# Ecological assessment of populations of *Juniperus phoenicea* L. in the Al-Akhdar mountainous landscape of libya

Hanan F. Kabiel, Ahmad K. Hegazy, Lesley Lovett-Doust, Saud L. Al-Rowaily, and Abd El-Nasser S. Al Borki

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Hanan F. Kabiel<sup>a</sup>, Ahmad K. Hegazy<sup>a</sup>, Lesley Lovett-Doust<sup>b</sup>, Saud L. Al-Rowaily<sup>c</sup>, and Abd El-Nasser S. Al Borki<sup>d</sup>

<sup>a</sup>Department of Botany and Microbiology, Faculty of Science, Cairo University, Giza, Egypt; <sup>b</sup>Department of Biology and Chemistry, Nipissing University, North Bay, ON, Canada; <sup>c</sup>Department of Plant Production, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia; <sup>d</sup>Department of Botany, Faculty of Science and Arts, University of Benghazi, Agdabia, Libya

#### ABSTRACT

The Phoenician juniper (Juniperus phoenicea L.) has long been common in the Al-Akhdar mountain range in NE Libya. Juniper trees in the region are showing signs of dieback, and minimal successful seedling recruitment has occurred. We investigate this effect at the level of individual trees, tree size classes, and populations. At ten sites, population size, growth and reproductive traits, and percentage dieback in different size classes were evaluated. Elevation, distance from the sea, and the interaction between these factors had a significant effect on tree volume, and number of branches, number of female cones per branch and number of female cones per tree. Two groups of populations were recognized: the first (Sites 2, 3, and 4) were near the coast. They appeared to be more recently established, with no individuals in the largest size class, and, at this point, less severe dieback. In contrast, in the rest of the sites there were no individuals (Sites 1, 5, 6, and 9) or very few individuals (Sites 7, 8, and 10) in the smallest size class, indicating widespread failure of recruitment in recent years. Mature female cones containing seeds were abundant at these sites (except for Site 8); therefore, this was not attributable to failure of sexual reproduction, but rather was associated with higher levels of intra- and inter-specific competition, combined with more variable rainfall in recent years. Smaller trees are particularly susceptible to dieback throughout the region. Conservation initiatives should include restrictions on clearing these natural forests for conversion to agriculture and, where harvesting is necessary, preferential harvesting of larger individuals.

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Dieback; failed seedling recruitment; *Juniperus phoenicea*; landscape; resistance; unstable size structure; variable rainfall

# Introduction

The Phoenician juniper (*Juniperus phoenicea* L., family Cupressaceae) is a Mediterranean tree typically found in coastal sites in the Mediterranean Basin, ranging from Portugal and the Canary Islands to Turkey and the northwest mountains of Saudi Arabia (Fisher 1997; Meloni et al. 2006). In Libya, *J. phoenicea* is abundant in the Al Akhdar mountain region (Hegazy et al. 2011). Plants in all habitat types show signs of dieback, a phenomenon that has become increasingly severe over the past ten years (A. K. Hegazy, unpublished). In a recent study on Caprera Island, in Italy, Linaldeddu et al. (2012) attributed dieback in

J. phoenicea to infection by the anamorphic (asexual) ascomycete, Diplodia africana Damm 60 & Crous. In that study, leaves on affected branches turned yellow, then dull red and finally brown. This sequence of change in leaf color was not observed in the present study, nor have other researchers reported such a phenomenon in the region. Instead what is seen is that leaves turn from green to brown, starting at the tip of upper branches, indicating a response to drought. In other studies, dieback in Phoenician juniper in Libya and the Arabian 65 Peninsula similarly showed no sign of fungal infection and has been attributed to drought, induced by climate change and associated changes in microclimate (Fisher and Gardner 1995; Gardner and Fisher 1996; Fisher 2000; Hegazy 2010).

Changes in population size structure and functional attributes can provide valuable insight on the status of a plant population (Wang et al. 2004; Hegazy et al. 2008). Size class distribution provides an indication of population stability and recruitment, as well as mortality rates; a stable size class distribution would include large numbers of juveniles, with progressively fewer members in larger-sized or older cohorts. The presence of a stable size or age-structure together with flexibility in traits related to persistence and regeneration, *i.e.* evidence of intraspecific variability in functional traits indicating phenotypic plasticity and/or potential local adaptation (Lovett-Doust, 1981; Hegazy 1994, 2001), in response to habitat heterogeneity would suggest that a plant population will survive, grow and reproduce even under changing climate conditions. It also provides a useful indication of the species' potential for ecological success (Lavorel 1999; Boucher et al. 2013; Read et al. 2013). Indeed, phenotypic variation is often associated with greater efficiency of resource use, greater microsite occupancy, and plays a fundamental role in community structure and dynamics (Bolnick et al. 2011; Dantas et al. 2013).

Phenotypic variation is often found in populations occurring along environmental gradients such as rainfall, temperature, slope or aspect. Altitudinal gradients are often important determinants of phenotypic variation in plant populations (e.g., de Bello, Lepš, and Sebastià 2006; Read et al. 2013). All of our study sites were either in the coastal region, on the windward (North-facing) slopes of the Al-Akhdar mountain range in NE Libya or in wadis (valleys or channels in the desert that collect (often sporadic) precipitation from adjacent uplands) on the North-facing slopes. As our initial hypothesis, we predicted that there would be a simple relationship between tree growth (in terms of vegetative and reproductive vigor) and altitude. This was based on the typical regional pattern of orographic rain. That is, when the onshore-moving moist air mass encounters a mountain range, there will be precipitation as a result of the fact that air cools as it ascends the windward side of a slope. This in turn reduces its capacity to hold water vapor, so rain falls on the windward slope, and the leeward slope is typically dry, as warming air descends. If this hypothesis is supported, there will be a significant positive relationship between altitude and plant vigor. A second potential factor was that of distance from the Mediterranean coast. As moistureladen air moves inland from the coast, and encounters successive mountain ranges, more and more of the moisture will have been lost. As a result, we would predict a decline in plant vigor with distance from the sea, controlling for altitude.

In order to characterize patterns of growth and reproduction and to assess the ecological status of J. phoenicea in NE Libya, we studied: (1) population size structure at different elevations and distances from the sea; (2) variation in functional traits within and among populations; and (3) the extent of dieback within individual trees, size classes, populations, and at the level of the region as a whole.

