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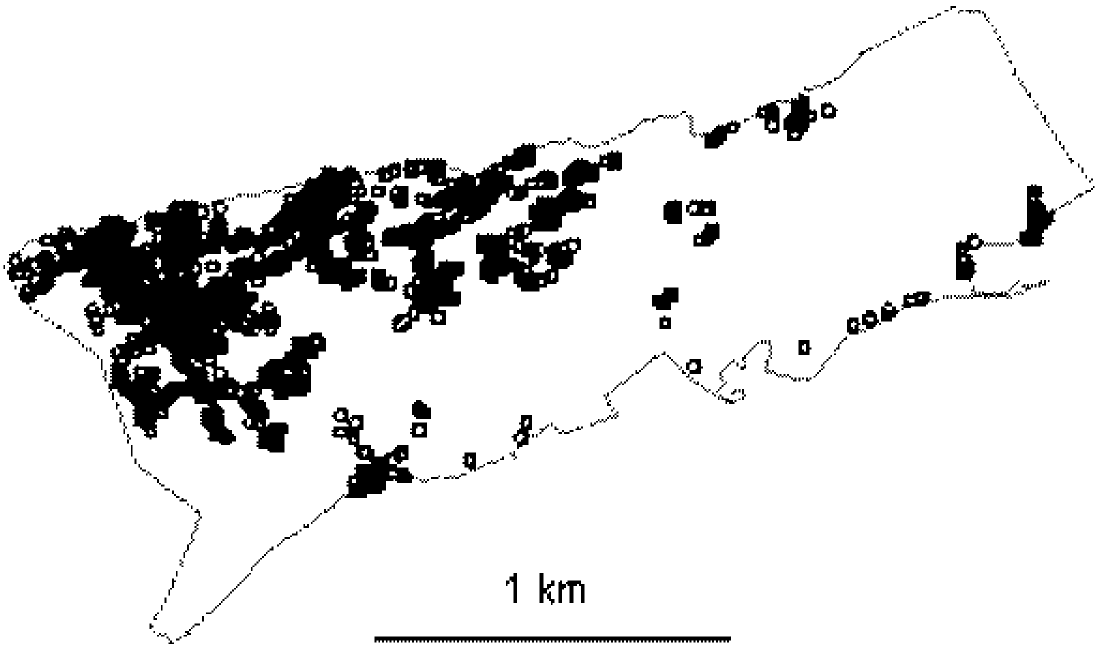

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STABILITY AND FLUCTUATIONS IN A THREE SPECIES SYSTEM: A PLANT WITH TWO VERY DIFFERENT POLLINATORS

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Abstract. We present a multi-species model of one plant with two nectivore pollinators. This system has mutualism between the plant and the pollinators, and competition for food between pollinators. Both pollinators are different: the large pollinator can consume all the nectar offered by the plant in each visit; it permits the genetic interchange between plants from different patches, so, it avoids self-fertilization problems. The small pollinator consumes only a fraction of the flower nectar but the seeds produced by its pollination are less successful. The model gives different dynamics depending on both the differences between the pollinators' growth rates and the inbreeding effect. Stronger oscillations occur when the growth rates are very different. Paradoxically, a high inbreeding causes an increment in populations. The three species system is biologically stable and the asymmetry between pollinator species results in different roles that enhance the global stability and persistence of the system.

Keywords: *Pollination; mutualism; population dynamics; competition; mathematical models*

Introduction

Ecological relationships usually involve several interacting species in a complex network. Notwithstanding this, most studies have focused on two species interactions. Predation, competition, parasitism, and mutualism are examples of systems extensively studied and modeled by ecologists as two species interactions. However, none of these relationships is in actual fact restricted to two species; competition for instance is clearly a three-element system because it involves at least two competitors and one resource that typically is another species, an ignored fact in the traditional Lotka-Volterra approximation [27].

Mutualism, in turn, is considered as a relationship in which both participants experience some net benefit. Mutualistic interactions occur along the entire range of biological organization and on all timescales [4, 17]. These two relations, mutualism and competition, are apparently opposed to each other, although they may be seen as extremes of an interaction gradient with different benefit:cost ratios [4]. In fact, many interactions are a mixture of different aspects that involve competitive, antagonistic and mutualistic interactions [38]. From the benefit:cost perspective, it becomes clear that the outcomes of mutualism and competition must be extremely variable, and not simply plus/plus or plus/minus interactions; in fact, outcomes of inter-specific relationships vary with the abiotic and biotic setting in which the interactions take place, and there are several factors that influence mutualism [16].

A common example of mutualism is pollination [3]. The plant-pollinator relationship happens between free-living species and, as in several mutualistic associations, one partner performs some action (a service) that benefits its associate receiving some payoff for doing so. These rewards may be some kind of food, for instance nectar [10, 21], pollen [11], or even seeds or ovules that the pollinator larvae eat [1, 9, 15]. Providing rewards has a cost for the plant. For example, up to 37% of the photosynthate that the milkweed, Asclepias syriaca, assimilates during flowering is used to produce nectar [33]. Nectar can contain as much as 5 $kJ.ml^{-1}$ of energy and provide half of the calories that a hummingbird needs [26].

The evolutionary significance of pollination has long been recognized [7, 23, 26, 29, 31]. These authors pointed out its role in the evolution of species by natural selection and, specially, its effect on morphological adaptations. A wide range of evidence suggests that some flower traits are specific adaptations that facilitate or enhance the pollinator visits [8, 41]. Pollinators have a great influence in gene flux between plant populations, affecting their genetic structure [8, 40, 41] and changing the seed production and the number of established seedlings [20, 31].

Despite the obvious two-side benefit in the plant-pollinator relationship, there is more in this system than one can see at a first glance.

The plant-pollinator relationship is rarely limited to a couple of species. It is more frequent to find processes, which involve several pollinators that compete between them for obtaining nectar, pollen or oviposition sites [5, 6, 13, 20] describe 23 species of pollinator insects for the Kiwi (*Actinidia deliciosa*) flowers, including 11 species of Diptera, five of Hymenoptera, and four of Coleoptera. [19] describes 11 species of bees, 12 species of butterflies and six species of flies that work as pollinators for the Mediterranean shrub *Lavandula latifolia*. According to [11], *Hormathophylla spinosa* (Cruciferae) has at least 70 visiting species of insects, grouped in two guilds: nectarivores (mainly small flies and ants) and pollinivores (bees and hover flies). Although insects are the main pollinator animals, many other animals may do so as well. For example humming-birds, bats, small rodents and marsupials are all well known pollinators (see for example [11, 25]).

It is then clear that during the process of pollination, several pollinators may interact among themselves. It is highly probable that different nectarivores or pollinivores compete for food, introducing a new element in this ecological web. The central questions then are, why don't single plant species have a single pollinator, for instance the most efficient, or the "cheapest" in terms of energy costs? How can a multispecies system be stable in ecological and evolutionary terms? Or, does a "best" pollinator exist?

In this paper we use a mathematical model to study the population dynamics of two pollinators and their resource, and the viability of this system in an attempt to provide answers to the above questions.

The mathematical model

We considered the simplest multi-species model: one plant and two pollinators. However, both pollinators differ in terms of home range, pollination efficiency and energy cost for the plant. In this way, the system represents mutualistic interactions (pollinator and plant) and competition (pollinators). The mutualism has different characteristics depending on the pollinator but competition is limited to resource exploitation because we assume no interference (never two pollinators visit the same flower simultaneously)

The model has the following assumptions:

a) There is only one species of plant, which bears one flower per individual per year. This restriction is unrealistic but necessary to avoid introducing effects of clumping or repeated visits to the same plant.

b) This flower produces a certain amount of nectar, which is renewed daily.

c) The plant is spatially distributed in patches.

d) There is a large pollinator (e.g.: a hummingbird), which has a wide movement range (can fly between patches), and consumes the total amount of nectar in each flower visit.

e) There is a small pollinator (e.g.: an insect), which has a limited movement range, and cannot fly between patches. This small pollinator consumes only a fraction *1/f* of the nectar of each flower it visits.

f) The flowers pollinated by the large pollinator produce in the next generation stronger and more numerous plants, due to a more intense genetic exchange between flowers from different patches.

g) Pollination is limited to either pollinator. Only the pollinators pollinate the plants, pollination by wind or any other means is neglected.

h) The plants reproduce annually and have discrete generations.

i) Pollinators reproduce annually but have overlapping generations.

Let A be the density of the small pollinator's population, P the density of the large pollinator's population, N_t the total number of flowers, at generation t. We have the following system of discrete equations representing the competition between pollinators:

$$P_{t+1} = P_t \times \left[1 + r \times \left(1 - \frac{A_t}{f \times N_t} - \frac{P_t}{N_t} \right) \right] \qquad \text{(Eq.1)}$$

$$A_{t+1} = A_t \times \left[1 + s \times \left(1 - \frac{A_t}{f \times N_t} - \frac{P_t}{N_t} \right) \right] \qquad \text{(Eq.2)}$$

Where r and s are the intrinsic grow rates for the large and small pollinators respectively. We assume that r, is smaller than s, given the well known relationship between growth rate and body size [32].

Regarding the flowers, we make a distinction between those pollinated by either the small or the large pollinator. This is due to the fact that the plants would not persist if only visited by the small pollinator, because of genetic inbreeding depression. So, if we represent the number of flowers pollinated by the small and the large pollinator respectively, n_A and n_P, we have the following equations

$$n_{A,t+1} = e_A \times (\alpha \times n_{A,t} + n_{P,t}) \times c_A \times c_{sat} \ , \qquad \text{(Eq.3)}$$

$$n_{P,t+1} = e_P \times (n_{A,t} + n_{P,t}) \times c_P \times c_{sat} \ , \qquad \text{(Eq.4)}$$

Were e_A and e_P are the germination ability and survival rate of seedlings. These parameters are the product of four multiplying factors: flower fecundity, seeds survival, seeds germination ability, and seedling survival. Implicitly, this term introduces the assumption of a non-homogenous environment, because a plant being pollinated by the large pollinator has an advantage when exposed to a fluctuating or heterogeneous environment. Coefficients c_A and c_P are the ratio of A and P to the total $A+P$.

The term c_{sat} is a saturation function given by:

$$c_{sat} = 1 - \exp(-d\frac{A+P}{N}) \qquad \text{(Eq.5)}$$

Where we have used N_t, the total number of plants

$$N_t = n_{A,t} + n_{P,t} \qquad \text{(Eq.6)}$$

The function c_{sat} acts in such a way that the number of flowers in each generation does not depend on the pollinator density except when that density is low compared to the flower abundance.

The parameter α ranges between 0 and 1, indicating the negative effect of inbreeding on plants; smaller a represent stronger negative effects.

We ran the model for five thousand generations starting from an initial situation where the population of the small pollinator is an order of magnitude larger than the one for the large pollinator. This assumption is coherent with the biological characteristics of the pollinators and the accepted relationship between body size and population abundance. Initially, the number of plants pollinated by each pollinator was fixed and equal (two thousand).

We used several different values for a and for the *s/r* relation; all the other parameters were kept constant: $e_A = 1.2$, $e_P = 1.4$, $d = 3.0$

Results

Both pollinators have an equilibrium point that satisfies $A^*/f + P^* = N$.

For plants, the equilibrium depends on several system parameters, in fact:

$$n_A^* = e_A(\alpha n_A^* + n_P^*)c_A c_{sat} \qquad \text{(Eq.7)}$$

$$n_P^* = e_P(n_A^* + n_P^*)c_P c_{sat} \qquad \text{(Eq.8)}$$

Stars in Eq. 7 and 8 symbolize fixed points (equilibria).

If we neglect c_{sat} variations, this is a homogeneous linear system that has a non-trivial solution only when its determinant is zero. In that case n_A^* is proportional to n_P^*, their values depend on A^* and P^*, and the system reaches a fixed equilibrium point.

Simulations show that there is no case where the actual model can present chaotic behavior, but it can converge with a monotonous or oscillatory behavior depending on the relationships among α, r and s.

When the *s/r* relation is 2 (case of light asymmetry between pollinators), the system converges rapidly for high α (*Figs. 1* and *2*) as well as for low α (*Figs. 3* and *4*), and populations remain constant after 50 generations. When α has the smaller value, populations show a slight oscillatory behavior (*Fig. 3*). This is not surprising because the use of difference equations introduces an implicit delay [14]. Despite this transient dynamics, a soft asymmetry between pollinators ensures stability and a fast convergence.

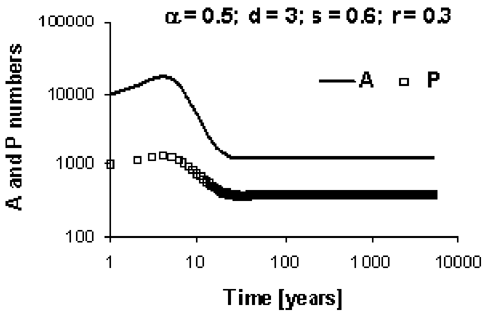

Figure 1. *Dynamics of the two pollinator populations under conditions of slight inbreeding and low asymmetry: α = 0.5 and s/r = 2. After 50 years, the system remains at stable equilibrium.*

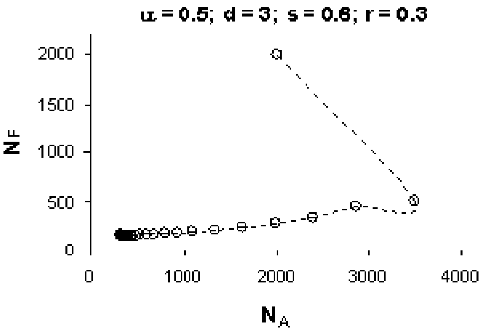

Figure 2. *Phase space of plants pollinated by each pollinator for the conditions of slight inbreeding and low asymmetry. The starting point is [2000, 2000] and there is an attractor at [331, 163].*

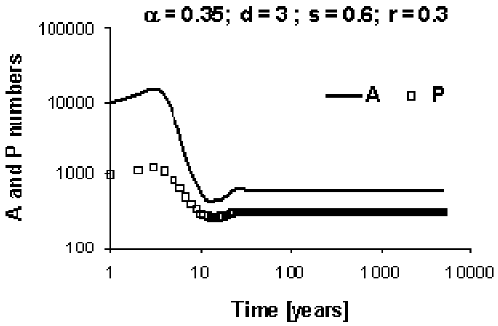

Figure 3. *Dynamics of the two pollinator populations under conditions of slight inbreeding and high asymmetry: α = 0.5 and s/r = 8. The system shows damped oscillations before reaching the stable equilibrium.*

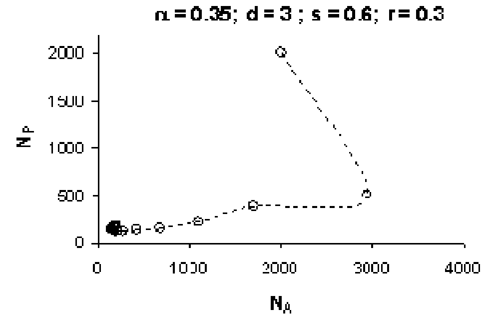

Figure 4. *Phase space of plants pollinated by each pollinator for the conditions of slight inbreeding and high asymmetry. The starting point is [2000, 2000] and there is an attractor at [729, 486] that is reached after 100 generations of damped oscillations.*

When the ratio *s/r* = 8, that is, when the growing rate of the small pollinator is practically one order of magnitude greater than that for the large pollinator (a strong asymmetry), oscillations became more pronounced. For a large α the system stills converges towards equilibrium; exponentially after 100 generations or less (*Figs. 5* and *6*). But for small α (strong effect of inbreeding) the system converges very slowly towards an exponential growth of populations, through oscillations (*Figs. 7* and *8*). In this latter case, we can see that, surprisingly, a high inbreeding causes a population increment. If this behavior is transient, it is long lasting (i.e.: “supertransient”); at less 5000 years.

