrophulariaceae	2	4
7	Cyperaceae	2	4
8	Primulaceae	2	3
9	Polygonaceae	2	3
10	Cupressaceae	1	3
11	Apiaceae and Lamiaceae	Two each (4)	One each (4)
12	Caprifoliaceae, Convallariaceae, Ericaeae and Euphorbiaceae	One each (4)	Two each (8)
13	Alliaceae, Araceae, Asparagaceae, Berberidaceae, Betulaceae, Brassicaceae, Campanulaceae, Elaeagnaceae, Ephedraceae, Equisetaceae, Fumariaceae, Iridaceae, Morinaceae, Oleaceae, Orchidaceae, Pinaceae, Rubiaceae, Salicaceae, saxifragaceae, Umbeliferae and Violaceae	21(One each)	21(One each)
Total	37 Families	64 Genera	89 Species

Appendix IV*:* Pearson correlation coefficients among the vegetative and reproductive traits of *J. indica*.

Traits	Abbreviation	TrDiam	Ht	CanAr	DrWtPl	NoFr
Trunk diameter $^{+}$	TrDiam	1 (*n* = 131)				
Plant height^{+}	Ht	**0.580****(*n* = 129)	1 (*n* = 129)			
Canopy area $^{++}$	CanAr	**0.344**** (*n* = 124)	-0.074 (*n* = 122)	1 (*n* = 127)		
Leaf dry weight per plant $^{+}$	DrWtPl	**0.538**** (*n* = 53)	0.252 (*n* = 52)	**0.932**** (*n* = 51)	1 (*n* = 56)	
Number of fruits per plant^{+}	NoFr	**0.439**** (*n* = 87)	**0.339**** (*n* = 87)	0.100 (*n* = 82)	0.332 (*n* = 33)	1 (*n* = 88)

$^{+}$ The values were log transformed before analysis.

$^{++}$ The values were square root transformed before analysis.