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# Materials and methods

# **Study species**

The Phoenician juniper (Juniperus phoenicea L., Family: Cupressaceae) is a keystone species in the Al Akhdar mountainous region, found in various habitat types from wadis to slopes but it is absent from the mountain peaks (Hegazy et al. 2011). This tree has scale-like leaves 110 known locally as "Araar." The whole plant is aromatic and is used as a source of essential oils (El-Darier and El-Mogaspi 2009). This species is recognized as the sole source of the tricyclic sesquiterpene, thujopsene; the heartwood contains an estimated 2.2% of this substance (Runeburg et al. 1960). Infusions of branches and fruits are used as an antiseptic, stomach tonic and diuretic, and in the treatment of cystitis and urinary infections. It is also applied 115 topically to treat dermatitis (A. K. Hegazy, unpublished). The species, like many members of the juniper family, has low palatability to ruminant herbivores, in part due to the strong flavor of terpenes (Rogosic et al. 2006), and in part because the antibacterial properties of these secondary compounds may disturb functioning of the ruminant gut flora (Schwartz, Regelin, and Nagy 1980). In some areas the species is used for construction, fence posts and 120 firewood, and the aromatic, succulent female cones are used in cooking and alcoholic beverages around the Mediterranean basin. The species is the most widespread and common juniper in the Mediterranean region, so the IUCN has classified it as of "Least Concern" (http://www.iucnredlist.org/details/42244/0). However, Phoenician juniper is a dominant species in dry and stony substrates, as well as in coastal dunes where sand movement 125 may cause burial of stems. It is also present in many protected areas, so it provides a useful sensitive and general indicator of changing environmental conditions in the region. In Cyprus the species was designated "Tree of the Year" in 2009 (Ministry of Agriculture, Natural Resources and Environment, Department of Forestry, Cyprus 2009). In Cyprus, logging is not allowed in the juniper forests; the Department of Forests, in the Ministry 130 of Agriculture, Natural Resources and Environment also emphasized the sensitivity of this species to fire; it does not regenerate well; instead, after a forest fire, planting of Phoenician juniper is necessary to ensure its regeneration and survival.

It may be useful to briefly address the current state of taxonomy related to J. phoenicia, such that the identity of our study species is clear (see Figure 1). Recent systematics work (Adams et al. 2013; Adams 2014) has addressed the distribution of what has to this point been called J. phoenicia var. turbinata. This variety (or for some authors, subspecies) was distinguished by female cones that were oval, in contrast to the globose cones of J. phoenicia var. phoenicia. Based on genetic studies Adams proposed that J. phoenicia ssp. eu-mediterranea was synonymous with J. phoenicia ssp. turbinata. He went on to argue that J. phoenicia ssp. (or var.) turbinata should be given distinct species status, thus re-naming the "Phoenician juniper" with oval cones as J. turbinata. This proposition was based on DNA fragment comparison of two samples of J. Phoenicia (var. phoenicia) from Spain, and J. phoenicia ssp. turbinata from various locations around the Mediterranean basin, with the only North African samples being from Morocco. It would be useful to carry out more thorough characterization of genetic diversity in "Phoenician juniper" populations around the Mediterranean basin to clarify whether any variation is varietal, regional, or ecotypic, or truly represents the level of genetic differentiation necessary to assign distinct species or subspecies. Much of the other taxonomic discussion has centered on the relative concentrations of different essential oils including terpenes, but such secondary compounds vary seasonally

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**Figure 1.** The North-facing slope of the Al Akhdar mountainous region at Ras El-Helal (site number 4) showing vegetation that is predominantly made up of *Juniperus phoenicia*. Insert: a cone-bearing branch of *Juniperus phoenicia* showing the globose (spherical) female cones, characteristic of *Juniperus phoenicia* (or *Juniperus phoenicia* var. phoenicia) depending on the taxonomy applied.

in individuals, and between individuals in populations, and, therefore, they do not offer a very strong basis for taxonomic distinctions. All of the populations surveyed in the present study in NE Libya had globose female cones (see insert in Figure 1). As a result, we are comfortable assigning these populations to the taxon *Juniperus phoenicia* var. *phoenicia*.

# Regional climate and soil parameters

Climate parameters for three weather stations, representing conditions across the region were obtained from the Libyan National Meteorological Center. At the three weather stations, soil samples were taken at a depth of 0–20 cm. Three replicate samples were taken in March 2013 from each study site, air dried, and sieved through a 2-mm sieve prior to analysis. Physical and chemical characteristics of the soil were analyzed (following Piper 1950; Jackson 1973; Allen et al. 1974; Black 1982; Isaac and Johnson 1983; Forester 1995). Particle size distribution and soil texture were determined by the pipette method (Piper 1950; Forester 1995). Soil organic matter content was determined according to Black (1982). Mineral ions were measured by atomic absorption spectroscopy (Varian, spectra AA220).

## Study sites

The Al-Akhdar mountainous region is in NE Libya, reaching 878 m above sea level (a.s.l.) and characterized by a Mediterranean climate, with cool rainy winters and hot dry summers (El-Tantawi 2005). The locations of the ten study sites are shown in Figure 2 and described in Table 1. Sites 1–6 are <4 km from the coast, while Sites 7–10 are >15 km from the coast. Sites 1, 7, and 10 are in wadis, the others represent North-facing mountain slopes. The population located at the highest elevation (Site 10, Wadi Moussa) was at 628 m elevation.

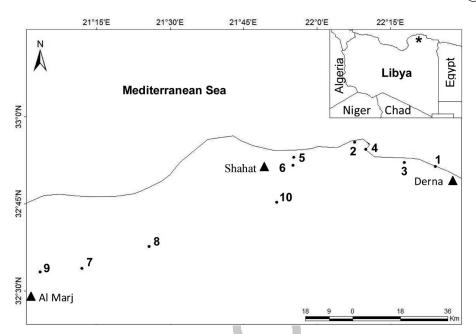
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**Figure 2.** Map of the study area in NE Libya, showing the location of the ten study sites in the Al Akhdar mountainous region. Site numbers are assigned in order of increasing elevation above sea level; see Table 1 for detailed information on each site. The three climate stations, Derna, Al Marj, and Shahat, corresponding to the environmental data in Table 2, are shown as triangles. The asterisk in the inset map indicates the study area within Libya.