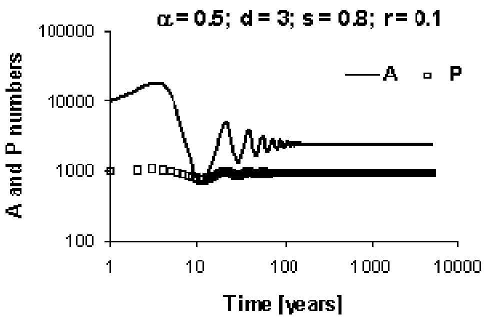

Figure 5. *Dynamics of the two pollinator populations under conditions of slight inbreeding and high asymmetry:* $\alpha = 0.5$ *and s/r = 8. The system shows damped oscillations before reaching the stable equilibrium.*

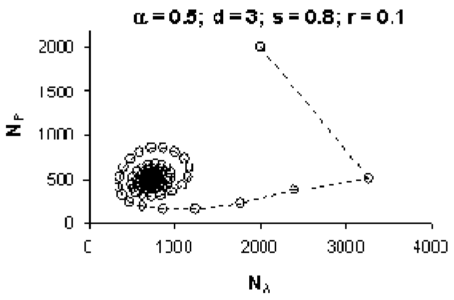

***Figure 6.** Phase space of plants pollinated by each pollinator for the conditions of slight inbreeding and high asymmetry. The starting point is [2000, 2000] and there is an attractor at [729, 486] which is reached after 100 generations of damped oscillations.*

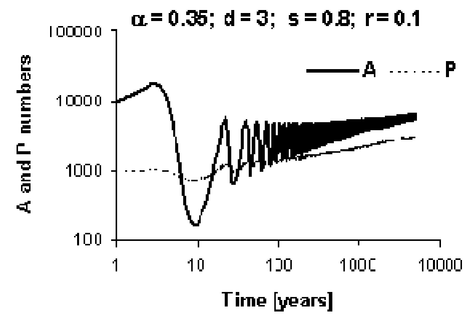

***Figure 7.** Dynamics of the two pollinator populations under conditions of strong inbreeding and high asymmetry: α = 0.35 and s/r = 8. The system shows a long transient oscillatory behavior that converge to an exponential grow of both populations.*

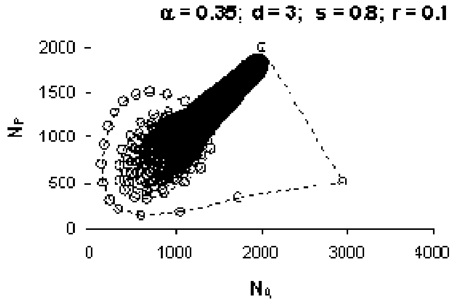

***Figure 8.** Phase space of plants pollinated by each pollinator for the conditions of strong inbreeding and high asymmetry. The starting point is [2000, 2000] and there is a long series of slightly damped oscillations. The tendency is to an exponential growth for both subpopulations.*

These different behaviors are summarized in *Table 1.*

Table 1. *Model behavior under different parameters sets.*

	Low s/r ratio (2.7)	**High s/r ratio (8.0)**
High α (0.5)	Asymptotically stable	Damped oscillations
Low α (0.35)	Stable with damped oscillations	Growing with damped oscillations

Discussion

Traditionally, modeling of mutualism has been reduced to the case of only two interacting species [24, 28], or has been based on individuals [35] due to the importance of the individual pollinator behavior. Some mathematical approaches to mutualism have been recently published [12, 22] but with different objectives than ours.

Our simple model shows a rich spectrum of dynamics. The plant-pollinator system is unstable with the small pollinators only, except for particular situations in which the inbreeding effect is canceled ($\alpha * e_A \geq 1$). On the other hand, large pollinators may coexist with the plant without the small pollinator but, due to its low growth rate, both populations would increase their numbers very slowly. It is clear in this context, that the introduction of a small pollinator benefits the large one because of causes an increase in the local plant populations. In this sense, our work shows the counterintuitive results in which pollinators may not compete but benefit form an indirect mutualistic relationship.

Our work shows also that realistic models of mutualisms need to involve the dynamics of three or more species. Attempts to do this suggest that the presence of a third species (predator or competitor) may stabilize some mutualistic interactions [3]. The persistence of assemblages of several pollinator species is important in the context of the conservation of biodiversity [36, 37]. It has been shown that there is a relation between pollination and the plant reproductive success, as well as a relation between pollinator's visits and the flowers' nectar offer [33, 34]. Most plants have life history traits that facilitate cross-pollination and ensure that beneficial genes can be transmitted relatively rapidly to successive generations [2]. Cross-pollination introduces genetic diversity into the population at a rate that enables the species to cope with a changing environment. This relationship between the pollination and the reproductive success of the plants has been broadly documented; for instance [30] have shown that sunflowers pollinated by bees, produce three times more seeds and four times more fruit by inflorescence than not pollinated ones. [13] found a similar effect in kiwi, where bees-pollinated plants bear fruits with weights 60% greater than not pollinated ones. [18] have shown that seed production was more broadly variable among wind-pollinated taxa than among animal-pollinated ones.

We cannot state that a "better" pollinator exists; the asymmetry between species results in different roles that enhance the global stability of the system. The least efficient pollinator brings a greater growth rate, while the pollinator with the slower rate of increase contributes to a better genetic exchange between plants and consequently, better chances of survival in non-homogeneous habitats.

The system shows a long range of stability for a wide variety of situations. Too intense inbreeding leads to fluctuations. The fluctuations are also induced by a strong difference in the growth rates of the two pollinators. When intense inbreeding coincides with the strong differences in growth rates, the system not only oscillates but also has growing populations. Therefore, a large population of a not efficient pollinator can benefit from a small population of a second kind of pollinators that gives the plant population higher genetic stability. That is, the presence of the second pollinator promotes the coexistence and beneficiates the first pollinator despite the competition between both.

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REFERENCES

[1] Addicott, J., Bao, T. (1999): Limiting the coasts of mutualism: Multiple modes of interaction between yuccas and yucca moths. – Proceedings of the Royal Society of London Series B Biological Sciences 266: 197-202.

[2] Aizen, M., Ezcurra, C. (1998): High incidence of plant-animal mutualisms in the woody flora of the temperate forest of southern South America: Biogeographical origin and present ecological significance. – Ecologia Austral 8: 217-236.

[3] Begon, M., Harper, J.L., Townsend, C.R. (1996): Ecology: individuals, populations and communities. Third edition. – Blackwell, Oxford.

[4] Bronstein, J.L. (1994): Our current understand. of mutualism. – Q. Rev. Biol. 69: 31-51.

[5] Brown, J., Kodric-Brown, A. (1979): Convergence, competition and mimicry in a temperate community of hummingbird-pollinated flowers. – Ecology 60: 1022-1035.

[6] Brown, J., Kodric-Brown, A., Whitman, T., Bond, H. (1981): Competition between hummingbirds and insects in the pollination of two species of shrubs. – Southwest Naturalist 26: 133-145.

[7] Darwin, C. (1859): El origen de las especies. Edition 1980. – EDAF, Madrid.

[8] Darwin, C. (1877): The various contrivances by which orchids are fertilised by insects. 2nd edition. – Appleton and Co., New York.

[9] Fleming, T., Holland, J. (1998): The evolution of obligate pollination mutualisms: Senita cactus and senita moth. – Oecologia 114: 368-375.

[10] Giurfa, M. (1991): La abeja recolectora de néctar. – Ciencia Hoy 2: 35-41.

[11] Gomez, J., Zamora, R. (1999): Generalization vs. specialization in the pollination system of *Hormathophylla spinosa* (Cruciferae). – Ecology 80: 796-805.

[12] Gomez, J., Zamora, R. (2000): Spatial variation in the selective scenarios of *Hormathophylla spinosa* (Cruciferae). – American Naturalist 155: 657-668.

[13] Greatti, M., Barbatini, R. (1997): Kiwi: uso de las abejas en la polinización. – Vida apícola 82: 27-31.

[14] Hassell, M., Jennings, J. (1975): The dynamics of competition and predation. – Edward Arnold, London.

[15] Hemborg, A., Despres, L. (1999): Oviposition by mut. seed-parasitic pollinators and its eff on annual fitness of single- and multi-flowered host plants. – Oecologia 120: 427-436.

[16] Herre, E., West, S. (1997): Conflict of interest in mutualism: documenting the elusive fig-wasp-seed tradeoff. – Proceed. of the Royal Society of London Ser. B 264: 1501-1507.

[17] Herre, E., Knowlton, N., Mueller, U., Rehener, A. (1999): The evolution of mutualisms: exploring the paths between conflict and cooperation. – TREE 14: 49-53.

[18] Herrera, C., Jordano, P., Guitian, J., Traveset, A. (1998): Annual variability in seed production by woody plants and the masting concept: Reassessment of principles and relationship to pollination and seed dispersal. – American Naturalist 152: 576-594.

[19] Herrera, C. (2000a): Flower-to-seedling consequences of different pollination regimes in an insect-pollinated shrub. – Ecology 81: 15-29.

[20] Herrera, C. (2000b): Individual differences in progeny viability in *Lavandula latifolia*: a long-term field study. – Ecology 81: 2170-2176.

[21] Irwin, R., Brody, A. (1998): Nectar robbing in Ipomopsis aggregata: Effects on pollinator behavior and plant fitness. – Oecologia 116: 519-527.

[22] Kameyama, T., Harrison, R., Yamamura, N. (1999): Persistence of a fig wasp population and evolution of dioecy in figs: A simulation study. – Res. on Pop. Ecology 41: 243-252.

[23] Knuth, P. (1906): Handbook of flower pollination. – Oxford University Press, Oxford.

[24] Kostitzin, V. (1934): Symbiose, parasitisme et évolution. – Hermann, Paris.

[25] Lamborn, E., Ollerton, J. (2000): Experimental assessment of the functional morphology of inflorescences of *Daucus carota* (Apiaceae): Testing the 'fly catcher effect'. – Functional Ecology 14: 445-454.

[26] López-Calleja, M., Fernández, M. (2001): ¿Usan los picaflores insectos sólo como fuente proteica? El caso de *Sephanoides sephanoides*. – In: I Reunión Binacional de Ecología Argentino-Chilena. ASAE, Bariloche.

[27] Margalef, R. (1995): Ecología. – Omega, Barcelona.

[28] May, R. (1981): Models for two interacting populations. – In: May R, (ed.) Theoretical Ecology. Pages 78-104. Sinauer Associates, Sunderland.

[29] Müller, H. (1883): The fertilization of flowers. – Macmillan, London,

[30] Muñoz Rogríguez, A. (1997): Girasol: polinización con ab. melíf. – Vida apíc. 84: 14-17.

[31] Pellmyr, O., Thompson, J., Brown, J., Harrison, R. (1996): Evolution of pollination and mutualism in the yucca moth lineage. – The American Naturalist 148: 827-847.

[32] Peters, R. (1983): The ecological implications of body size. – Cambridge University Press, Cambridge.

[33] Pleasants, J., Chaplin, S. (1983): Nectar production rates of *Asclepias quadrafolia*: causes and consequences of individual variations. – Oecologia 59: 232-238.

[34] Real, L., Rathcke, B. (1988): Patterns of individual variability in floral resources. – Ecology 69: 728-735.

[35] Real, L., Marschall, E., Roche, B. (1992): Individual behavior and pollination ecology: Implications for the spread of sexually transmitted plant diseases. – In: DeAngelis, D.L., Gross, L.J., (eds.) Individual-based models and approaches in ecology. Populations, communities and ecosystems. Pages 492-508. Chapman & Hall, New York - London.

[36] Robertson, A,. Kelly, D., Ladley, J., Sparrow, A. (1999): Effects of pollinator loss on endemic New Zealand mistletoes (Loranthaceae). – Conservation Biology 13: 499-508.

[37] Traveset, A. (1999): The importance of mutualisms for biodiversity conservation in insular ecosystems. – Revista Chilena de Historia Natural 72: 527-538.

[38] van Baalen, M., Jansen, V. (2001): Dangerous liasons: the ecology of private interest and common good. – Oikos 95: 211-224.

[39] Waser, N., Prise, M. (1983a): Pollinator behavior and natural selection for flower color in *Delphinium nelsonii*. – Nature 302: 422-424.

[40] Waser, N., Prise, M. (1983b): Optimal and actual outcrossing in plants, the nature of plant-pollinator interaction. – In: Jones, C.E., Little, R.J., (eds.) Handbook of Pollination Biology. Pages 341-359. Van Nostrand-Reinhold, New York.

[41] Waser, N. (1993): The adaptative nature of floral traits: ideas and evidence. – In: Real, L.A., (ed.) Pollination Biology. Pages 241-285. Academic Press, New York.

LOW MACRO-ARTHROPOD ABUNDANCE IN EXOTIC *EUCALYPTUS* PLANTATIONS IN THE MEDITERRANEAN

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Abstract: Exotic plantations of *Eucalyptus* are replacing large areas of extensive agroecosystems throughout the Mediterranean basin. A comparison of the abundance of ground macro-arthropods in some of major types of traditional land uses in southern Iberia (cork oak woodlands, olive groves, fallow fields, vineyards, and riparian vegetation) revealed that *Eucalyptus* plantations held the lowest abundances for most groups of arthropods. This indicates that the expansion of *Eucalyptus* plantations in the Mediterranean is very negative for ground arthropods, and for the many species of vertebrates that depend on them as prey.

Keywords: *Exotic plantations, landscape management, ground arthropods*

Introduction

Throughout the Mediterranean basin exotic *Eucalyptus* plantations are replacing large areas of native woodland and traditional extensive agroecosystems, such as olive grove or cork oak woodland [4]. However, since plantation forests tend to be unfavourable habitat for a wide range of animal taxa, particularly in the case of even-aged, single-species stands of exotic species, the expansion of these plantations may pose problems for the preservation of Mediterranean biodiversity [6, 13].

In spite of the generalized use of *Eucalyptus* in forestry in many parts of the World, there is little information on its impact on the invertebrate fauna, and most existing studies focus on the impact of single groups. In addition, the results of different studies carried out throughout the World do not allow a general conclusion about the influence of the plantations on the abundance of invertebrates. Although most studies report a negative influence on invertebrates (e.g. [5, 9]), a few found invertebrate communities in plantations that are similar in abundance or diversity to those of neighbouring natural or semi-natural habitats (e.g. [1, 10]). This diversity of results suggests that the response of arthropods to *Eucalyptus* plantations varies among taxa and depends on the geographic context and other environmental factors, so specific studies are needed to understand the impact of plantations in different ecological regions.

Although the high biodiversity value of the Mediterranean basin led to its classification as a Biodiversity Hotspot, little is known about how the expansion of *Eucalyptus* plantations may be affecting the epigeic invertebrate communities in the region. Such knowledge is important not only because of the intrinsic value of those communities, but also because of the many species that depend on them as prey. The objective of this study is to evaluate how the abundance of ground macro-arthropods in

Eucalyptus plantations in southern Iberia compares with that of some of the main land uses that they are replacing, namely cork oak woodlands, olive groves, vineyards, riparian vegetation and fallow farmland.

Methods

The study was conducted from March 1997 to February 1998 near Évora, Portugal (~ 8º 8´ W, 38º 29´ N), a region with a Mediterranean to sub-Mediterranean climate, with cool and rainy winters and dry hot summers.

Arthropods were sampled in the main land cover types of the area, which are also common throughout much of Mediterranean Iberia: (i) cork oak woodland, with sparse trees (*Quercus suber*) and an undergrowth of sparse shrubs and herbs; (ii) olive grove, with olive trees (*Olea europaea*) and herbs; (iii) fallow field, sparsely covered with grasses; (iv) vineyard, culture of *Vitis vinifera* on regularly plough and mostly bare ground; (v) riparian vegetation, well vegetated areas along stream banks; and (vi) Eucalyptus plantation, stands of *Eucalyptus globulus* with some *Cistus* shrubs growing on mostly bare ground. Although *Eucalyptus* has been used in the area since 1964, the trees of the studied plantation were 6 to 7 years old. This age is quite representative of the Eucalyptus plantations in the region, which are maintained on short rotations, mostly for the production of pulp for the paper industry.

Three sets of four pitfall traps (10.5 x 17.0 cm, 10 cm deep) were placed 50-100 meters apart in each of the six above listed habitats. Traps were partly filled with saltwater to preserve captured arthropods, and kept open for 6 days in March, June, July, September, and November 1997 and in February 1998, so we had a total of 432 trap days for each habitat. Samples were frozen and analyzed in the lab, where arthropods were identified at least to order. Numbers of individuals in each trap were counted and averaged for each habitat type.

The comparison of the abundances of arthropods in *Eucalyptus* plantations with those in other major land uses is based on the most common groups of typical ground-dwelling arthropods: Isopoda, Arachnida, Formicidae and Carabidae (*Table 1*). Arthropods that were not sufficiently represented to justify its own class were included in either “other coleoptera” or “other arthropods” (mainly Grylloidea, Chilopoda, Heteroptera, larvae of Lepidoptera, and Diptera). The comparison of the samples of the *Eucalyptus* plantation to those of other habitats was done using Scheffé-Test after significant differences between habitats were proven by the H-Test.

Results and Discussion

Ground macro-arthropods caught in the pitfall traps included Arachnida (Scorpiones, Solifugae, Araneae, Opiliones, Acari), Myriapoda (Chilopoda, Diplopoda), Crustacea (Isopoda) and Insecta (Saltatoria, Dermaptera, Blattodea, Hemiptera, Lepidoptera, Diptera, Hymenoptera, Coleoptera).

Riparian habitats had the highest abundance of all but two groups (Isopoda and Formicidae; *Table 1*), which may be explained by the greater moisture available in its soil. Cork oak woodlands held the next highest overall abundances, and the highest values for Arachnida, Formicidae, Carabidae and “other arthropods”. This may be due to the greater relative complexity of this habitat, and to the fact that it is presumably the most similar to the original natural vegetation of the region. *Eucalyptus* plantation had the lowest abundances of all groups of arthropods (*Table 1*), with the exception of Isopoda, which are detritivores and can probably benefit from the high amount of leaf-litter in them [11]. In most other groups, the abundance in *Eucalyptus* plantations was several times lower than that of the best habitats in the region.

Table 1. *Abundance of different ground macro-arthropod groups in the studied habitats (mean number of specimens per trap in each sampling session and standard error). Grey tones represent the rank from higher (light) to lower (dark) abundance. An * indicates a significant difference (p<0,05) in tests comparing the abundance between each land use and the Eucalyptus plantations.*

HABITAT	**Isopoda**	**Formicidae**	**Arachnida**	**Carabidae**	**Other Coleopt**	**Other Arthrop**	**Large Arthrop**
Riparian vegetation	2.36 (0.63)	1.93 (0.24)	13.66 (1.68)*	1.77 (0.35)*	4.32 (0.97)*	9.41 (1.47)*	1.23 (0.26)*
Oak woodland	1.26 (0.21)	3.44 (0.17)*	5.05 (0.43)*	1.35 (0.24)*	0.88 (0.13)	3.94 (0.81)	0.93 (0.17)
Olive grove	1.16 (0.28)	3.18 (0.25)*	4.73 (0.55)	0.55 (0.16)	0.47 (0.13)	3.63 (0.41)	0.61 (0.18)
Fallow field	0.23 (0.07)	3.08 (0.24)*	4.19 (0.41)	1.15 (0.27)*	2.56 (0.73)*	2.87 (0.35)	0.63 (0.12)
Vineyard	2.45 (0.67)	2.88 (0.24)*	4.66 (0.42)	0.55 (0.12)	0.43 (0.13)	2.64 (0.38)	0.50 (0.10)
***Eucalyptus* plantation**	2.70 (0.72)	1.46 (0.16)	2.01 (0.27)	0.17 (0.06)	0.37 (0.08)	1.94 (0.34)	0.45 (0,07)

One possible explanation for the low number of arthropods in *Eucalyptus* plantations is the usually low density and diversity of herbs and shrubs, which can be attributed to a variety of factors. Mechanical treatments of the soil prior to planting or at middle rotation are known to reduce the diversity, ground cover, and biomass of the undergrowth [3]. The release of phytotoxic substances by *Eucalyptus globulus*, is likely to have similar effects on the undergrowth [12]. Nutrient deficiencies may limit plant growth in older stands that have been used for multiple rotations, due to the export of nutrients by harvesting [7]. Finally, *Eucalyptus* are known to be very efficient competitors for water, which under certain circumstances may limit the development of undergrowth [8].

Another factor that may contribute to the low abundances of arthropods in the plantations is the direct effect of *Eucalyptus* leaves leachates on them, as it has been demonstrated that the leachates of E. *globulus* reduce the viability of certain arthropod species (e.g. [2]).

As many species of Mediterranean vertebrates prey on the larger ground arthropods, we estimated the abundance of individuals more than 1cm long in the six habitats. We found that these larger individuals are also least abundant in the *Eucalyptus* plantations (*Table 1*), which should negatively influence their potential as foraging habitats for many vertebrates.

The impact of the reduction of arthropods was less pronounced in the wetter sampled areas of the *Eucalyptus* plantations (*Fig. 1*). They had better developed undergrowth, presumably because of the greater availability of water, and a greater abundance of all groups of macro-arthropods, with the exception of Carabidae. The difference was significant in Isopoda and “other arthropods” (p<0,05; Wilcoxon-test). This is coherent with the results of studies carried out in plantations of E. *globulus* in northern Iberia, which has a much higher rainfall; the plantations had well developed undergrowth and a fauna of leaf-litter invertebrates similar in composition and density to that of nearby native forest [1].

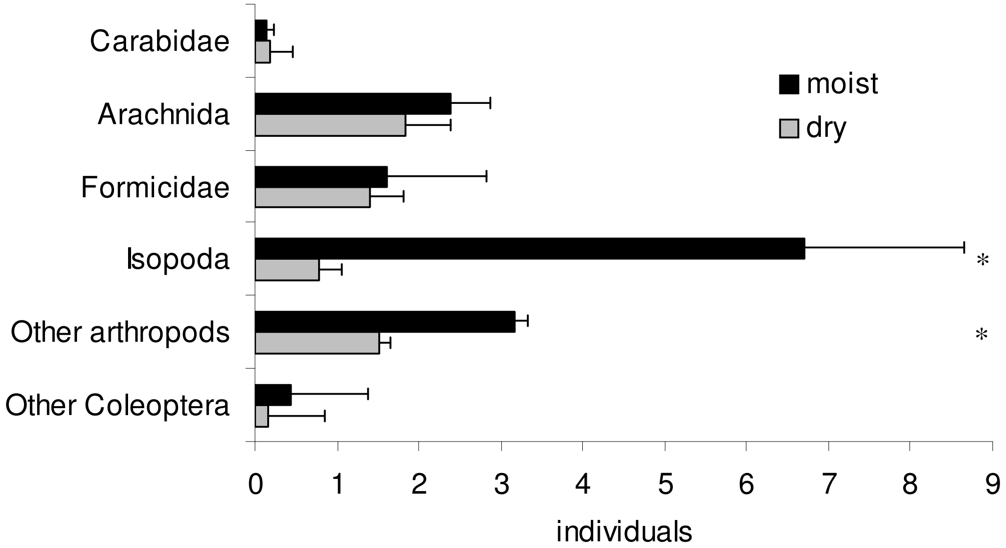

Figure 1. *Abundance of ground macro-arthropods in the moist and the dry sections of the Eucalyptus globulus plantation. Columns represent mean number of specimens per trap in each sampling session and lines the standard error. An * indicates a significant difference (p<0,05)*

The environmental factors that control the abundance of arthropods in exotic stands of *Eucalyptus* are diverse, although poorly understood. In addition to water availability, the age of the trees seems to be very important, as old plantations can harbour abundant faunas [10]. Consequently, our results can not be extrapolated to all *Eucalyptus* plantations in the Mediterranean, as we only studied young stands. However, they do suggest that these stands, which are by far the most widespread in the region, under the dry conditions typical of its climate, harbour small abundances of ground macro-arthropods. Consequently, the expansion of *Eucalyptus* plantations, at the expense of other traditional types of land use, should be seen as a challenge to the preservation of Mediterranean biodiversity.

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REFERENCES

[1] Bará, S., Rigueiro, A., Gil, M.C., Mansilla, P., Alonso, M. (1985): Efectos ecológicos del *Eucalyptus globulus* en Galicia. Estudio comparativo con *Pinus pinaster* y *Quercus robur*. – Instituto Nacional de Investigaciones Agrarias, Madrid.

[2] Canhoto, C., Laranjeira, C. (2007): Leachates of *Eucalyptus globulus* in intermittent streams affect water parameters and invertebrates. – International Review of Hydrobiology 92: 173-182.

[3] Carneiro, A., Fabião, A., Martins, M.C., Fabião, A., da Silva, M.A., Hilário, L., Lousã, M., Madeira, M. (2008): Effects of harrowing and fertilisation on understory vegetation

and timber production of a *Eucalyptus globulus* Labill. plantation in Central Portugal. – Forest Ecology and Management 255: 591-597.

[4] Carnus, J.M., Parrotta, J., Brockerhoff, E., Arbez, M., Jactel, H., Kremer, A., Lamb, D., O'Hara, K., Walters, B. (2006): Planted forests and biodiversity. – Journal of Forestry 104: 65-77.

[5] da Silva, P.M., Aguiar, C.A.S., Niemela, J., Sousa, J.P., Serrano, A.R.M. (2008): Diversity patterns of ground-beetles (Coleoptera: Carabidae) along a gradient of land-use disturbance. – Agriculture Ecosystems & Environment 124: 270-274.

[6] Hartley, M.J. (2002): Rationale and methods for conserving biodiversity in plantation forests. – Forest Ecology and Management 155: 81-95.

[7] Merino, A., Balboa, M.A., Soalleiro, R.R., Gonzalez, J.G.A. (2005): Nutrient exports under different harvesting regimes in fast-growing forest plantations in southern Europe. – Forest Ecology and Management 207: 325-339.

[8] Poore, M.E.D., Fries, C. (1985): The ecological effects of eucalyptus. – Food and Agriculture Organization, Rome.

[9] Ratsirarson, H., Robertson, H.G., Picker, M.D., van Noort, S. (2002): Indigenous forests versus exotic eucalypt and pine plantations: a comparison of leaf-litter invertebrate communities. – African Entomology 10: 93-99.

[10] Sax, D.F. (2002): Equal diversity in disparate species assemblages: a comparison of native and exotic woodlands in California. – Global Ecology and Biogeography 11: 49-57.

[11] Sousa, J.P., Vingada, J.V., Loureiro, S., da Gama, M.M., Soares, A. (1998): Effects of introduced exotic tree species on growth, consumption and assimilation rates of the soil detritivore *Porcellio dilatatus* (Crustacea : Isopoda). – Applied Soil Ecology 9: 399-403.

[12] Souto, X.C., Bolano, J.C., Gonzalez, L., Reigosa, M.J. (2001): Allelopathic effects of tree species on some soil microbial populations and herbaceous plants. – Biologia Plantarum 44: 269-275.

[13] Stephens, S.S.,Wagner, M.R. (2007): Forest plantations and biodiversity: A fresh perspective. – Journal of Forestry 105: 307-313.

COMMUNITY COMPOSITION AND TREE POPULATION STRUCTURE IN UNDISTURBED AND DISTURBED TROPICAL SEMI-EVERGREEN FOREST STANDS OF NORTH-EAST INDIA

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Abstract Species composition, density, importance value index, diversity, dominance and tree population structure were studied in disturbed and undisturbed stands of tropical semi-evergreen forest in Lunglei District of Mizoram, North-East India. Total number of species in the undisturbed stand was 67 while in the disturbed stand 63 species were recorded. Number of tree and shrub species in the undisturbed stand were higher (32 and 18) than the disturbed stand (17 and 16). However, the number of herbaceous species remained higher in the disturbed stand (30) compared to the undisturbed stand (17). *Castanopsis tribuloides* (Smith) was common in both stands, and showed dominance in the undisturbed stand with a density of 90 individuals' ha^{-1}. However *Schima wallichii* (DC.) Korth Choicy was dominant in the disturbed stand with a density of 125 individuals' ha^{-1}. In both stands higher dbh classes showed lower density than that of the lower and intermediate girth classes. In general, the undisturbed forest stand showed more density of trees in each dbh classes and the intermediate girth class in particular. The study reveals that the anthropogenic disturbance causes disruption of forest structure and changes community composition which ultimately leads to disruption of tree population structure.

Key words: *Dominance, Species diversity, Floristic composition, Forest structure, Girth class*

Introduction

The structure of plant as well as animal communities in many natural ecosystems is largely influenced by the disturbances, frequently occurring in the system naturally or due to anthropogenic activities [2, 6, 16, 33]. In many of these systems, disturbances change overall community structure [54, 60] which in turn can ultimately affect community and population dynamics. The importance of disturbance for maintaining community composition [15, 21, 43] and determining population dynamics [37, 49, 50, 58] has been well recognized in the tropical and extra-tropical systems. Disturbances also have profound effect on the regeneration of non-pioneer under storey trees [33]. [10] viewed disturbance as a negative force that destroys climax assemblages and brings instability in the system, while [28, 36, 42] considered it as a positive force that might increase species diversity in the community by preventing competitive exclusion by dominant species. Species richness has been related to the occurrence of natural disturbance by several authors [22, 28, 45].

Apart from disturbances, climate, especially precipitation also play significant role in species composition and structure [4, 11, 19]. The diameter distribution of trees has been often used to represent the population structure of forests [31, 40, 51].

Configuration of curves has also been correlated with successional status of forests [20, 51] and degree of tolerance to shade [63]. In this study, we assessed the overall species composition and tree population structure in an undisturbed and disturbed tropical forest stands of Mizoram, North-East India.

Materials and methods

Study Site

The study was carried out in two forest stands, one disturbed and the other relatively undisturbed stands of tropical semi-evergreen forest [9], located in Lunglei District, Mizoram, North-East India. The disturbed stand is located at Hminlokawn (latitude 22°51´30´´N and longitude 92° 47´0´´ E) *ca.* 9 km north of the undisturbed stand located at Zobawk (latitude 22^o 47´30´´ N and longitude 92° 47´50´´ E) and the sites are *ca.* 230 km south of Aizawl, the capital city of Mizoram. The undisturbed stand is about 25 years old natural forest stand (local source) which is preserved by the village community of Zobawk (*ca.* 16 km from the Lunglei town) from any type of anthropogenic interruption in the natural forest growth except negligible non timber forest products collection by the local community for their sustenance. While the disturbed stand is situated in the outskirt of Lunglei (*ca.* 8 km from the town), which is experiencing high degree of disturbance in terms of various anthropogenic activities such as fire wood collection, timber logging and grazing etc. Each of the selected forest stands covers an area of more than about 25 ha of land.

The climate is monsoonic under the direct influence of the south-west monsoon, with marked seasonal variation in temperature and rainfall. Depending on the variation in temperature and other climatic conditions, three seasons *viz.* winter (November-February), spring (March-May), rainy/summer season (Jun-October) are observed in the area as is in other parts of the state. The cold or winter season starts from November and lasts till February with comparatively lower temperature (11-23 °C) and very less rainfall. The rainy season or summer, the longest season (nearly 6 months) starts in the second part of May with interrupted showers, and incessant rain begins in June and continues till September and ends in the last part of October. During this season temperature remains high (temperature ranged between 22-34 °C). Normally July and August are the most precipitated months, receiving about 40% of the annual rainfall; while December and January are the driest months. During 2004 and 2005 the area received 4,076 mm and 2,773 mm of rainfall respectively [1]. The mean minimum and maximum temperature were 15.5 and 26.5 °C, respectively, and relative humidity varied between 41 and 89%.