# Population size structure and plant variation

Ten quadrats  $(10 \text{ m} \times 10 \text{ m})$  were established in the study area, in areas of high abundance of the study species, one in each population of *J. phoenicea* (for site descriptions see Table 1). The size (volume in m<sup>3</sup>) of each individual within each100 m<sup>2</sup> quadrat was estimated and vegetative and reproductive traits were measured (see the following section). Particular attention was given to the question of seedling

Elevation Distance from the (meters Mediterranean Site a.s.l.\*) coast (m) Location Site Habitat type 1 15 350 32° 51' 24.9" N; 22° 24' 14.4" E Wadi Karssa Wadi 2 22 1000 32° 55' 35.5" N; 22° 07' 44.8" E Ras El-Helal-Susa Road Mountain slope 32° 52' 08.2" N; 22° 17' 54.9" E 3 29 500 Susa-Derna Coastal Road Coastal plain 32° 54' 22.7" N; 22° 09' 58.5" E 4 48 150 Ras El-Helal Mountain slope Shahat -Susa Road 5 193 1600 32° 53' 01.9" N; 21° 55' 20.2" E Mountain slope 32° 51' 38.4" N; 21° 55' 05.8" E 6 345 4000 Shahat Mountain slope 7 364 22000 32° 33' 58.4" N; 21° 11' 58.4" E Al Ghareeb Wadi 8 387 19500 32° 37' 43.0" N; 21° 25' 45.4" E Oasr Libva Mountain slope 32° 33' 18.2" N; 21° 03' 24.6" E 9 407 21000 ElMarj-El Beida Road Mountain slope 32° 45′ 17.2″ N; 21° 51′ 46.7″ E 10 628 16500 Wadi Moussa Wadi

**Table 1.** Study sites: location and habitat types in the study of ten populations of *Juniperus phoenicea* in the Al-Akhdar mountain region.

Note: All mountain slopes were North-facing in aspect.

\*a.s.l. = above sea level.

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recruitment, and each site was carefully searched for evidence of seedlings. Vegetative traits measured included tree volume (m<sup>3</sup>), tree height (m), and number of branches per individual tree. Reproductive traits were assessed in terms of the number of mature female cones per branch, the total number of female cones per individual and the percentage of all plants in the sample area of each population that were reproductive (bearing female cones).

To calculate tree volume the following equation representing the volume of an ellipsoid was used:  $\pi/6(R_1R_2R_3)$ , where  $R_1$  = the height, and R2 and R3 = the major and minor diameters of the crown (www.onlineconversion.com/object volume ellipsoid.htm). Trees were categorized into size classes according to volume: In the first 190 instance they were divided into six size classes, corresponding to:  $A1 = < 1 \text{ m}^3$ , A2 = $1-5 \text{ m}^3$ ,  $B1 = 5-10 \text{ m}^3$ ,  $B2 = 10-15 \text{ m}^3$ ,  $C1 = 15-25 \text{ m}^3$ ,  $C2 = >25 \text{ m}^3$ . It is noteworthy that no seedlings or juveniles  $(<0.5 \text{ m}^3)$  of *J. phoenicea* were found anywhere in this study. To facilitate comparisons between sites these categories were combined to generate three size classes, corresponding to:  $A = < 5 \text{ m}^3$ ,  $B = 5-15 \text{ m}^3$ ,  $C = > 15 \text{ m}^3$ . 195 The percentage contribution of each size class was calculated, and the tree density and mean trait values were determined for each population. Tree density was determined by tallying the number of trees in each (standardized) study area. The coefficient of variation (CV, or ratio of the standard deviation to the mean) was estimated. Relationships between traits and elevation, and CV and elevation were plotted. A 200 multivariate regression was carried out (using a generalized linear model that assumed a normal distribution and identity link function for % data, and a Poisson model for tallies of branch number, cone number, and so forth. This was intended to test the effects of elevation, distance from the sea, and their interactions on vegetative and reproductive traits. For this analysis sites were clustered into three groups according 205 to elevation (<200, 201-400, and >400 m above sea level) and three groups according to distance from the sea (<5 km, 5–20 km, and >20 km from the coast).

# Plant health

To assess the health of J. phoenicia trees at different elevations, individuals within each of the ten study sites (one  $10 \times 10$  m<sup>2</sup> guadrat per site) were classified as either healthy, 210 or showing various levels of dieback. Dieback was characterized in terms of leaves that had changed from green to brown. The degree of dieback in each individual tree was assessed by visual inspection according to five categories: (1) healthy; (2) 10% dieback; (3) 25% dieback; (4) 50% dieback; and (5) 100% dieback, or complete death of the tree. We used these categories because they were relatively easy to discriminate 215 and were reliably assessed by different observers. Potential threats to the natural woodland, and future needs were also discussed with local residents (A. K. Hegazy, unpublished).

# **Community composition**

Within J. phoenicea populations, the presence/absence of associated perennial plants was 220 recorded. The diversity of associated species was grouped in four altitudinal ranges: <100 m, 100–300 m, 300–500 m, and >500 m above sea level.

# **Results**

# **Climate and soil parameters**

Climate parameters for the three weather stations are presented in Table 2 (LNMC 2012). 225 Average annual temperature at the three weather stations over the period 2006-09 was not very variable within a site from year-to-year (there was a low coefficient of variation), but the three sites all differed significantly, with Derna, on the coastal plain, being the warmest at an average of 20.8°C, followed by Al Marj at 18.1°C and Shahat at 16.7°C. Average temperatures therefore decreased with altitude. Records of total annual rainfall at each site 230 from 2006-09 shows high variability from year-to year at a site (high coefficient of variation), and years of higher or lower rainfall did not coincide at different weather stations. Total precipitation appeared to increase with altitude, but only the value for Shahat (the highest site at 490.5 mm) differed significantly from the other two sites. The short-term pattern over these four years seems to involve low predictability of rainfall, rather than a 235

Table 2. Temperature, rainfall, and soil parameters for weather stations at Derna, Al Mari, and Shahat, located along an altitudinal gradient within the Al-Akhdar region where populations of Juniperus phoenicea occur.