Methodology

To study the community composition and other phytosociological characteristics of the vegetation at the selected sites, thorough field surveys were conducted during June 2002- May 2003 for site selection and quadrat study. Phytosociological attributes of each species were studied by randomly laying 50 quadrats of 10 x 10 m^2 sizes, for trees (≥10 cm dbh.) and 5 x 5 m^2 quadrats for shrubs, 20 quadrates of 1 x 1 m^2 for herbaceous species at each of the selected forest stands. Total 240 quadrats were laid (120 quadrates at each sites) covering entire study area to minimize sampling error. The size and the number of quadrats were determined following [30, 38]. Floristic composition, density, diversity, dominance, distribution and tree population structure were studied according to [38, 39, 56]. From the relative values the importance value index (IVI) was calculated according to [12]. All the plants encountered in the quadrates were identified with the help of herbaria and different flora viz. Flora of Assam, Flora of British India and Flora of Arunachal Pradesh etc. The data collected were also used to compute community indices such as Sorensen's similarity index [59], Shannon-Wiener's diversity index [55], and Pielou's evenness index [46].

Results

Both stands showed high floristic composition (*Tables 1-2*). IVI distribution curve (*Figure 1*) showed that the disturbed stand had higher dominance or low evenness while the undisturbed stand had lower dominance or higher evenness among trees and shrubs. Total number of species in the undisturbed stand was 67 while in the disturbed stand 63 species were recorded. Total density of plants was 2,84,510 individuals ha^{-1} and 1,04,030 individuals ha^{-1} in the disturbed and undisturbed stands respectively. Higher density in the disturbed stand is due to the predominance of herbaceous species with high densities. Number of tree species in the undisturbed stand was 32 while in the disturbed stand it was 17. Number of shrub species was higher in the undisturbed stand (18) than in the disturbed stand (16). Nonetheless, the number of herbaceous species remained higher in the disturbed stand (30) than in the undisturbed stand (17). *Castanopsis tribuloides* was common in both stands, and showed dominance in the undisturbed stand with a density of 90 individuals' ha^{-1}. *Schima wallichii* was dominant in the disturbed stand with a density of 125 individuals' ha^{-1}. The canopy of the forest is composed of both evergreen and deciduous broad-leaved trees, whose height reached not more than 25 m. The species were distributed in four strata, *viz.*, canopy layer, sub-canopy layer, shrub layer and herbaceous layer.

The canopy layer (height>10 m) was dominated by *Castanopsis tribuloides* in the undisturbed stand and *Schima wallichii* in the disturbed stand. *Castanopsis tribuloides* is also present in large numbers in the disturbed stand. In the undisturbed stand *Engelhardtia spicata, Cinnamomum obtusifolium*, *Macaranga indica*, *Elaeocarpus robusta* and *Sapium baccatum* were more in number after *Castanopsis tribuloides*. In the disturbed stand this layer consists of *Schima wallichii, Castanopsis tribuloides* and *Macaranga indica*. The sub-canopy layer (3-10 m height) was composed mainly of *Cinnamomum glaucescens, Alseodaphne petiolaris*, and *Helicia robusta* besides few other species in the undisturbed stand whereas in the disturbed stand *Wendlandia grandis*, *Cinnamomum glaucescens,* and *Eurya symplocina* were the main component species. The canopy and sub-canopy trees together forms a continuous dense cover in the undisturbed stand, except for few gaps formed here and there due to natural disturbances like tree falls etc. In the disturbed stand, the continuity was interrupted by different sizes of gaps created by tree falls mostly due to anthropogenic activities.

The shrub layer was dominated by *Derris wallichii* in the disturbed stand, and by *Pinanga gracilis* in the undisturbed stand. *Rubus birmanicus, Rhynchotechum ellipticum, Morinda angustifolia, Maesa montana* and *Milletia pachycarpa* were also present in large number in the disturbed stand. *Areca triandra, Acacia pinnata, Smilax ovalifolia, Derris wallichii, Calamus guruba* and *Lonicera macaranda* are the main component species besides *Pinanga gracilis* in the shrub layer of the undisturbed stand. Lianas and other woody climbers and twiners were quite common in both forest stands.

Table 1. *Density (plants ha^{-1}) and importance value index (IVI) of component species in the two tropical semi-evergreen forest stands. (Species rating based on IVI shown in parenthesis)*

Species	**Undisturbed**			**Disturbed**		
	Density	**TBC**	**IVI**	**Density**	**TBC**	**IVI**
Trees						
Actinodaphne obovata (Nees) Bl.	15	52.65	3.72 (32)	-	-	-
Alseodaphne petiolaris (Mwiaan.) Hook. f.	40	232.46	10.99 (6)	-	-	-
Callicarpa arborea Roxb.	10	26.39	2.28 (37)	-	-	-
Castanopsis indica Roxb. ex Lindley	10	149.63	5.08 (24)	-	-	-
Castanopsis tribuloides Smith	90	688.60	27.70 (1)	60	583.19	35.13 (20)
Celtis australis L.	15	61.90	3.91 (29)	25	71.96	9.21 (9)
Cinnamomum glaucescens (Nees) Meissn	70	358.04	16.83 (3)	-	-	-
Cinnamomum obtusifoluim (Roxb.) Nees.	45	170.33	10.42 (8)	-	-	-
Cinnamomum zeylanicum Bl.	30	114.73	6.63 (19)	-	-	-
Diospyros toposia Buch-Ham.	5	46.43	1.90 (40)	-	-	-
Drimycarpus racemosus Hook.	5	6.06	0.98 (49)	10	33.39	3.87 (23)
Dysoxylum macrocarpum Roxb.	30	86.12	7.01 (16)	-	-	-
Dysoxylum procera Hiem.	10	25.88	2.27 (39)	-	-	-
Elaeocarpus robusta var.*grandis F. Muell*	40	205.2	10.88 (7)	5	14.33	1.84 (29)
Engelhardtia spicata Bl.	15	109.99	5.02 (25)	25	137.33	12.73 (6)
Eriobotrya bengalensis (Roxb.) Hook. f.	10	116.42	4.33 (26)	-	-	-
Eugenia cumini (L.) Druce	5	22.99	1.36 (44)	-	-	-
Eurya symplocina Bl.	-	-	-	35	123.81	13.03 (5)
Ficus prostrata Wall. ex. Miquel	-	-	-	10	84.67	5.90 (16)
Garcinia morella (Gaertn.) Desr.	5	21.80	1.34 (45)	-	-	-
Glycosmis pentaphylla (Retz.) Correa	-	-	-	10	38.71	4.08 (21)
Helicia robusta Roxb.	25	66.56	5.21 (22)	-	-	-
Ilex godajam (Colebr.) Wall. Ex. Hook. f.	20	164.02	7.09 (15)	-	-	-
Leea indica (Burm. f.) Merr.	5	4.08	0.93 (50)	-	-	-
Litsea polyantha Juss.	10	15.99	1.72 (42)	15	40.15	5.41 (18)
Litsea semecarpifolia (Wall.) Hook. f.	15	50.04	3.15 (34)	-	-	-
Macaranga indica Wight.	35	141.22	8.07 (13)	45	206.93	18.88 (3)
Mitragyna diversifolia (Hook. f.)	-	-	-	10	33.54	3.87 (22)
Myristica linifolia (Roxb.) Warburg.	20	82.29	5.40 (21)	-	-	-
Olea dioca Roxb.	-	-	-	5	7.36	1.56 (30)
Phoebe attenuata (Nees.) Nees.	10	92.06	3.78 (30)	-	-	-
Quercus xylocarpa Kurz.	40	316.26	12.37 (4)	-	-	-
Randia wallichii Hook. f.	-	-	-	10	39.08	4.09 (20)
Rhus sucedánea L.	-	-	-	10	28.37	3.66 (25)
Sapium baccatum Roxb.	55	494.47	18.43 (2)	-	-	-
Schima wallichii (DC.) Korth. Choicy	35	220.91	9.88 (10)	125	933.45	60.31 (1)
Styrax polysperma Clarke in Hook. f.	30	173.49	8.48 (12)	-	-	-
Styrax serrulatum Roxb.	15	16.26	2.90 (36)	10	25.29	3.55 (26)
Syzygium sp.	-	-	-	25	68.54	9.07 (11)
Wendlandia grandis (Hook. f.) Cowan.	10	25.98	2.27 (38)	-	-	-
Shrubs						
Acacia caesia L.	10	0.25	1.17 (46)	45	0.94	9.14 (10)
Acacia pinnata L.	55	0.09	5.14 (23)	-	-	-
Acmenosperma claviflorum Roxb. Kausal.	-	-	-	5	2.21	1.36 (31)
Anodendron paniculatum A. DC.	20	0.26	3.38 (33)	-	-	-
Areca triandra Roxb. Wx Buch. Ham.	70	1.49	7.19 (14)	-	-	-
Arenga pinnata (Wurmb) Merr	20	4.34	2.95 (35)	-	-	-
Bauhinia anguina (Roxb.)H. Ohadhi	25	2.33	3.75 (31)	35	1.62	5.56 (27)
Bauhinia tenuiflora G. Watt ex C. B. Clarke (basionym)	-	-	-	20	0.07	4.31 (19)
Calamus guruba Ham.	80	1.70	9.39 (11)	-	-	-

Caryota urens L.	15	0.20	1.50 (43)	-	-	-
Derris wallichii Prain	85	13.77	9.99 (9)	15	10.26	17.00 (4)
Entada pursaetha DC.	-	-	-	30	2.02	6.93 (15)
Litsea angustifolia Wall. Ex. Hook. F.	45	1.72	5.56 (20)	15	0.15	3.82 (24)
Lonicera sp.	50	5.09	6.99 (17)	-	-	-
Maesa montana A. DC.	-	-	-	50	1.23	8.88 (12)
Melastoma nepalensis Lodd.	-	-	-	5	0.09	1.27 (32)
Millitia sp.	10	0.18	1.17 (47)	40	0.52	7.86 (13)
Morinda angustifolia Roxb.	-	-	-	40	0.64	7.09 (14)
Phlogacanthus tubiflorum Nees	10	0.01	1.17 (48)	-	-	-
Pinanga gracilis (Rox.) Bl.	105	2.86	11.05 (5)	-	-	-
Rhynchotechum ellipticum (Wall. ex. D. Dietr) A. DC.	40	0.22	4.17 (27)	65	0.18	10.32 (8)
Rubus birmanicus Hook. f.	-	-	-	85	0.42	12.31 (7)
Sauraria sp.	10	3.45	1.76 (41)	-	-	-
Schefflera venulosa (Wight & Arn.) Harms.	-	-	-	5	0.09	1.27 (33)
Smilax ovalifolia Roxb.	65	0.16	6.83 (18)	-	-	-
Spatholobus roxburghii Benth.	40	0.19	4.17 (28)	-	-	-
Tabernaemontana divaricota (L.) R.Br.ex.Roem &Schdult	-	-	-	15	0.29	3.05 (28)

- indicates species absence

The number of herbaceous species differed between the two stands. The undisturbed stand harbors lesser number of herb species as compared to the disturbed stand. *Hedychium coccineum* is common in both stands, but it is out-numbered by *Selaginella sp*. and *Digitaria adscendens* in the disturbed stand. The ground vegetation is very less and sparse under canopy whereas it is dense in the forest floor of gaps.

The density of tree seedlings and saplings also varied between the two stands (*Table 3*). The saplings of *Castanopsis tribuloides* were abundant in the undisturbed stand followed by saplings of *Cinnamomum glaucescens*. In the disturbed stand, saplings of *Schima wallichii* were abundant, followed by saplings of *Wendlandia grandis* and *Castanopsis tribuloides*.

Shannon's Species Diversity Index shows that the undisturbed stand have higher diversity (H′=3.1461) as compared to that of the disturbed stand (H′ =2.2881). Pielou's Evenness Index also reveals that the undisturbed stand has more consistency in species distribution. Undisturbed stand had higher evenness index (E=0.7565) while the disturbed stand had lower evenness (E= 0.6488) (*Table 4*). Sorensen's similarity indices between the two stands revealed that these stands have similarities to some extent in species composition which is more prominent in shrub component than the trees (*Table 4*).

In both stands higher dbh classes showed lower density (*Table 7 and Figure 2*) than that of the lower and intermediate girth classes. A similar trend of straight line relationship between density and diameter (negative exponential curve) was observed in the undisturbed and disturbed forest stands. However, intermediate girth class (21-20cm) showed more density than that of lower dbh (10-20 cm) in the two stands (Figure 2). In both forest stands, trees with intermediate dbh class (21-20 cm) showed highest density per ha than all other dbh classes. Individuals of the lowest dbh class (10-20 cm) showed higher tree density per hectare after intermediate dbh class in both undisturbed and disturbed forest stands followed by other dbh classes in the order of 31-40 cm > 41-50 cm > 51-60 cm in the two stands. In the disturbed stand high dbh (51-60 cm) individuals were found to be as much as those of the undisturbed stand. In general, the undisturbed forest stand showed more density of trees in each dbh classes, particularly the intermediate girth class, except the individuals with highest girth class (61-70 cm) was absent in this stand which were found in the disturbed stand only.

Table 2. *Density (plants ha^{-1} x 10^{3}) and importance value index (IVI) of herbs component species in the two (Undisturbed and disturbed) tropical semi-evergreen forest stands.*

Species	**Undisturbed**		**Disturbed**	
	Density	**IVI**	**Density**	**IVI**
Ageratum conyzoides L.	-	-	1.70	3.23
Bidens pilosa L.	-	-	1.50	18.48
Blumea lanceolaria (Roxb.) Druce	-	-	0.35	7.32
Cissampelos pareira L.	0.05	2.50	0.15	1.97
Commelina benghalensis L.	0.40	10.29	-	-
Curculigo capitulate (Lour) Kuntze	0.60	29.78	0.10	2.80
Cynodon dactylon (syn) *Panicum dactylon* (L) pers.	-	-	0.75	8.37
Cyperus rotundus L.	-	-	0.75	12.97
Dalbergia tamariscifolia Cav.	0.55	19.35	-	-
Dioscoria alata L.	-	-	0.85	9.16
Digitaria adscendens (HBK) Henr.	-	-	2.95	12.67
Diplazium esculentum (Retz) Sw.	0.30	5.13	-	-
Dryopteris sp.	-	-	0.20	1.99
Eupatorium adenophorum Spreng	-	-	0.30	4.27
Eupatorium riparium Regel	-	-	1.10	10.68
Eupatorium odoratum L.	-	-	0.85	17.56
Gleichenia sp.	0.90	15.39	-	-
Hedychium coccineum Buch.-Ham.ex.Sm.	2.40	99.93	1.05	23.82
Hedyotis scandens Roxb.	0.15	5.66	0.20	3.63
Imperata cylindrical (L.) Beauv.	-	-	2.00	10.85
Ipomea sp.	-	-	0.30	3.85
Lygodium flexosum (L.) Sw.	-	-	0.10	2.20
Mikania micrantha HBK	-	-	0.50	6.94
Oxalis corniculata L.	-	-	0.10	1.24
Panicum sp.	0.55	11.21	0.70	5.66
Paspalum sp.	-	-	2.35	15.64
Passiflora nepalensis Wall.	-	-	0.10	2.95
Phrynium capitatum Willd.	1.55	29.43	-	-
Piper longum L.	0.35	10.25	0.65	14.95
Polygonum sp.	-	-	0.45	7.06
Pteridium sp.	0.40	16.83	-	-
Pueraria subspicata (Benth.) Maesen	0.30	9.46	0.05	1.26
Scoparia dulcis L.	-	-	1.70	12.29
Selaginella sp.	0.30	6.79	4.40	27.27
Themeda arundinacea Roxb.	0.10	2.94	-	-
Thysanolaena maxima O.ktze	1.25	21.76	1.45	16.80
Urena lobota L.	-	-	0.70	13.41

- indicates species absence

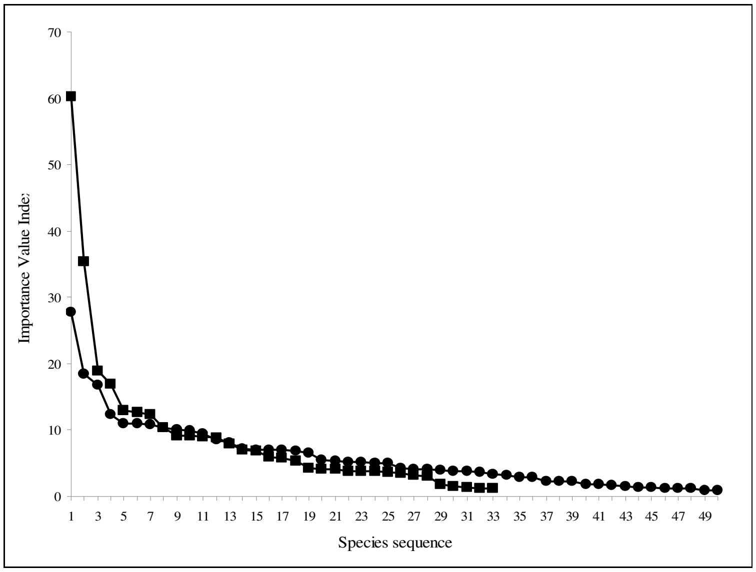

Figure 1. *IVI distribution among tress and shrubs in the disturbed (■) and undisturbed (●) forest stands*