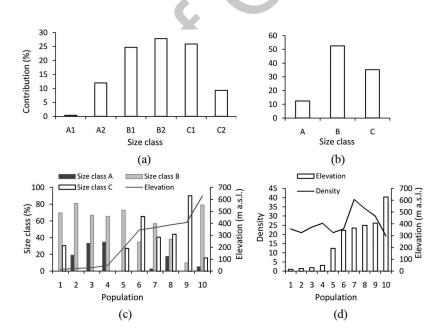
Station	Derna	Al Marj	Shahat
Altitude (m above sea level)	30	325	355
Habitat	Coastal plain	Mountain slope	Mountain slope
Distance from the Mediterranean coast (km)	5	40	10
Average temperature (°C/month)			
2006	20.5	18.6	15.9
2007	20.5	18.6	16.7
2008	21.3	19.2	17.2
2009	21.0	-	17.0
Mean	20.8 <sup>ª</sup>	18.1 <sup>b</sup>	16.7 <sup>c</sup>
Coefficient of Variation	0.02	0.02	0.03
Standard Error	0.09	0.12	0.14
Total annual rainfall (mm/Year)			
2006	276.8	360.9	416.6
2007	262.0	429.9	527.6
2008	326.7	229.9	466.3
2009	201.8	419.1	551.4
Mean	266.8ª	359.9ª	490.5 <sup>b</sup>
Coefficient of Variation	0.19	0.25	0.12
Standard Error	12.9	22.9	15.2
Particle size (%)			
Coarse sand	12	10	16
Fine sand	61	16	6
Silt	15	25	36
Clay	12	49	42
Textural class	Sandy loam	Loamy	Loamy clay
Organic matter %	0.2	6.2	6.9
lon concentration (ppm)			
Fe+++	3.76	16.40	36.30
HC03-	237.9	189.1	213.5
CI–	4553.7	53.3	71.0
SO4——	806.4	182.4	182.4
Ca++	238.0	74.0	64.0
Mg++	74.4	42.7	47.6
Na+	2730.1	20.7	29.9
K+	117.3	11.7	23.5

Note: See Figure 1 for site locations. Statistically significant differences between means for annual averages for temperature and rainfall are indicated with different superscripts. These three weather stations do not directly correspond to particular study sites. The data simply indicate the variability of environmental parameters in the region.

simple trend of progressively increasing drought (Table 2). Soil texture was a sandy loam in the coastal plain, transitioning to loam, and loamy clay at higher altitudes. Organic matter content and iron (Fe<sup>3+</sup>) increased with altitude (Table 2). Salt content, in ppm (HCO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>,  $SO_4^{2-}$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Na^+$ , and  $K^+$ ) was greatest in the coastal plain, decreasing in soils further inland with Shahat, at about 10 km from the sea, usually being more saline than Al Marj located at 40 km from the sea coast.

# Regional and population-level size structure

In the region as a whole, the dominant size class was group "B", made up of mid-sized individuals (>50% contribution, see Figure 3a and b). The smallest size class, A, was found in only six of the ten study sites and made up only 12.35% of plants in the region as a whole (Figure 3a–c). Small plants (Size class A) were abundant (33–34%) in only two sites: Site 3 on the Susa-Derna Coastal Road on the coastal plain (at 29 m altitude), and Site 4 in Ras El-Helal on the lower mountain slope (at 48 m, Figure 3c). Both sites are near the coast. At these sites tree densities are also higher than they are at the other coastal sites, at twenty-four and twenty-six individuals per 100 m<sup>2</sup> respectively (Figure 3d). In contrast, populations in the wadis (Sites 1, 7, and 10) had few plants in the small size class (0–5.26%) and small plants were absent altogether from Sites 5, 6, and 9 on the mountain slope (Figure 3c). The largest



**Figure 3.** Size class structure of *Juniperus phoenicea* L. assessed in terms of estimated tree volume in m<sup>3</sup>; (a) pooled for all populations studied in the Al-Akhdar mountainous region, showing six size classes; (b) pooled for all populations studied in the Al-Akhdar mountainous region, summarized in terms of three size classes; (c) shows the frequency of each of the three size classes in each of the ten study sites, labeled 1–10; and (d) shows the relationship between tree density and elevation, noting that the sites are numbered 1–10 in order of their increasing elevation. Where there are 6 size classes (in Figure 3a) these correspond to:  $A1 = < 1 \text{ m}^3$ ,  $A2 = 1-5 \text{ m}^3$ ,  $B1 = 5-10 \text{ m}^3$ ,  $B2 = 10-15 \text{ m}^3$ ,  $C1 = 15-25 \text{ m}^3$ ,  $C2 => 25 \text{ m}^3$ . Where there are three size classes (in Figure 3b, c) these correspond to:  $A = < 5 \text{ m}^3$ ,  $B = 5-15 \text{ m}^3$ ,  $C = > 15 \text{ m}^3$ . See Table 1 for site locations.

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size class (>15 m<sup>3</sup>, size class "C") made up about 35% of J. phoenicea in the region overall (Figure 3a and b). These large plants made up 40–90% of Populations 6, 7, 8, and 9, located at 345-407 m. At these sites plant density was also higher ranging from 23-39 individuals per 100 m<sup>2</sup>(Figure 3d). Size class C is absent altogether from Populations 2, 3, and 4 which are in the coastal zone at <48 m, but these large plants are present at Population 1, a wadi site at 15 m (Wadi Karssa) where they make up about 30.43% of the population (Figure 3c and d).

#### Phenotypic variation

Altitude and distance from the sea were themselves correlated with an R<sup>2</sup> of 0.66, significant 2.60 at (p < 0.05). Tree volume was significantly influenced by elevation, distance from the sea and the interaction between these two factors, at p < 0.001 (Table 3). The general trend was that tree volume was greatest at mid altitudes. There was no significant relationship between elevation and tree height, but a significant effect of distance from the sea (p < 0.01, Table 3). Site 9 was an outlier with trees of an average height (6.48 m  $\pm$  0.05 s.e.) that was almost twice that of 265 trees elsewhere (Figure 4a). The number of branches per individual increased significantly with elevation and distance from the sea, and the interaction between these factors was also significant all at p < 0.001 (Table 3). Greatest numbers of branches per individual were seen in site 10, where the average was  $31.37 (\pm 0.35 \text{ s.e.})$  branches per individual (Figure 4b).

	Chi-Square	df	<i>p</i> -value	Significance
(a) Volume				
Elevation	50.143	2	< 0.000	***
Distance	35.406	2	< 0.000	***
Elevation*Distance	47.721	1	< 0.000	***
(b) Height				
Elevation	1.269	2	0.530	N.S.
Distance	8.697	2	0.013	**
Elevation*Distance	12.343	1	< 0.000	***
(c) Number of branches per t	ree			
Elevation	117.587	2	< 0.000	***
Distance	109.739	2	< 0.000	***
Elevation*Distance	19.845	1	< 0.000	***
(d) Number of female cones	per branch			
Elevation	345.460	2	< 0.000	***
Distance	268.919	2	< 0.000	***
Elevation*Distance	210.439	1	< 0.000	***
(e) Number of female cones	per tree			
Elevation	12922.840	2	< 0.000	***
Distance	6121.815	2	< 0.000	***
Elevation*Distance	7062.227	1	< 0.000	***
(f) Fruiting plants (%)				
Elevation	6.473	2	0.039	*
Distance	9.128	2	0.010	*
Elevation*Distance	1.235	1	0.266	N.S.
(g) Dieback (%)				
Elevation	4.400	2	0.111	N.S.
Distance	10.558	2	0.005	**
Elevation*Distance	7.120	1	0.008	**

Table 3. Generalized linear models testing the effect of elevation and distance from the sea on the vegetative and reproductive traits of Juniperus phoenicea, and the percent of dieback.

Note: Models based on a normal distribution were used in the analysis of the percent of plants that were fruiting, and percent dieback, whereas a Poisson distribution with log function was used for other variables.

The statistical significance of relationships is indicated as follows: N.S. = not statistically significant; \*p < 0.05; \*\*p < 0.01; \*\*\**p* < 0.001.

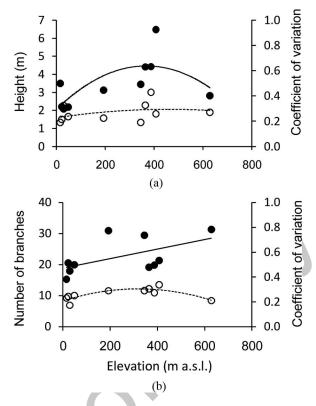


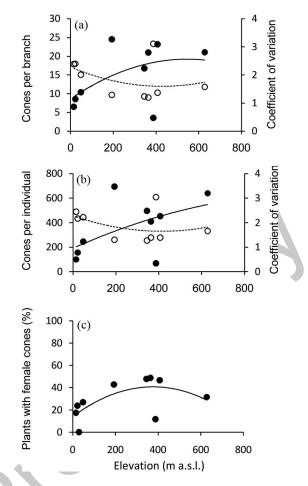
Figure 4. Vegetative traits (closed circles) and the corresponding coefficients of variation (open circles) in Juniperus phoenicea L. populations at different elevations within the Al-Akhdar mountainous region. (a) Mean height (m) plotted against elevation; and (b) Number of branches per individual plotted against elevation.

With regard to reproductive traits, the number of female cones per branch and total num-270 ber of female cones per individual were all significantly correlated with elevation, distance from the sea and the interaction between these factors, at p < 0.001 (Table 3, Figure 5). The overall result for % of trees that were fruiting showed a weak, but significant relationship with elevation and distance from the sea at p < 0.05, with no significant interaction effect. The percentage of fruiting seemed to be highest at mid-altitudes (Figure 5c). An outlier 275 was Population 8, at 387 m, where all three attributes were particularly low, with an average of 3.5 cones per branch, 67.64 cones per tree and 11.8% of trees producing cones (Figure 5). At the lowest elevation, in Population 1 (Wadi Karssa) at 15 m, trees produced an average of 6.5 cones per branch, 99.6 cones per tree, and 17.4% of trees bore cones. At the highest elevation, in Population 10 (Wadi Moussa), we found an average of  $21.05 (\pm 1.74 \text{ s.e.})$  cones per branch, 640 ( $\pm$ 56.01 s.e.) cones per tree, and 31.6% of the trees bore cones (Figure 5). The coefficient of variation for reproductive traits decreased somewhat with elevation (Figure 5).

# Evidence of foliage dieback

All size classes of junipers showed some degree of dieback (Figure 6a and b). Overall, dieback (in terms of the percent of plants that showed 50-100% dieback) was greatest in the small (A1 and A2) and medium (B1 and B2) size classes (Figure 6a). All trees in size

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**Figure 5.** Reproductive traits (closed circles) and the corresponding coefficients of variation (open circles) in *Juniperus phoenicea* L. populations at different elevations within the Al-Akhdar mountainous region. (a) Number of female cones per branch; (b) Number of female cones per individual; and (c) Percentage of plants bearing female cones.

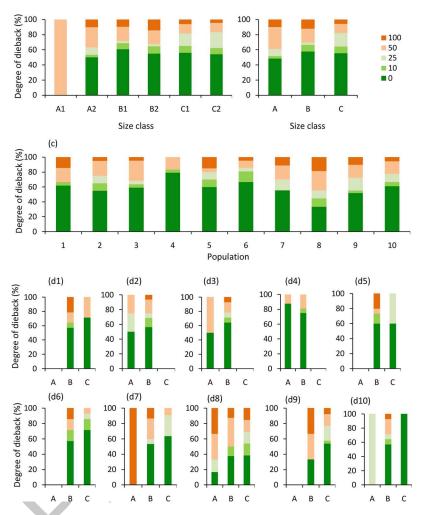
class A1 showed 50% dieback; this went down to 26.7% in size class A2, but complete dieback had occurred in 10% of the trees. Complete dieback increased to 12% in size class B then decreased to 5% in size class C (Figure 6b). In size class C, most of the damaged trees showed 10-25% dieback.

There was no statistically significant relationship between elevation (which is in turn associated with rainfall and inversely associated with temperature) and percent dieback. However, distance from the sea and the interaction between elevation and distance from the sea were significant at p < 0.01 (Table 3). The greatest proportion of healthy plants (79.17%) was recorded in Population 4, at 48 m, while the lowest proportion of healthy plants (33.33%) was found in Population 8, at 387 m. Figure 6c shows elevated dieback in Populations 7, 8, and 9; sites which are more than 15 km inland, at elevations ranging from 364–407 m. There, the smallest trees were most affected (size class A) with none surviving in Population 7 (Figure 6d, 7–9). On the other hand, the greatest percentages of intact individuals in size class A were found in Population 4 (87.5%), which is located near the sea coast at 48 m elevation

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**Figure 6.** Patterns of foliage dieback in *Juniperus phoenicea* plants: (a) pooled for all populations studied in the Al-Akhdar mountainous region, divided into six size classes; (b) pooled for all populations studied in the Al-Akhdar mountainous region, divided into three size classes; (c) shows patterns of dieback for each population, numbered according to ranking along the elevation gradient; and (d) shows, separately for each population, the pattern of dieback partitioned into three size classes. Where there are six size classes (in Figure 5a) these correspond to:  $A1 = < 1 \text{ m}^3$ ,  $A2 = 1-5 \text{ m}^3$ ,  $B1 = 5-10 \text{ m}^3$ ,  $B2 = 10-15 \text{ m}^3$ ,  $C1 = 15-25 \text{ m}^3$ ,  $C2 = > 25 \text{ m}^3$ . Where there are three size classes (in Figure 5b–d) these correspond to:  $A = < 5 \text{ m}^3$ ,  $B = 5-15 \text{ m}^3$ ,  $C = > 15 \text{ m}^3$ . The shading in each histogram corresponds to recognition of five distinct levels of intensity of dieback; dark green corresponds to no evidence of dieback; mid green to 0-10% dieback; light orange to 25-50% dieback; and dark orange to 100% (complete dieback). The boundaries for these categories were chosen based upon consistent and reliable (concord-ant) assessment by the investigators. See Table 1 for site locations.