Table 3. *Density of tree seedlings (plants ha^{-1} x 10^{3}) and saplings (plants ha-1) during peak growth period (August- September, 2005) in the undisturbed and disturbed forest stands*

Species	**Undisturbed**		**Disturbed**	
	Seedlings	**Sapling**	**Seedlings**	**Sapling**
A. petilorais	-	30	-	-
C. tribuloides	1.85	35	1.60	35
C. australis	0.10	10	0.40	30
C. glaucescens	0.25	30	0.85	25
C. obtusifoluim	0.45	15	-	15
C. zeylanicum	1.65	20	0.70	-
D. racemosus	-	-	0.10	-
D. macrocarpum	0.40	10	-	-
E. robusta	0.35	5	-	10
E. spicata	0.25	5	0.30	30
E. cumini	1.10	25	1.05	40
E. symplocina	-	-	0.15	10
L. indica	0.05	-	-	5
L. polyantha	-	5	-	-
M. indica	-	-	1.20	15
M. linifolia	0.75	20	-	-
O. peniculata	-	-	-	5
P. attenuata	-	-	-	5
R. wallichii	-	-	-	30
R. sucedánea	-	-	-	5
S. baccatum	-	15	-	-
S. wallichii	1.60	10	7.90	95
S. polysperma	0.15	-	-	-
S. serrulatum	-	-	-	10
S. saligna	-	-	0.20	-
W. grandis	-	5	0.80	50

- indicates species absence

Table 4. *Community indices of trees and shrubs in the two forest stands*

Community indices	Source of variation	Index value
Sorensen's similarity index	Trees	36.73
	Shrubs	41.18
Shannon's diversity index	Undisturbed	3.1461
	Disturbed	2.2881
Pielou's evenness index	Undisturbed	0.7565
	Disturbed	0.6488

Table 5. *DBH distribution of tree species in the undisturbed stand*

Species	**Family**	**Dbh class**						**Total**
		10-20	**21-30**	**31-40**	**41-50**	**51-60**	**61-70**	
A. obovata	Lauraceae	1	2	-	-	-	-	3
A. petiolaris	Lauraceae	3	3	-	2	-	-	8
C. arborea	Verbenaceae	1	2	-	-	-	-	3
C. indica	Fagaceae	-	-	-	2	-	-	2
C. tribuloides	Fagaceae	3	6	5	3	1	-	18
C. australis	Ulmaceae	1	2	-	-	-	-	3
C. glaucescens	Lauraceae	7	2	4	-	-	-	13
C. obtusifoluim	Lauraceae	3	5	1	-	-	-	9
C. zeylanicum	Lauraceae	3	2	1	-	-	-	6
D. toposia	Ebenaceae	-	-	1	-	-	-	1
D. racemosus	Anacardiaceae	1	-	-	-	-	-	1
D. macrocarpum	Meliaceae	4	2	-	-	-	-	6
D. procera	Meliaceae	1	1	-	-	-	-	2
E. robusta	Tiliaceae	1	5	2	-	-	-	8
E. spicata	Juglandaceae	-	1	2	-	-	-	3
E. bengalensis	Rosaceae	-	-	1	1	-	-	2
E. cumini	Myrtaceae	-	1	-	-	-	-	1
G. morella	Guttiferae	-	1	-	-	-	-	1
H. robusta	Proteaceae	3	2	-	-	-	-	5
I. godajam	Ilicineae	-	2	1	1	-	-	4
L. indica	Ampelidaceae	1	-	-	-	-	-	1
L. polyantha	Lauraceae	2	-	-	-	-	-	2
L. semecarpifolia	Lauraceae	1	2	-	-	-	-	3
M. indica	Euphorbiaceae	2	4	1	-	-	-	7
M. linifolia	Myristicaceae	1	2	1	-	-	-	4
P. attenuata	Lauraceae	-	1	-	1	-	-	2
Q. xylocarpa	Fagaceae	3	-	3	2	-	-	8
S.. baccatum	Euphorbiaceae	3	3	2	1	2	-	11
S. wallichii	Theaceae	2	3	-	2	-	-	7
S. polysperma	Styraceae	2	2	1	1	-	-	6
S. serrulatum	Styraceae	3	-	-	-	-	-	3
W. grandis	Rubiaceae	1	1	-	-	-	-	2
Total number of trees		**53**	**57**	**26**	**16**	**3**	**-**	**155**

Table 6. *DBH distribution of tree species in the disturbed stand*

Species	**Family**	**Dbh class**						**Total**
		10-20	**21-30**	**31-40**	**41-50**	**51-60**	**61-70**	
C. tribuloides	Fagaceae	4	3	1	2	1	1	12
C. australis	Ulmaceae	3	2	-	-	-	-	5
D. racemosus	Anacardiaceae	1	1	-	-	-	-	2
E. robusta	Tiliaceae	1	-	-	-	-	-	1
E. spicata	Juglandaceae	1	2	2	-	-	-	5
E. symplocina	Theaceae	3	4	-	-	-	-	7
F. prostrata	Moraceae	-	-	2	-	-	-	2
G. pentaphylla	Rubiaceae	1	1	-	-	-	-	2
L. polyantha	Lauraceae	2	1	-	-	-	-	3
M. indica	Euphorbiaceae	2	5	2	-	-	-	9
M. diversifolia	Rubiaceae	1	1	-	-	-	-	2
O. dioca	Oleaceae	1	-	-	-	-	-	1
R. wallichi	Rubiaceae	-	2	-	-	-	-	2
R. sucedanea	Anacardiaceae	1	1	-	-	-	-	2
S. wallichii	Theaceae	6	11	4	2	2	-	25
S. serrulatum	Styraceae	1	1	-	-	-	-	2
S. saligna	Myrtaceae	3	2	-	-	-	-	5
Total number of species		**31**	**37**	**11**	**4**	**3**	**1**	**87**

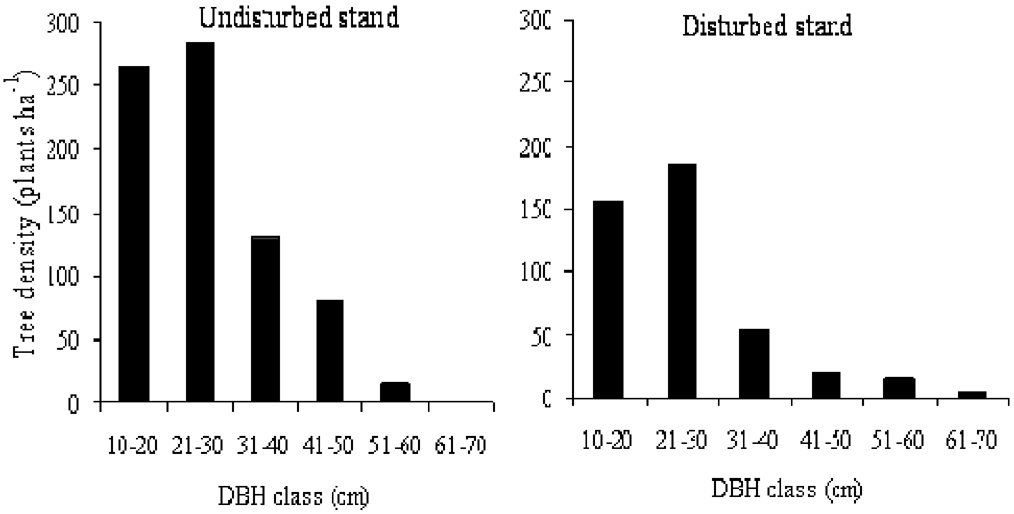

Figure 2*. Density-diameter distribution of trees in the two forest stands.*

***Table 7**. Species wise density diameter distribution of tree species in the two stands*

Species	**Undisturbed**					**Disturbed**					
	Dbh (cm)					**Dbh (cm)**					
	15	**25**	**35**	**45**	**55**	**15**	**25**	**35**	**45**	**55**	**65**
A. obovata	5	10	-	-	-	-	-	-	-	-	-
A. petiolaris	15	15	-	10	-	-	-	-	-	-	-
C. arborea	5	10	-	-	-	-	-	-	-	-	-
C. indica	-	-	-	10	-	-	-	-	-	-	-
C. tribuloides	10	30	25	15	5	20	15	5	10	5	5
C. australis	5	10	-	-	-	15	10	-	-	-	-
C. glaucescens	35	10	-	20	-	-	-	-	-	-	-
C.obtusifoluim	15	25	5	-	-	-	-	-	-	-	-
C. zeylanicum	15	10	5	-	-	-	-	-	-	-	-
D. toposia	-	-	5	-	-	-	-	-	-	-	-
D. racemosus	5	-	-	-	-	5	5	-	-	-	-
D.macrocarpum	20	10	-	-	-	-	-	-	-	-	-
D. procera	5	5		-	-	-	-	-	-	-	-
E. robusta	5	25	10	-	-	5	-	-	-	-	-
E. spicata	-	5	10	-	-	5	10	10	-	-	-
E. bengalensis	-	-	5	5	-	-	-	-	-	-	-
E. cumini	-	5	-	-	-	-	-	-	-	-	-
E. symplocina	-	-	-	-	-	15	20	-	-	-	-
F. prostrata	-	-	-	-	-	-	-	10	-	-	-
G. morella	-	5	-	-	-	-	-	-	-	-	-
G. pentaphylla	-	-	-	-	-	5	5	-	-	-	-
H. robusta	15	10	-	-	-	-	-	-	-	-	-
I. godajam	-	10	5	5	-	-	-	-	-	-	-
L. indica	5	-	-	-	-	-	-	-	-	-	-
L. polyantha	10	-	-	-	-	10	5	-	-	-	-
L. semecarpifolia	5	10	-	-	-	-	-	-	-	-	-
M. indica	10	20	5	-	-	10	15	10	-	-	-
M. diversifolia	-	-	-	-	-	5	5	-	-	-	-
M. linifolia	5	10	5	-	-	-	-	-	-	-	-
O. dioica	-	-	-	-	-	5	-	-	-	-	-
P. attenuata	-	5	-	5	-	-	-	-	-	-	-
Q. xylocarpa	15	-	15	10	-	-	-	-	-	-	-
R. wallichii	-	-	-	-	-	-	10	-	-	-	-
R. sucedanea	-	-	-	-	-	5	5	-	-	-	-
S. baccatum	15	15	10	5	10	-	-	10	-	-	-
S. wallichii	10	15	-	10	-	30	55	20	-	10	-
S. polysperma	10	10	5	5	-	-	-	-	-	-	-
S. serrulatum	15	-	-	-	-	5	5	-	-	-	-
S. saligna	-	-	-	-	-	15	10	-	-	-	-
W. grandis	5	5	-	-	-	-	-	-	-	-	-

- indicates species absence

Discussion

Disturbance whether of natural or anthropogenic activities, leads to creation of different niches for the establishment and onward growth of tree seedlings. The results of the present study are in conformity with the findings of [48], in general, that community composition changes with disturbance. The importance of disturbance for maintaining community composition has been studied in a variety of ecosystems [15, 26, 41]. In many of these systems either increasing or decreasing disturbance changes overall community structure [54, 60]. In both stands species diversity and richness is very high as compared to other tropical forests [47].

Treefall gaps, created due to lopping and felling of trees for fuel wood, fodder forage and grazing [32], offer specialized regeneration conditions to prevailing spatial and microenvironmental heterogeneity [3]. Canopy-gaps, created by disturbances, and resulting spatial variability in under-storey light conditions have played a prominent role in expansion of forest diversity [7, 53, 57]. Such heterogeneities have been considered by many workers to be of fundamental importance in the maintenance and promotion of high tree diversity in tropical forest communities [2, 14, 28]. Our study, however, doesn’t have conformity with these views. The undisturbed stand showed higher species richness and diversity as compared to that of the stand undergoing disturbance, as far as tree populations are concerned. Density and frequency are also higher in the undisturbed stand. The findings are, rather, in conformity with the view held by [10], who viewed disturbance as a negative force that destroys climax assemblages and brings instability in the system. The results also conforms the findings of [48], who found that species diversity and abundance markedly declined from undisturbed to the disturbed stands, in their study on the community composition and tree population structure of three forest stands of different degree of disturbance in the sub-tropical broad-leaved forest of Meghalaya, India.

Theoretical analyses often predict a peak in species diversity at intermediate disturbance intensity or frequency [28], although characteristic of the disturbance and the system may influence this response [44]. Thus, the disturbed stand of the present study can be considered as of high disturbance intensity as it contains lower species diversity and tree density particularly of intermediate girth class. It may be argued that the characteristics of the system and type of disturbance might be responsible for this trend [48].

Variation in the density of forest understories across sites is important for the maintenance of tree diversity as they can compete with the seedlings of trees [25, 65, 67] and could lead to ambiguous evidence for the importance of canopy gaps in maintaining tree diversity [5, 23]. [2], in their study on changes in species richness and abundance with experimental disturbance in old field plant communities, observed that the impact of disturbance on species richness depends largely on the nature of the dominance and rates of successional or seasonal change of the community. They also concluded that disturbances have positive impact in maintaining species richness in herbaceous systems.

Herbaceous species increased in number in the disturbed stand, and outnumbered the tree and shrub species in the disturbed stand supporting the view of [2]. [62] found that space created by small canopy gaps and environmental historical factors were of comparable importance for controlling herb species composition and distribution while space was of somewhat greater importance for woody plants, and that the past human land use hand a strong impact on species composition on the Barro Colorado Island of

Panama. [17] also described that the allocation and growth of herbaceous plants can be altered by previous land use, in their experiment with 12 herbaceous plant species in southern Appalachian forest stands of USA. Thus, the higher total species diversity and lower tree species composition in the disturbed stand may be attributed to the stand characteristic allowing the light-demanding herbaceous species to increase, and in turn suppressing the tree seedlings due to competition with the herbaceous species.

The presence of certain pioneer tree species, which are expected to be abundant only in the disturbed or degraded sites (e.g., *Schima wallichii*), in the undisturbed stand may be due to similarity of the stand with the disturbed stand, enhancing the species to invade and establish in it, in the past though no such record has been found. According to [24], exotic species tended to invade the biodiversity '*hot spots'* rich in native species, at a higher degree due to similarity of two sites in resource availability [61], propagule supply [34], disturbance in the past [8], or internal heterogeneity [13]. The presence of pioneer species in undisturbed stand may also be explained by the abundance-occupancy relationship of [18], who described that species increasing in abundance tend to increase in occupancy. *Schima wallichii* have high abundance in the vicinity of the undisturbed stand, and thus have high chance of occupying the undisturbed stand. The undisturbed stand shows high trees and shrubs diversity, where as herbaceous communities are lesser in number as compared to the disturbed stand. The predominance of herbaceous species in the disturbed stand could be attributed to the higher penetration of sun light in the forest floor. The diameter distribution of trees has often been used to represent population structure of forests [31, 40, 51].

Straight line relationship between density and diameter (negative exponential curve) in both stands is in conformity with that of [48, 52]. However, the lower density of lower diameter class (10-20 cm dbh) as compared to intermediate girth class (21-30 dbh) give the appearance of a positively skewed distribution curve, in both stands and can be explained as the result of selective felling of lower as well as higher girth classes in the disturbed stand, while in the undisturbed stand it can be interpreted as due to low tree mortality and lower removal rate across the intermediate diameter classes [51, 66] and lower recruitment of component tree species. The lower density of the higher dbh classes of trees in the undisturbed stand, as compared to intermediate or lower dbh classes, can be attributed to the relatively high mortality of large canopy trees [20, 35]. The above characteristics of girth classes also suggests that the forest is still growing and yet to reach to its climax stage as it is only of about 25 years old forest. The argument is further supported by the presence of highest girth class trees in the disturbed forest stand which might have been a climax forest prior to disturbance.