(Figure 4d, d4). As part of the field study local residents were interviewed; they attributed dieback in *J. phoenicea* to a series of severe drought years (A. K. Hegazy, unpublished). An additional threat is the clearing of trees, particularly in wadis, in order to plant barley as feed for livestock. There is also considerable urban encroachment, especially in villages adjoining juniper woodlands. Root harvesting for fuel and wood-cutting to make fence posts offer further challenges for the juniper populations in this region (A. K. Hegazy, unpublished).

# **Community composition**

*Juniperus phoenicea* occupies a range of habitats within the Al Akhdar mountainous region, from wadis to open areas on the north-facing mountain slope, and at different elevations. It is therefore not surprising that different communities of perennial species were found growing with J. phoenicea at different elevations (Table 4). Three species were always associated with J. phoenicea, namely Phillyrea angustifolia L., Pistacia lentiscus L., and Rhamnus lycioides L. Four additional species were always found in association in the three lower altitude zones (i.e., Zones 1, 2, and 3): Ceratonia siliqua L., Cistussa lyifolius L., Phlomis floccosa

	Elevation zone				
Species	1	2	3	4	
Trees					
Ceratonia siliqua L.	+	+	+		
Olea europa L.		+	+		
Pinus halepensis Mill.	+		+		
Shrubs					
Arbutus pavarii Pump	+		+	+	
Asparagus aphyllus L.			+		
Calicotome spinosa (L.) Link			+		
Capparis spinosa subsp orientalis			+		
Cistus parviflorus Lam.			+	+	
Cistus salvifolius L.	+	+	+		
Cupressus sempervirens L. var. horizontalis		I.	+	+	
Genista acanthoclada DC			+		
Nerium oleander L.			+		
			+		
Nicotiana glauca R.C. Graham			+		
Ononis hispida Desf	+				
Periploca angustifolia Labill.	+				
Phillyrea angustifolia L.	+	+	+	+	
Phillyrea latifolia L.	+		+		
Pistacia lentiscus L.	+	+	+	+	
Rhamnus lycioides L.	+	+	+	+	
Rhamnus oleoides L.	+	+			
Rhus tripartita (Ueria) Grande	+		+		
Rosmarinus officinalis L.	+	+	+		
Rubus sanctus Shreber			+	+	
Scarcopoterium spinosum (L.) Spach.		+	+		
Sub shrubs					
Erica sicula Guss.			+		
Fagonia cretica L.		+			
Phlomis floccosa D.Don.	+	+	+		
Satyreja thymbra L.		+	+	+	
Thymus capitatus (L.) Hoffm & Link		'	+		
Thymus algeriensis Boiss. Et Reut			+		
Herbs					
Asphodelus fistulosus L.	+		+		
Ballota pseudo-dictamnus (L.) Benth	Т		+		
Cichorium spinosum L.			+		
Echium angustifolium Mill.			+		
		1	+		
Eryngium campestre L.		+			
Gymnocarpus decandrus Forssk.		+			
Hypericum triquetrifoliumTurra.	45	+	+	-	
Total number of species	15	15	31	8	
Percentage of the total number of species	21.7	21.7	44.9	11.6	

**Table 4.** Lists of perennial species associated with *Juniperus phoenicea* populations at different elevation zones within the Al-Akhdarregion.

*Note*: 1 = < 100 m (corresponding to Sites: 1, 2, 3, and 4); 2 = 100-300 m (corresponding to Site: 5); 3 = 300-500 m (corresponding to Sites: 6, 7, 8, and 9); 4 = > 500 m (corresponding to Site: 10) in terms of meters above sea level. Identification of species was made according to Jafri and El-Gadi (1977–1993).

D. Don, and Rosmarinus officinalis L. Species such as Cistus parviflorus Lam, Cupressus 315 sempervirens L. var. horizontalis, and Rubus sanctus Shreber. appear at higher elevations (>300 m, Table 4). The greatest diversity of associated perennial species (31) occurred in the 300-500 m elevation zone (Zone 3), where about 45% of all associated species occurred; of the associated species encountered in this study, ten occurred only in this zone. In contrast Zone 4 (>500 m) was the least diverse, containing only eight associated perennials. 320

## Discussion

#### **Population structure**

No seedlings of J. phoenicea were found anywhere in this study; indeed individuals in the smallest size class (A1, volume  $<1 \text{ m}^3$ ) were very rare overall in the region (Figure 3a). Size class A ( $<5 \text{ m}^3$ ) was entirely absent from Sites 1, 5, 6, and 9, and very low in abundance in Sites 7 and 10; this despite the fact that Sites 1, 7, and 10 are wadis, where water supplies might be expected to be higher than on exposed slopes (Figure 3c). The absence or low abundance of juveniles is not attributable to low production of female cones at these sites (Figure 5). The low abundance of plants  $<5 \text{ m}^3$  in volume, including seedlings, suggests environmental conditions in the region have been unsuitable for seedling establishment 330 in recent years or that young plants are more susceptible to grazing. The problem of seedling recruitment requires further investigation, including assessment of seed set and seed viability, status of the seed bank, water requirements for germination, competition for light, and the availability of "safe sites" for germination as well as assessment of the relative palatability of different life history stages (Hegazy et al. 2014). However, it looks like a situation 335 common to many long-lived Middle Eastern trees; in some populations (especially Sites 9 and 6) the Phoenician juniper seems to have over-matured populations and there is some indication of loss of the regeneration niche (Hegazy and Lovett-Doust 2015 in prep<sub>1</sub>).

None of the populations in this study had a stable size structure; all of the sites showed significant under-representation of the smallest size class (Figure 3c), and in some cases 340 individuals in the largest (presumably oldest) size class made the greatest contribution to the population (Sites 6, 8, and 9). These Sites, located in elevation Zone 3 (see Table 4) also contained higher densities of J. phoenicea (Figure 3d), as well as having the greatest diversity of associated perennials (Table 4), so it is possible that seedling recruitment is suppressed at these sites through both intra- and inter-specific competition. Similar 345 patterns of low recruitment have been reported in other juniper species. For example, Juniperus communis L. was studied in the Mediterranean mountains of Spain; there low recruitment was attributed to climatic stress imposed by summer drought (García-Mora, Gallego-Fernández, and García-Novo 1999; Gauquelin et al. 1999). In contrast interspecific competition was cited as the explanation for the decline of Juniperus phoenicia in Spain, where Pistacia lentiscus became increasingly dominant in mixed stands, over time (Armas and Pugnaire 2009).