The present study reveals that the anthropogenic disturbance causes disruption of forest structure and changes species composition which ultimately leads to reduction of tree species, density and frequency which is a major forest component. The increase in species number, density and frequency of herbaceous community due to disturbances causes reduction of tree seed ling establishment growth which ultimately leads to reduction of trees with lower girth class. Thus, it can be suggested that proper management strategies can be adopted for judicious utilization of forest resources including controlled selective felling may reduce the forest degeneration process and enhance sustainable forest production.

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REFERENCES

[1] Anonymous (2006): Statistical Handbook of Mizoram. – Government of Mizoram.

[2] Armesto, J.J., Pickett, S.T.A. (1985): Experiments on disturbance in old field plant communities: Impact on species richness and abundance. – Ecology 66: 230-240.

[3] Barik, S.K., H.N. Pandey, R.S. Tripathi, Rao, P. (1992): Microenvironmental variability and species diversity in treefall gaps in a sub-tropical broadleaved forest. – Vegetatio 103: 31-40.

[4] Beard, J.S. (1944): Climax vegetation in tropical America. – Ecology 25: 127-158.

[5] Beckage, B., Clark, J.S., Clinton, B.D., Haines, B.L. (2000): A long-term study of tree seedling recruitment in Southern Appalachian forests: the effects of canopy gaps and shrub under-stories. – Canadian Journal of Forest Research 30: 1617-1631.

[6] Bennett, L.T., Adams, A. (2004): Assessment of ecological effects due to forest harvesting: approaches and statistical issues. – Journal of Applied Ecology 41: 585-598.

[7] Brokaw, N., Busing, R.T. (2000): Niche versus chance and tree diversity in forest gaps. – Trends in Ecology and Evolution 15: 183-188.

[8] Brown, R.L., Pete, R.K. (2003): Diversity and invisibility of Southern Appalachian plant communities. – Ecology 84: 32-39

[9] Champion, H.G., Seth, S.K. (1968): A revised survey of the forests types of India. – Government of India Publication, Delhi. pp. 251-267

[10] Clements, F.E. (1936): Nature and structure of the climax. – Journal of Ecology 24: 252-284.

[11] Condit, R., Aguilar, S., Hernandez, A., Perez, R., Lao, S., Angehr, G., Hubbell, S.P., Foster, R.B. (2004): Tropical forest dynamics across a raifall gradient and the impact of an El Ninodry season. – Journal of Tropical Ecology 20: 51-72.

[12] Curtis, J.T. (1953): The Vegetation of Wisconsin: An Ordination of Plant Communities. – University of Wisconsin Press, Madison, pp 657.

[13] Davies, K.F., Chessons, P., Harrison, S., B Inouye,.D., Melbourne, B.A., Rice, K.J. (2005): Spatial heterogeneity explains the scale dependence of the native exotic diversity relationship. – Journal of Ecology 86: 1602-1610.

[14] Denslow, J.S. (1980): Gap partitioning among tropical rain forest trees. – Biotropica 12: 47-55.

[15] Elderd, B.D. (2003): Changing flow regimes: its impacts on riparian vegetation and a common Riparian species, Mimulus guttatus. – Ecological Applications 13: 1610-1625.

[16] Elderd, B.D., Doak, D.F. (2006): Comparing the direct and community-mediated effects of disturbance on plant population dynamics: flooding, herbivory and Mimulus guttatus. – Journal of Ecology 94: 656-669.

[17] Fraterrigo, J.M., Turner, M.G., Pearson, S.M. (2006): Previous land use alters plant allocation and growth in forest herbs. – Journal of Ecology 94: 548-557.

[18] Gaston, K.J., Blackburn, T.M., Greenwood, J.J.D., Gregory, R.D., Quinn, R.M., Lawton, J.H. (2000): Abundance occupancy relationship. – Journal of Applied Ecology 37(1): 39-59.

[19] Gentry, A.H. (1982): Patterns of neotropical plant species diversity. – In: Hecht, M.K, Wallace, B., Prance, G.T. (eds.), Evolutionary Biology, Plenum Press, New York, NY, USA, Vol. 15. pp. 1-84.

[20] Goff, F.G., West, D.C. (1975): Canopy-under storey interaction effects on forest population structure. – Forest Science 21: 98-108.

[21] Gross, K., Lockwood, J.R., Frost, C.C., Morris, W.F. (1998): Modelling controlled burning amd trampling reduction for conservation of Hudsonia Montana. – Conservation Biology 12:1291-1301.

[22] Grubb, P.J. (1977): The maintenance of species richness in plant communities: the importance of the regeneration niche. – Biological Review 52: 107-145.

[23] Gutierrez, A.G., Armesti, J.J., Aravena, J.C. (2004): Disturbance and regeneration dynamics of an old-growth North Patagonian rain forest in Chiloe Island, Chile. – Journal of Ecology 92: 598-608.

[24] Harrison, S., Grace, J.B. Davies, K.F., Safford, H.D., Viers, J.H. (2006): Invasion in a diversity hotspot: exotic cover and native richness in the Californian serpentine flora. – Ecology, 87(3): 695-703.

[25] HilleRisLambers, J., Clark, J.S. (2003): Effects of dispersal, shrubs and density-dependent mortality on seed and seedling distributions in temperate forests. – Canadian Journal of Ecology 33: 783-795.

[26] Hobbs, R.J., Mooney, H.A. (1991): Effects of rainfall variability and gopher disturbance on serpentine annual grassland dynamics. – Ecology 72: 59-68.

[27] Hooker, J.D. (1872-97): The Flora of British India, 7 Vols. – London.

[28] Huston, M.A. (1979): A general hypothesis of species diversity. – American Naturalist 113: 81-101.

[29] Kanjilal, U.N., Kanjilal, P.C., Das, A., De, R.N., Bor, N.L. (1934-40): Flora of Assam. 5 Vols. – Government Press, Shillong.

[30] Kershaw, K.A. (1973): Quantitative and Dynamic Plant Ecology. 2nd. ed. – New York, Elsevier.

[31] Khan, M.L., Rai, J.P.N., Tripathi, R.S. (1987): Population structure of some tree species in disturbed and protected subtropical forests of northeast India. – Acta Oecologia 8: 247-255.

[32] Kumar, M., Sharma, C.M., Rajwar, G.S. (2004): A study on the community structure and diversity of a sub-tropical forest of Garwhal Himalaya. – Indian Forester 130(2): 207-214.

[33] Kwit, C., Platt, W.J. (2003): Disturbance history influences regeneration of non- pioneer under storey trees. – Ecology 84(10): 2575-2578.

[34] Levine, J. (2000): Species diversity and biological invasions relating local process to community pattern. – Science 288: 852-854.

[35] Lorimer, C.G., Dahir, S.E., Nordhiem, E.V. (2001): Tree mortality rates and longetivity in mature and old growth hemlock-hardwood forests. – Journal of Ecology 89: 960-971.

[36] Lubchenco, J. (1978): Plant species diversity in a marine inter-tidal community: importance of herbivore food preference and algal competative abilities. – American Nature 112: 23-29.

[37] Menges, E.S. (1990): Population viability analysis for an endangered plant. – Conservation Biology 4: 52-62.

[38] Misra, R. (1968): Ecology work book. – Oxford and IBH, Calcutta.

[39] Mueller-Dombois, D., Ellenberg, H. (1974): Aims and Methods of Vegetation Ecology. – John Wiley and Sons, Inc., New York.

[40] Newton, P.F., Smith, V.G. (1988): Diameter distributional trends within mixed Black-spruce/Balsam-fir and pure Black spruce stand types. – Forest Ecology and Management 25: 123-138.

[41] Paine, R.T., Levin, S.A. (1981) Intertidal landscapes: disturbance and the dynamics of pattern. – Ecological Monographs 51: 145-178.

[42] Paine, R.T. (1966): Food web complexity and species diversity. – American Naturalist 100: 65-75.

[43] Pascarella, J.B., Horvitz, C.C. (1998): Hurricane disturbance and the population dynamics of a tropical under-storey shrub: Megamatrix elasticity ananlysis. – Ecology 79: 547-563.

[44] Peet, R.K., Glenn-Lewin, D.C., Wolf, J.W. (1983): Prediction of man's impact on plant species diversity. A challenge for vegetation science. – In: Holzner W, MJA Werger & I. Ikusima (eds.), Man's impact on vegetation. Dr. W. Junk, The Hague, The Netherlands, pp. 41-54.
[45] Pickett, S.T.A. (1980): Non-equilibrium coexistence of plants. – Bulletin of Torrey Botanical Club 107: 238-248.
[46] Pielou, E.C. (1966): The measurement of diversity in different types of biological collections. – Journal of Theoretical Biology 13: 131-144.
[47] Prance, G.T., Beentje, H., Dransfield, J., Johns, R. (2000): The tropical flora remains under collected. – Annals of the Missouri Botanical Garden 87: 67-71.
[48] Rao, P., Barik, S.K., Pandey, H.N., Tripathi, R.S. (1990): Community composition and tree population structure in a sub-tropical broad-leaved forest along a disturbance gradient. – Vegetatio 88: 151-162.
[49] Rao, P., Barik, S.K., Pandey, H.N., Tripathi, R.S. (1997): Tree seed germination and seedling establishment in treefall gaps and understorey in a sub-tropical forest of North-East India. – Australian Journal of Ecology 22: 136-145.
[50] Sahoo, U.K., Tomar, J.M.S., Upadhyaya, K. (2008): Phytosociological analysis of Pinus kesiya stands exposed to varying intensities of disturbance at Umiam Watershed in Northeast India. – International Journal of Ecology and Environmental Sciences 34(4): 337-346.
[51] Saxena, A.K., Singh, S.P., Singh, J.S. (1984): Population structure of forests of Kumaun Himalaya: implications for management. – Journal of Environment Management 19: 307-324.
[52] Schmeiz, D.A., Lindsey, A.A. (1965): Size-class structure of old growth forests in Indiana. – Forest Science 11: 258-264.
[53] Schnitzer, S.A., Parren, M.P.E., Bongers, F. (2004): Recruitment of Lianas into logging gaps and the effects of pre-harvesting climber cutting. – Forest Ecology and Management 190: 87-98.
[54] Shaforth, P.B., Stromberg, J.C., Patten, D.T. (2002): Riparian vegetation response to altered disturbance and stress regimes. – Ecological Applications 12: 107-123.
[55] Shannon, C.E., Wienner, W. (1963): The Mathematical Theory of Communication. – University of IIIinois, Urana, III, p.125.
[56] Shimwell, D.W. (1971): The description and classification of vegetation. – Sidgwick and Jackson Ltd, London.
[57] Shugart, H.H. (1984): A Theory of Forest Dynamics: the Ecological Implications of Forest Succession Models. – Springer-Verlag, Berlin.
[58] Smith, M., Caswell, H., Mettler-Cherry, P. (2005): Stochastic flood and precipitation regimes and the population dynamics of a threatened floodplain plant. – Ecological Applications 15: 1036-1052.
[59] Sorensen, T. (1948): A method of establishing groups of equal amplitude in plant sociology based on similarity of species content. – Det. Kong. Danske Vidensk. Selsk BiologySkr (Copenhagen) 5: 1-34.
[60] Sousa, W. (1979): Disturbance in marine intertidal boulder fields: the monequillibrium maintenance of species diversity. – Ecology 60: 1225-1239.
[61] Stohlgren, T.J., Binley, D., Chong, G.W., Kalkhan, M.A., Schell, L.D., Bull, K.A., Otsuki, Y., Newman, G., Bashkin, M., Son, Y. (1999): Exotic species invade hot spots of native plant diversity. – Ecological Monographs 69: 25-46.
[62] Svenning, J.C. (2000): Small canopy gaps influence plant distribution in the rain forest understory. – Biotropica 32: 252-261.
[63] UNESCO/UNEP/FAO (1978): Tropical forest ecosystems: A state-of-knowledge report. – Paris, UNESCO.
[64] Valencia, R., Foster, R.B., Villa, V., Condit, R., Svenning, J.C., Hernandez, H., Romoleroux, K., Losos, E., Magard, E., Balslev, H. (2004): Tree species distributions and

local habitat variation in the Amazon: large forest plot in eastern Ecuador. – Journal of Ecology 92: 214-229.

[65] Veblen, T.T. (1982): Growth patterns of *Chusquea* bamboos in the understorey of Chilean *Notho-fagus* forests and their influences in forest dynamics. – Bulletin of the Torrey Botanical Club 109: 474-487.

[66] West, D.C., Shugart, H.H. Jr., Ranny, J.W. (1981): Population structure of forest over a large area. – Forest Science 27: 701-710.

[67] Yamamoto, S., Nishimura, N., Matsui, K. (1995): Natural disturbance and tree species co-existence in an old-growth beech-dwarf bamboo forest, southwestern Japan. – Journal of Vegetation Science 6: 875-886.

THE EFFECT OF SALINITY ON THE GROWTH OF THE HALOPHYTE *ATRIPLEX HORTENSIS* (CHENOPODIACEAE)

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Abstract. The effect of salt stress on the growth of the Atriplex hortensis was determined by growing plants in soils receiving a one-time irrigation of nutrient solution containing low, medium, and high levels of NaCl. Atriplex plants were grown in sand culture under controlled greenhouse conditions. The experiment was compared to two varieties of A. hortensis. Growth and yield were progressively declined by increasing NaCl concentrations. Atriplex hortensis, an annual halophyte, exhibits significant reduction in height and biomass under saline stress conditions. The photosynthetic activity decreases when plants are grown under saline conditions leading to reduced growth and productivity. Low levels of salinity (5 g/l NaCl) did not cause substantial inhibition of growth but increasing concentrations of salt induced a progressive decline in length and weight of the plants. Salt stress induced a significant decrease in leaf area, but it had no significant effect on leaf water content. The results of present study indicate no differences in salinity tolerance in both Atriplex plants. Another possible conclusion is that improved tolerance to salt stress may be accomplished by decline in growth and photosynthetic activity. Based on these findings the tolerant Atriplex can be grown in moderately NaCl-contaminated soils.

Keywords: *Atriplex; salt stress; biomass production; chlorophyll concentration; water content; stomatal conductance.*

Introduction

Salinity is one of the most important environmental factors limiting crop production of marginal agricultural soils in many parts of the world. Salinity effects on plants include ion toxicity, osmotic stress, mineral deficiencies, physiological and biochemical perturbations, and combinations of these stresses [31, 67, 68, 74, 102]. Salt ion toxicity has numerous deleterious effects on plants such as denaturing cytosolic enzymes [68]. Many investigations on quantification of salt tolerance of plant species have been based on experiments in which NaCl was the predominant salt [18, 42, 44]. Salt stress affects many aspects of plant metabolism and, as a result, growth and yields are reduced. Excess salt in the soil solution may adversely affect plant growth either through osmotic inhibition of water uptake by roots or specific ion effects. High concentrations of salts have detrimental effects on plant growth [23, 63] and excessive concentrations kill growing plants [20]. Halophytes are distinguished from glycophytes by their tolerance of saline conditions. The use of halophytic plants in pasture and fodder production on saline soils is the only economically feasible solution available [40]. Halophytes are plants that grow naturally in saline environments, such as salt marshes, salt spans and salt deserts [36]. Halophytic species differ widely in the extent to which they accumulate ions and their overall degree of salt tolerance [25, 26]. *Atriplex* species (saltbushes) are dominant in many arid and semi-arid regions of the world, particularly in habitats that combine relatively high soil salinity with aridity [60, 75, 77]. Several species belonging to the genus Atriplex are well adapted to harsh environmental conditions and therefore constitute a useful material for the identification of physiological mechanisms and genes involved in abiotic stress resistance [14, 90, 100]. Atriplex is a halophyte saltbush species highly resistant to drought [50], salinity [7], and heavy-metal stress [55]. Atriplex spp. are among a group of halophytes that complete their life cycle at high salinity levels and have the ability to accumulate high concentrations of micronutrients much greater than the required minimum [82, 101]. Atriplex spp. has increased biomass production with salt increments in the growth medium ranging from 5 to 10 g l^{-1} NaCl [41, 72]. A similar promotion of growth has also been reported for other halophytic species [9, 18].