#### Phenotypic variation

Examination of the profile of each population in the study indicates that climatic, edaphic, and biotic factors influence both population and individual attributes.

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In the present study, some sites have small and medium-sized plants, but have no plants in the largest size class (Sites 2, 3, and 4, located in the coastal region). These sites may have been more recently established. Supporting evidence would be the overall lower density of *J. phoenicea* recorded at these sites and the lower number of cone-bearing individuals, cones per plant and cones per branch. Obviously, as smaller plants, these individuals are also shorter and have fewer branches per tree (Figure 4).

In terms of the number of branches and total number of fruits per individual, greater values were observed at middle and high elevations; this is not surprising, since they were larger individuals. Indeed these findings are a direct reflection of the relative abundance of the three size classes at these sites (see the aforementioned). Variability in vegetative traits was high, both within and among populations (CV between 0.17–0.54) with greatest variation at the mid-elevations. Variability in reproductive traits was even greater (CV within populations ranged from 1.19–3.11), and tended to decrease with increasing elevation. Variations among populations were the highest for the reproductive traits.

Variation within populations will be greater than variation among populations when local habitats are heterogeneous, or where populations extend along an environmental gradient (e.g. Albert et al. 2010; Moreira, Tavsanoglu, and Pausas 2012; Dantas 2013). Habitat heterogeneity in the Al Akhdar is a function of the regional landscape where the surface geology is complex (see, e.g. Elfigih and Melad 2013) sites are at different elevations, and at different distances from the sea, and plants within a site (as well as at different sites) may be growing with very different water supplies, at different slope positions, aspect, soil types, and so forth. The Al Akhdar region is not a simple, single mountain range with a foot, slope, and top. Rather, a series of mountain ridges at mid-level elevations form a complex mountainous landscape, which turn may generate a more complex relationship between plant traits and altitude, pair As a result the initial hypothesis that altitude alone would be the major determinant of plant growth and reproduction was rejected.

Within and among-site variability is high in the present study with regard to reproductive traits (CV ranged from 1.2 to 3.1) as compared to vegetative traits (CV ranged from 0.2 to 0.4). This is a reflection of the contrasting size-structures (and presumably age-structures) within different populations, combined with contrasting conditions at each site. Trees in the "coastal" populations (Sites 2, 3, and 4) are distinctive in being short, but with a comparable number of branches per tree to trees in Sites 7, 8, and 9. This suggests that the species differs in growth form in these two areas, possibly as a result of the more unstable substrate of the coastal dunes, where there may be a more prostrate growth form, with occasional sand burial and greater stimulation of lateral branching. Based on the assessments of tree volume, few plants in these coastal sites are big enough to reproduce. The coastal populations appear to be more recently established, while other populations consist mostly of larger, mature trees that are failing entirely to recruit juveniles (1, 5, 6, and 9), or doing very poorly in terms of juvenile recruitment (7, 8, and 10).

Phenotypic (functional) traits reflect interactions between plants and their environment (Grassein, Till-Bottraud, and Lavorel 2010). Albert et al. (2010) and Dantas et al. (2013) both showed the behavior of individuals and populations in plant species differ depending on local conditions, and that mean population trait values in each environmental condition or distribution of trait values along a gradient, should be used instead of any single "representative" mean trait value. This seems appropriate in the present case due to the complex microhabitat heterogeneity where adjacent sites may differ in elevation and water

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availability, creating significant variability within and among populations. Also, the rainfall and temperature gradients, from coastline to mountains, run in opposite directions, yet both are important determinants of plant growth; optimal conditions are therefore likely to occur at mid-altitudes. In the present study, elevation and proximity to the sea coast had signifi-405 cant effects on most of the study traits, specifically tree volume, the number of branches per tree, the number of female cones per branch, and the number of female cones per tree. On the other hand, tree height was not significantly affected by elevation. The percent of trees bearing cones in a population was only slightly (p < 0.05) affected by elevation and distance from the sea, and was not affected by the interaction between these factors. It was, instead a 410 function of the size distribution of trees in the population. Two sites were conspicuous outliers in terms of having zero, or a low percent of trees bearing cones. At Site 3 none of the trees bore female cones, and at Site 8 only about 10% bore female cones. Site 8 was distinctive in that it had the highest level of dieback stress, with only 33% of plants being healthy (showing no evidence of dieback). The population also contained a higher proportion of 415 small and midsized trees, many of which may have been pre-reproductive. At Site 3, there were no large plants (>15 m<sup>3</sup>), and plants were short in height, but relatively well-branched. It is possible that this younger population, experiencing relatively high temperatures combined with low rainfall (using the nearby Derna Climate Station as an indicator) were sub-optimal for tree growth and reproduction.

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## Ecological status and conservation

In terms of conservation status, J. phoenicia is regarded as being at lower risk of extinction ("Least Concern" category, IUCN, 2013). Moreover as a drought-tolerant species (Martínez-Ferri et al. 2000), J. phoenicea is found in nearly all habitat types within the mountainous landscape, as indicated by the wide range of plant communities in which it is present (see Table 4) and is typically viewed as having high potential for seedling establishment (Pausas 1999). At the same time the shallow root system, with alow root/shoot ratio and high susceptibility to disturbance (Pausas 1999), makes the species more sensitive to climate warming than many associated perennials.

The dieback phenomenon described in the present study does not appear to be associated 430 with fungal infection by Diplodia africana Damm & Crous, as was the case for J. phoenicea L. on Caprera Island, in Italy (Linaldeddu et al. 2012). Dieback in Libya more likely reflects a combination of the impacts of limited water supply and/or overharvesting. This latter inference is based on conversations with local inhabitants who disclosed that they dig up large volumes of juniper roots for use mostly as fuel (A. K. Hegazy, unpublished). Following a 435 series of dry years, with variable precipitation, small-sized trees, with their (presumably) shallower and less extensive root systems, would be more susceptible to dieback than larger trees (Figure 6b).

Dieback was not associated with elevation but rather by distance from the sea and the interaction between distance from the sea and elevation (p < 0.01, Table 3). As previously 440 mentioned, the site with the lowest proportion of healthy trees was Site 8, Qasr Libya, located on a north-facing slope at a mid-level altitude, comparable to Al Marj where rainfall is approximately 360 mm/year. Site 8 also had the second-highest density of J. phoenicea. This altitude corresponds to vegetation Zone 3, where the diversity of associated perennials is greatest. It is possible that the severe dieback effect at this site is due to a combination of 445

intra- and inter-specific competition, intensified by a series of drought years (e.g., see Ohno et al. 2010; Hosseini et al. 2013). In future studies it would be very useful to carry out controlled experiments where junipers are subjected to controlled levels of drought and inter- and intra-specific competition in order to better understand the proximate causes of dieback.

Phoenician junipers occupy a variety of habitats, at different elevations. This is reflected in the distinct, diverse groups of associated perennial species, especially in Zone 3, the 300– 500 m altitude range corresponding to Populations 6, 7, 8, and 9, which contained the greatest diversity of associated perennial species (Table 4). It is apparent that Populations 7, 8, and 9 also suffered most dieback, especially of the youngest size class. At site 7, all of the trees that were  $<5 \text{ m}^3$  in volume were suffering 100% dieback; in site 8, only 10% of the smallest size class were healthy; and in the case of Site 9, the smallest size class was absent altogether. These are the sites with the highest densities of *J. phoenicia*, as well as being locations where there is the greatest diversity of perennials. Thus intra- or interspecific competition may increase the level of dieback in conjunction with potential effects of more variable rainfall. Interactions among drought, competition, growth, reproduction, and dieback clearly need more investigation.

In a comparative study of several juniper species in the Arabian Peninsula, extensive dieback was reported at altitudes below 2000 m, with less dieback occurring at altitudes above 2400–2500 m. Species in these woodlands included *J. phoenicea* L., *J. procera* Hochst. ex Endl., and *J. excelsa* M. Bieb. subsp. *Polycarpos* (K. Koch) Takhtajan (Fisher 2000). Similarly, Sigl, Strunk, and Barth (2006) reported that juniper woodlands throughout the Peninsula are all showing extensive dieback and only few signs of regeneration at lower altitudes. Sigl, Strunk, and Barth (2006) suggested global climate change, microclimate changes due to overgrazing, and periodic droughts in the region are causing the extensive dieback phenomenon. Presumably overgrazing could help in reducing the number of "safe sites" for juniper regeneration.

In contrast, in the present study no simple trend was evident for dieback in relation to either elevation or habitat type (wadis, slopes, or coastal plain). However, there was a significant effect of distance from the coast, and the interaction between distance from the coast and altitude (p < 0.01). This was the opposite pattern to that reported by Fisher (2000) in Arabia, that is, in the present study dieback was greater further inland, where, typically, altitudes were greater. Our results may be explained by the finding that the sites on the coastal plain, that are also, by definition, at low altitudes (Sites 2, 3, and 4) contain the highest percentage of individuals in size class "A" (small trees), with 50% or more of these being healthy. Coastal sites may be more subject to intermediate levels of substrate disturbance, resulting in more recently established populations that lack large individuals. In contrast the wadis and higher elevation sites in our study had few, if any, plants in the smallest size class, and were dominated by mid-sized and large trees. The high elevation sites with low dieback in the study by Sigl, Strunk, and Barth (2006) on the Arabian Peninsula were at a much higher elevations(>2400 m) than the "high elevation" sites (>300 m) studied here, in Libya. It is possible that the high elevation sites in Arabia are in "cloud forest" regions where humidity is consistently high, so less drought-induced dieback occurs there.

Climate change, resulting in severe drought or cold has also been identified as a likely cause of widespread regional-scale forest mortality worldwide (Matzner, Byer, and Brown, 2004; Knapp and Soule 2005; Fensham and Fairfax 2007; McDowell et al. 2008; Koepke,

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Kolb, and Adams 2010). Dieback in juniper woodlands has also been attributed to the long term change in conditions associated with global warming that result in periodic droughts and changing microclimates (Fisher and Gardner 1995; Gardner and Fisher 1996; Fisher 2000; El-Bana et al. 2010).

In the present study each population is experiencing conditions including rainfall and temperature that are correlated with altitude and distance from the sea, which themselves are significantly correlated in this coastal region. When additional stressors are experienced (e.g., intra- and interspecific competition, soil conditions, grazing, and human impacts), plant dieback may be induced. It might be proposed that a series of years of reduced rainfall may be partially offset by additional surface water supplies (e.g., those that might be expected in wadis). However, the three wadi sites (1, 7, and 10) did not appear to have heal-thier plants. Indeed, at Site 1, there were no small-sized trees; at Site 7, all the small trees had suffered 100% dieback; and at Site 10, all the small trees were showing 25% dieback. Wadis are an important resource from the perspective of use by humans and animals and it is possible that, despite the greater supply of surface water that would be expected there, competing activities (including complete clearing of trees for agriculture, settlement, or wood, and root cutting for fuel as well as water diversion), all act against the juniper population, and that they have a particularly severe effect on the smallest size class (as shown in Figures 6a, b, and d).

## Conclusions

The results of this study do not support the model of a significant positive relationship between population vigor and altitude, seen in studies on the Arabian Peninsula (Fisher 2000; Sigl, <u>Strunk, and Barth</u> 2006). However, elevation and/or distance from the sea and/ or the interaction between them significantly affect individual and population traits. All of the sites in NE Libya show poor to nonexistent juvenile recruitment and unstable population size structures. *Juniperus phoenicea* is a relatively long-lived species so population decline is not immediately apparent, but low recruitment and disproportionately high juvenile dieback and mortality indicate a serious problem is developing, in terms of populations being no longer self-sustaining. The lack of seedlings and the low number of plants in small size classes is not attributable to failure to reproduce, as female cones are being produced at all sites (except for Site 3). Rather, in the inland sites in particular, seedlings are not establishing. This may be due either to differential herbivory, or poor growth and survivorship.

It is clear that trees in the largest size class are showing less severe dieback (Figure 6). This may be attributable to their larger (deeper) root systems and ability to be more resistant to drought. However, if drought conditions persist, these aging trees will become progressively more severely impacted.

Conservation efforts should include control of urbanization and conversion of natural habitats to agriculture, along with promotion of sustainable use of the juniper trees. Such initiatives may require government legislation (as exists in, for example, Cyprus) as well as raised awareness in local communities regarding the ecological value of the juniper wood-land. It is important to manage the system to ensure recruitment of juveniles and the establishment of a stable size- and age structure in these natural woodlands, in the first instance through further investigation of the proximate causes of failed regeneration, and in terms of actions, through active plantings, and conservation and protection of surface water supplies.

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