It is suggested that *Atriplex spp*. may be more suitable for revegetating very saline soils and also be a good source of productive feed [33, 76].

These plants could be promising, since *Atriplex* species have special bladders in the leaves that act as salt sinks for the removal of the excess of salt [47]. In the arid zones and other dry lands, halophytic plants often dominate because of their tolerance to drought and salinity [24]. *Atriplex* spp. are among the most salt-tolerant higher plants. They have adapted to salinity by tolerating salts internally and/or by excreting salt [41, 62]. However, the presence of high salt levels does not seem to be required for optimal growth. It is reported that soil salinity suppresses shoot growth more than the root growth [57, 81]. [23] reported that salinity causes reduction in leaf area as well as in rate of photosynthesis, which together result in reduced crop growth and yield. Also, high concentration of salt tends to slow down or stop root elongation [45] and causes reduction in root production [23].

In general, low salinity levels do not appear to have a deleterious effect on the growth of *Atriplex* spp. and may actually stimulate growth [3, 15, 59, 104]. However, high salinity levels may cause a reduction in total growth of *Atriplex* spp., especially in leaf biomass [6, 28, 66, 80, 85, 95, 97].

In this research, growth parameters such as dry mass, leaf area, plant height and root elongation of *Atriplex* plant were determined in order to get a general view of the effects of salinity on the overall growth of *A. hortensis.* Chlorophyll concentration, water content and stomatal conductance of plants were measured in order to understand how salinity affects the physiology of plants. Among Chenopodiaceae the genus *Atriplex* is the most studied, probably because *Atriplex* species are used for rehabilitation of saline soils.

Materials and Methods

Plants

Seeds of *Atriplex hortensis* were taken from a botanic garden: Denmark House, Pymoor, Ely, Cambridgeshire (CN seeds). Plants were grown in a greenhouse at 28/20 °C (day/night) under a photoperiod of 16 h.

Atriplex seeds were planted in pots of 14 cm diameter and 25 cm depth; each pot contained 3,5 kg soil. The soil characteristics were as follows: sandy loam in texture, sand 52.3%; silt 10.5%; clay 12%; pH 8.75 and organic matter 1.5%. Ten seeds per pot and six replicates were used for each treatment. Irrigation was applied on a two days basis to achieve soil water field capacity level. Treatments were as follows:

Control and Salt Treatment:
a- control (0 NaCl) b- 5 g/l NaCl
c- 10 g/l NaCl d- 15 g/l NaCl

Growth parameters of Atriplex

The plant height (cm), root system length (cm) and leaf area (cm^2) were measured (after 3 months). Plants were washed with distilled water and separated into shoots and roots. The dry weight (dw) was obtained after oven drying the plants at 60 °C for 48 hours. The dry weights of the root and shoot systems were also determined.

The effect of salinity on physiology parameters was studied in terms of water content, chlorophyll concentration and stomatal conductance.

Plant pigments

Chlorophyll a and b were estimated spectrophotometrically [64], after acetone extraction of the pigments from fresh leaves. Chlorophyll concentration was determined with four replicate plants. A leaf sample of 0.1 g was ground and extracted with 5 mL of 80% (v/v) acetone in the dark. The slurry was filtered and absorbancies were determined at 645 and 663 nm.

Statistical Analysis

The pot experiment was set up in randomized complete block design replicated five times. ANOVA (SAS version 10.0) was employed for statistical analysis of data. Statistical significance was defined as $P < 0.05$

Results

Effect of salinity on leaf growth

Increasing NaCl concentrations in the irrigation solution significantly decreased total leaf area of both *Atriplex* varieties (*Fig. 1*). The leaf area of both varieties was nearly equal concentration in green and red, respectively. At 15 g/l NaCl total leaf area was reduced by 13% (red) and 47% (green) compared with the controls.

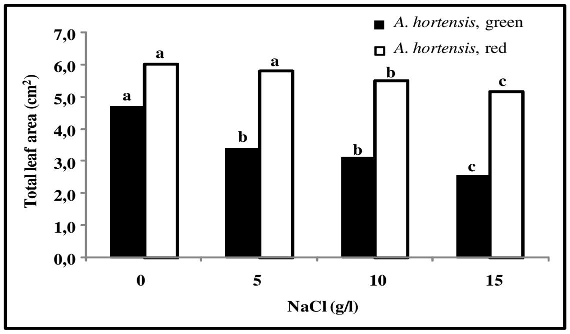

Figure 1. *The effect of salinity on total leaf area in the two Atriplex varieties green and red. Different letters represent a significant difference (P<0.05) between treatment*

Effect of salinity on dry weight

Shoot dry weights at all stages of development were reduced progressively with increasing NaCl concentrations while reversibly.

Increasing concentration of salt in soil significantly retarded ($p < 0.05$) dry weight of stems and roots *(Table. 1).* Dry weight significantly decreased ($p < 0.05$) for shoots (leaves + stems), roots and total biomass of plants in response to increasing concentration of salt *(Table. 1).* Percentage relative weight of tissues of salinized plants compared to those of control plants were computed as (salinized tissue dry weight/control dry weight) x100. Dry weight values of tissues given in Tab. 1 were used for the calculation of percentage relative weight of tissues. Values of percentage relative weight varied from 88 to 52 % for shoots, from 80 to 39 % for roots and from 87 to 50 % for total biomass in response to increasing soil salinity from 5 to 15 g/l. In experiment, dry plant weight decreased dramatically with the increasing NaCl concentration. The greatest dry plant weight of *Atriplex* was obtained with the first treatment in all range of salinity treatments. The general tendency was that increasing concentrations of salt induced a progressive decline in the length of shoots and in the weight of roots, stems and leaves.

Table 1. *Effect of salinity on root and shoot dry weights of Atriplex hortensis. Different letters represent a significant difference (P<0.05) between treatments.*

Treatment	**Shoot**		**Root**		**Total weight**	
	(g/plant)	(%)	(g/plant)	(%)	(g/plant)	(%)
***A hortensis* varieties green**						
0 g NaCl/l	14,545 a	100	2,253 a	100	16,798 a	100
5 g NaCl/l	12,838 b	88	1,758 b	78	14,596 b	87
10 g Nacl/l	9,702 c	67	1,295 c	57	10,997 c	65
15 g NaCl/l	7,538 d	52	0,889 d	39	8,426 d	50
$LDS_{0,05}$	0,278	-	0,066	-	0,271	-
***A hortensis* varieties red**						
0 g NaCl/l	14,355 a	100	2,091 a	100	16,447 a	100
5 g NaCl/l	12,538 b	87	1,680 b	80	14,218 b	86
10 g Nacl/l	10, 139 c	71	1,301 c	62	11,440 c	70
15 g NaCl/l	7,460 d	52	0,936 d	45	8,396 d	51
$LDS_{0,05}$	0,247	-	0,054	-	0,247	-

Mean from five replicate pots are given and different letters in the same column are significantly different at the level of 0.05.

Effect of salinity on chlorophyll concentration

Salinity stress (5, 10 and 15 g/l NaCl) resulted in significantly progressive decline the photosynthetic pigments (chlorophyll a and b). Similarly, Chl b concentration decreased with 20% at NaCl concentrations of 5 g/l. Red variety had significantly more Chl a and Chl b than green variety at all NaCl concentrations. The strongest effect of salt occurred with two varieties between 10 and 15 g/l NaCl.

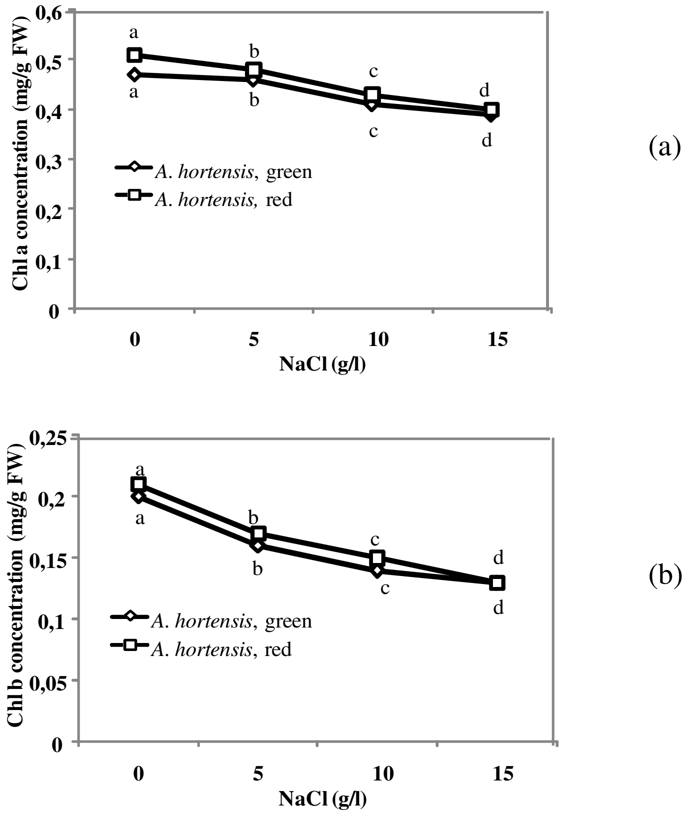

Figure 2. *Effects of increasing NaCl concentration in the irrigation on chlorophyll a (a) and chlorophyll b (b) in the two Atriplex varieties green and red.*

Effect of salinity on growth

Plant height decreased with increasing salinity for the both varieties (*Fig. 3a*). Plant height decreased in the plants supplemented with NaCl and their rate of loss was proportional to the concentration of the NaCl. Stem height decreased rapidly at 100 cm in 15 g/l NaCl concentration for two varieties. The plant height of both varieties was nearly equal at all NaCl concentration, suggesting no difference in varietal response to NaCl.

Increasing concentration of salt in soil significantly retarded ($p < 0.05$) elongation of stems and roots (*Fig. 3b*). Root growth of two *Atriplex* varieties responded differently to increasing salinity. As salinity level increased from control (0 g/l) to (15 g/l), green variety exhibited a trend of increasing in root mass. In contrast, root elongation of red variety decreased linearly with increasing salinity.

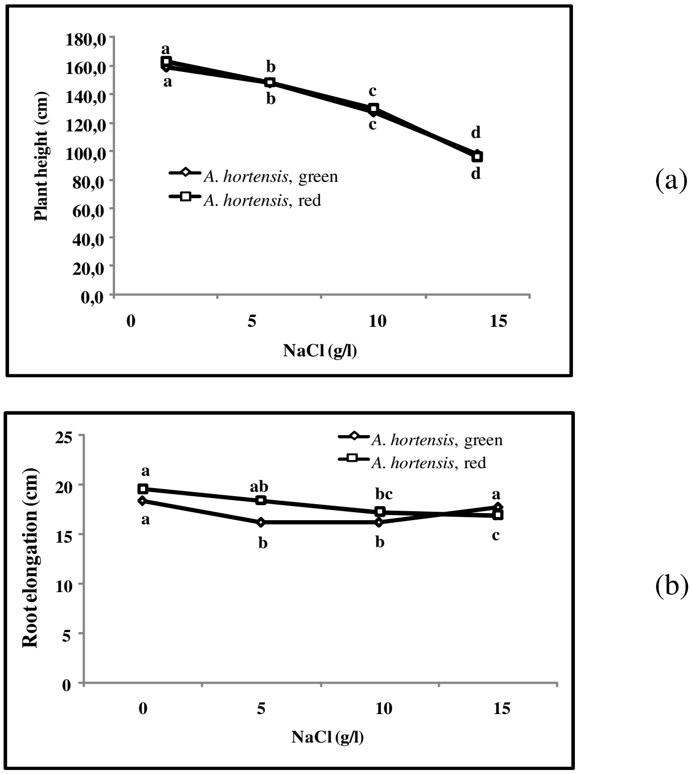

Figure 3. *Effects of increasing NaCl concentration in the irrigation on plant height (a) and root elongation (b) in the two Atriplex varieties green and red*

Effect of salinity on water content

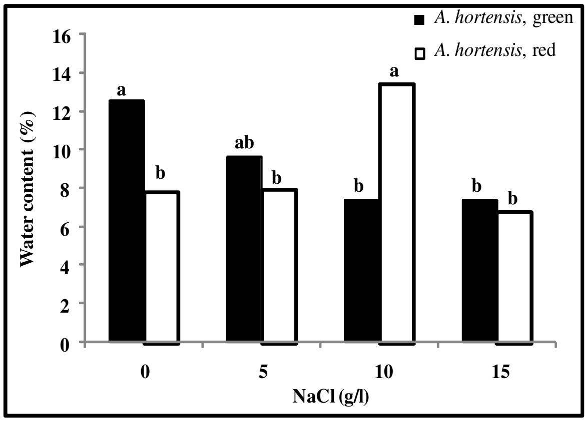

Figure 4. *The effect of salinity on total leaf area in the two Atriplex varieties green and red. Different letters represent a significant difference (P<0.05) between treatments*

The water content of leaves of *A. hortensis* red variety was highest in plants grown at 10 g/l NaCl, followed by the leaves grown at 5 g/l and the lowest water content was in leaves grown at 15 g/l NaCl *(Fig. 4)*. Further increase in salinity caused a reduction in water content to a very low value at higher salinities.

Effect of salinity on stomatal conductance

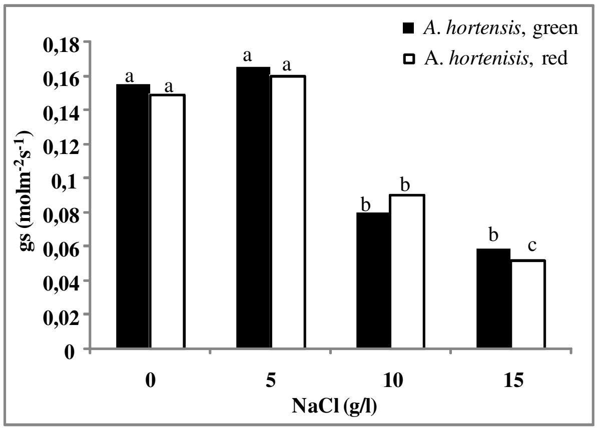

Figure 5. *Effects of increasing NaCl concentration on stomatal conductance of the two Atriplex hortensis varieties green and red.*

Stomatal conductance *(Fig. 5)* of both varieties decreased by about 50% with increasing external NaCl concentration. Mean stomatal conductance (*g*s) measures ranged from 0,16 molm^{-2} s^{-1} in low salinity treatments up to 0,06 molm^{-2} s^{-1} in high salinity treatments.

Discussion

Atriplex hortensis is highly tolerant of salinity. Halophytic *Atriplex* species show stimulation of growth at NaCl concentrations that are inhibitory to non-halophytes [77]. Saline soils and saline irrigations constitute a serious production problem for vegetable crops as saline conditions are known to suppress plant growth [87]. Increases in salinity lead to a reduction and/or delay in growth of both halophyte and glycophyte plants [37, 39, 96]. An important finding was that plants could survive at 15 g l^{-1} salinity and this might be a suitable concentration to use to select highly salt-tolerant plants. Studies have shown that *Atriplex* spp., such as *A. nummularia*, *A. griffithii* and *A. hortensis*, could survive under highly saline conditions; with optimal growth occurring at 5 to 10 gl^{-1} NaCl [41, 82, 101]. Other researchers have not observed growth stimulation by NaCl in *S. virginicus* [12, 70, 71].

Halophytes such as *Atriplex* spp. show a stimulation of growth at NaCl concentrations that are inhibitory to the growth of non-halophytes [77]. [3] showed that the growth of both *Atriplex inflata* F. Muell. and *A. nummularia* Lindl. was greater at 600 mm NaCl than in nutrient controls. *Atriplex* spp. vary in their degree of salt tolerance [80]. *Atriplex halimus* L. had the least decrease in dry mass production (40%) at 750 mm NaCl, whereas *A. calotheca* (Rafn.) Rafn. and Fries. (67%) and *A. nitens* Schkuhr (80%) had greater decreases but all three species were able to survive in this salt treatment [80]. [93] determined that *A. spongiosa* F. Muell. was able to grow in medium containing over 600 mm NaCl, with dry mass production decreasing by 50% at 800 mm NaCl. Other chenopod halophytes, such as *Halosarcia pergranulata* [91], *Suaeda fruticosa* [41] and *Sarcocornia fruticosa* [84], have growth optima at moderate to high salinities.

In general, low salinity levels do not appear to have a deleterious effect on the growth of *Atriplex* spp. and may actually stimulate growth [3, 15, 59]. Many species of *Atriplex* are valued as livestock forage when herbage availability is low especially in arid environments and salt-affected area [34] because they have high content of crude protein, vitamins (A, C and D) and minerals such as chromium [61, 86].

In this study we show that the presence of medium-high concentrations of NaCl in the growth solution induced more deleterious effects on growth. These results fit with those previously reported in *Atriplex prostrata* [21]. Most species tested had their maximum biomass production in low salinity treatments. These results broadly match those obtained by [17, 25, 27, 29, 30, 38] who reported that low NaCl concentrations stimulate growth of some halophytic species, but an excess of salt decreases growth and biomass production. [56] reported that in most halophytic species growth decreases gradually with the increase of salt rate in the culture medium above a critical threshold specific to each species. Reduction of plant growth under saline conditions is a common phenomenon [4] but such reduction occurs differently in different plant organs. For example, in the present experiment, root dry weight was reduced more than shoot dry weight by salt stress. In contrast, [35] observed that salt stress inhibited the growth of shoot more than root in *Brassica* species.

Succulence is anatomical adaptation, which, by increasing the vacuolar volume, permits the accumulation of large amounts of water and dissolved ions in both shoots and roots [68]. Exposure to salinity concentrations has been shown to increase the tissue water content of halophytes [42, 51]. In halophytes, water content and the ability to make osmotic adjustments have been seen as important determinants of growth response [9, 10, 46, 58]. In the present study salinity stress resulted in decreased chlorophyll content. Saline stress led to leaf chlorosis, which ultimately resulted in significant photoinhibition and photodestruction of chlorophyll pigments. Similar results have been reported for other legumes [1, 92, 94].

The effect of salt stress on *Atriplex* leaf growth and shoot development is similar to its effect on sorghum [11] and lettuce [49]. Measurements of chlorophyll fluorescence provide quantitative information about photosynthesis through noninvasive means [98]. Salinity responses of chlorophyll a fluorescence have been studied in barley (*Hordeum vulgare* L.), [8, 48], rice (*Oryza sativa* L.), [54] and sorghum [53, 88, 89]. An increase in chlorophyll content has been thought to be due to the accumulation of NaCl in the chloroplast [43]. [65] also concluded that salt stress induced an increase in the chlorophyll content, which could be due to an increase in the number of chloroplasts in stressed leaves. The decreased in chlorophyll content under salinity stress is a commonly reported phenomenon and in various studies, because of its adverse effects on membrane stability [2, 5]. [73] found that the chlorophyll content reduction of leaves started to occur in plants grown at 100 mM NaCl and higher concentrations. These reductions could be attributed to the effect of salinity that causes inhibition of synthesis of chlorophyll or accelerating its degradation [83].

In general, salinity can reduce plant growth or damage the plants through: (i) osmotic effect (causing water deficit), (ii) toxic effects of ions and (iii) imbalance of the uptake of essential nutrients. These modes of action may operate on the cellular as well as on higher organizational levels and influence all the aspects of plant metabolism [23, 45].

All these results agree with those previously reported for *A. griffithii* where the growth of plants cultivated in the absence or in the presence of 90 m*M* Na^+ was similar even after 90 days [41]. Previous studies in *Atriplex amnicola* also indicated that this plant increased growth after additions of NaCl to the growth medium up to 25-50 m*M* but then growth declined as salt concentration was increased [6].

Results for reduction of shoot growth and leaf area development of *A.hortensis* with increasing salt concentration are in conformity with finding of [16], who reported that growth in Kenaf (*Hibiscus cannabinus*) under moderate salt stress was affected primarily through a reduction in elongation of stem and leaf area development.

There were very clear effects of NaCl on stomatal conductance in the present experiment, across the whole range of salinity similar to results published for *S. pectinata* by [32]. In other species, salinity decreases assimilation through reductions in leaf area [69, 79] and stomatal conductance [13, 78]. Exposure of halophytes to increasing salinity may result in partial closure of the stomata, in order to limit both transpiration and the transport of salts to the leaves [99]. Salinity is known to inhibit photosynthesis in a number of plant species [19, 22, 52, 103].

Conclusion

In summary our results show that *A. hortensis* is a highly salt tolerant annual halophyte, this plant has the ability to complete its life cycle under very high saline media. Their growth may be stimulated by the presence of salts in the growth medium. In conclusion, results presented here show that salinity reduces leaf growth and stomatal conductance variables. The remarkable reduction of total plant leaf area is likely to affect whole plant photosynthesis, contributing to the low biomass production. Chlorophyll fluorescence appears to be a useful indicator of salt stress at high NaCl concentrations. Finally, in this study, salinity stress results in a clear stunting of plant growth, which results in a considerable decrease in the dry weights of shoots and roots. Increasing salinity is accompanied also by significant reductions in stomatal conductance, plant height and root length. Increased research on the selection of halophytic species which have an economic utilization may enable the rehabilitation and revegetation of salt-affected lands given that the appropriate soil and irrigation management is applied.

REFERENCES

[1] Al-Khanjari, S, Al-Kathiri, A, Esechie, H.A. (2002): Variation in chlorophyll meter readings, nodulation and dry matter yields of alfalfa (Medicago sativa L.) cultivars differing in salt tolerance. – Crop Res. 24: 350-356.

[2] Al-Sobhi, O.A., Al-Zahrani, H.S., Al-Ahmadi, S.B. (2005): Effect of salinity on Chlorophyll & carbohydrate contents of Calotropis procera seedlings. – King Fasil University J. (Accepted, in press).

[3] Ashby, W.C., Beable, N.C.W. (1957): Studies in halophytes. Salinity factors in the growth of Australian salt bushes. – Ecology 38: 344-352.

[4] Ashraf, M., Harris, P.J. (2004): Potential biochemical indicators of salinity tolerance in plants. – Plant Sci. 166: 3-16.

[5] Ashraf, M.Y., Bhatti, A.S. (2000): Effect of salinity on growth and chlorophyll content in rice. – Pak. J. Ind. Res. 43: 130-131.

[6] Aslam, Z., Jeschke, W.D., Barrett-Lennard, E.G., Setter, T.L., Watkin, E., Greenway, H. (1986): Effects of external NaCl on the growth of Atriplex amnicola and the ion relations and carbohydrate status of leaves. – Plant. Cell. Environ. 9: 571-580.

[7] Bajji, M., Kinet, J.M., Lutts, S. (1998): Salt stress effects on roots and leaves of Atriplex halimus L. and their corresponding callus cultures. – Plant. Science 137: 131-42.

[8] Belkhodja, R., Morales, F., Abadia, A., Gomez-Aparisi, J., Abadia, J. (1994): Chlorophyll fluorescence as a possible tool for salinity tolerance screening in barley (Hordeum vulgare, L.). – Plant Physiol. 104: 667-673.

[9] Ben Amor, N., Ben Hamed, K., Debez, A., Grignon Abdelly, C. (2005): Physiological and antioxidant responses of the perennial halophyte Crithmum maritimum to salinity. – Plant. Sci. 168: 889-899.

[10] Benlloch-Gonzalez, M., Fournier, J.M., Ramos, J., Benlloch, M. (2005): Strategies underlying salt tolerance in halophytes are present in Cynara cardunculus. – Plant. Sci. 168: 653-659.

[11] Bernstein, N., Lauchli, A., Silk, W.K. (1993a): Kinematics and dynamics of sorghum (Sorghum bicolor L.) leaf development at various Na/Ca salinities: I. Elongation growth. – Plant. Physiol. 103: 1107-1114.

[12] Blits, K.C., Gallagher, J.L. (1991): Morphological and physiological responses to increased salinity in marsh and dune ecotypes of Sporobolus virginicus (L) Kunth. – Oecologia 87: 330-335.

[13] Brugnoli, E., Lauteri, M. (1991): Effects of salinity on stomatal conductance, photosynthetic capacity, and carbon isotope discrimination of salt-tolerant (Gossypium hirusutum L.) and salt-sensitive (Phaseolus vulgaris L.) C3 non-halophytes. – Plant. Physiology. 95: 628-635.

[14] Cabello-Hurtado, F., Ramos, J. (2004): Isolation and functional analysis of the glycerol permease activity of two nodulin-like intrinsic proteins from salt stressed roots of the halophyte Atriplex nummularia. – Plant. Science 166: 633-640.

[15] Chatterton, N.J., McKell, C.M. (1969): Atriplex polycarpa. I. Germination and growth as affected by sodium chloride in water cultures. – Agronomy J. 61: 451-453.

[16] Curtis, P.S., Lauchli, A. (1986): The role of leaf area development and photosynthetic capacity in determining growth of kenaf under moderate salt stress. – Austral. J. Plant Physiol 13: 553-565.

[17] Daoud, S., Koyro, H-W., Harrouni, M.C. (2001): Salinity tolerance of beta vulgaris ssp. Maritima. I. Biomass production and osmotic adjustment. (to appear in H. Lieth, Ed. "Cash Crop halophytes : Potentials, pilot Projects, Basic and Applied Research on Halophytes and saline irrigation. – "Ten years after the Al Ain meeting" T:VS Series, Kluwer academic publishers group (the Netherlands).

[18] Debez, A., Hamed, K.B., Grignon, C., Abdelly, C. (2004): Salinity effects on germination, growth, and seed production of the halophyte Cakile maritime. – Plant and soil 262: 179-189.

[19] Dionisio-Sese, M.L., Tobita, S. (2000): Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance. – Journal of Plant Physiology 157: 54-58.

[20] Donahue, R.L., Miller, R.W., Shickluna, J.C. (1983): Soils: An Introduction to Soils and Plant Growth. – Englewood Cliffs, New Jersey, Prentice-Hall.

[21] Egan, T.P., Ungar, I.A. (1998): The effect of different salts of sodium and potassium on the growth of Atriplex prostrate (Chenopodiaceae). – J. Plant Nutr. 21: 2193-2205.

[22] Flowers, T.J., Yeo, A.R. (1981): Variability in the resistance of sodium chloride salinity within rice (Oryza sativa L.) varieties. – New Phytologist 88: 363-373.

[23] Garg, B.K., Gupta, I.C. (1997): Saline Wastelands Environment and Plant Growth. – Jodhpur: Scientific Publishers.

[24] Gihad, E.A., El Shaer, H.M. (1992): Utilization of halophytes by livestock on rangelands: problems and prospects. In: V.R, Squires and A.T., Ayoub (Editors), Halophytes as a Resource for Livestock and for Rehabilitation of Degraded Lands. – Kluwer Academic, Dordrecht, pp. 77-96.

[25] Glenn, E., O'Leary, J.W. (1984): Relationship between salt accumulation and water content of dicotyledonous halophytes. – Plant. Cell. Environment. 7:253-261.

[26] Glenn, E., Pfister, R., Brown, J., Thompson, T., O'Leary, J. (1996): Na and K accumulation and salt tolerance of Atriplex canescens (Chenopodiaceae) genotypes. – American Journal of Botany. 83: 997-1005.

[27] Gorham, J. (1996): Mechanism of salt tolerance of halophytes. – In: Choukr-Allah R, Malcolm CV, Hamdy A, eds. Halophytes and biosaline agriculture. New York: Marcel Dekker, Inc., 31-53.

[28] Greenway, H. (1968): Growth stimulation by high chloride concentrations in halophytes. – Israel Journal of Botany 17: 169-177.

[29] Harrouni, M.C., Daoud, S., Koyro, H-W. (2001): Effect of seawater irrigation on biomass production and ion composition of 7 halophytic species in Morocco. (to appear – in: H. Lieth, Ed. "Cash Crop halophytes : Potentials, pilot Projects, Basic and Applied Research on Halophytes and saline irrigation. Ten years after the Al Ain meeting" T:VS Series, Kluwer academic publishers group, the Netherlands).

[30] Harrouni, M.C., Daoud, S., El Alami, A., Debbagh, B., Chokr-Allah, R., Bengaddour, R. (1999): Responses of some halophytes to seawater irrigation in Morocco. Halophyte uses in different climates II, pp. 57-75. Edited by A. Hamdy, H. Lieth, M. Todorovic and M. Moschenko.

[31] Hasegawa, P.M., Bressan, R.A., Zhu, J.K., Bohnert, H.J. (2000): Plant cellular and molecular responses to high salinity. – Annu. Rev. Plant Physiol. Mol. Biol. 51: 463-499.

[32] Heckathorn, S.A., Delucia, E.H. (1991): Effect of leaf rolling on gas exchange and leaf temperature of Andropogon gerardii and Spartina pectinata. – Bot. Gaz. 152: 263-268.

[33] Hopkins, D.L., Nicholson, A. (1999): Meat quality of Squires, V. R. and A. L. Ayoub, 1992. Halophytes as a wether lambs grazed on saltbush (A. nummularia) plus supplements or Lucerne (Medicago sativa). – Meat Sci. 51: 91-95.

[34] Houerou, H.N.K., Le Houerou, H.N., Choukr, Allah R., Malcolm, C.V., Hamdy, A. (1995): Forage halophytes in the Mediterranean Basin. Halophytes and Biosaline Agricultures: 115-136, isolation of antifungal proteins and peptides. – Pak. J. Bot. 39 (1): 211-221.

[35] Jamil, A., Shahid, M., Khan, M.M., Ashraf, M. (2007): Screening of some medicinal plants for

[36] Jefferies, R.L. (1981) Osmotic adjustment and the response of halophytic plants to salinity. – Bio Science 31(1): 42-46.

[37] Katembe, W.J., Ungar, I.A., Mitchell, J.P. (1998): Effect of salinity on germination and seedling growth of two Atriplex species (Chenopodiaceae). – Ann of Botany 82: 167-175.

[38] Kelly, P.M., Jones, P.D., Sear, C.B., Cherry, B.S.G., Tavakol, R.K. (1982): Variations in surface air temperatures: Part 2. Arctic regions 1881−1890. – Monthly Weather Review 110: 71-83.

[39] Khan, M.A., Ungar, I.A. (1984): The effect of salinity and temperature on germination of polymorphic seeds and growth of Atriplex triangularis. – Am. J. of Botany 71: 481-489.

[40] Khan, M.A., Duke, N.C. (2001): Halophytes - A resource for the future. – Wetlands Ecolo. Manag. 6: 455-456.

[41] Khan, M.A., Ungar, I.A., Showalter, A.M. (2000): Effects of salinity on growth, water relations and ion accumulation of the subtropical perennial halophyte, Atriplex griffithii var. stocksii. – Ann. Bot. 85: 225-232.

[42] Khan, M.A., Ungar, I.A., Showalter, A.M. (2005): Salt stimulation and tolerance in an intertidal stem-succulent halophyte. – J. Plant Nutri. 28: 1365-1374.

[43] Kirst, G.O. (1989): Salinity tolerance of eukaryotic marine algae. – Annu Rev Plant Physiol Plant Mol Biol 40: 21-53

[44] Koyro, H.W. (2006): Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte Plantago coronopus (L.). – Environ. Exp. Bot. 56: 136-146.

[45] Kramer, P.J. (1983): Water Relations of Plants. – New York, Academic Press.

[46] Kurkova, E.B., Kalinkina, L.G., Baburina, O.K., Myasoedov, N.A., Naumova, T.G. (2002): Responses of Seidlitzia rosmarinus to salt stress. – Biol. Bull. 29(3): 221-228.

[47] Laeuchi, A., Luettge, U. (2002): (Eds.) Salinity. – Kluwer Academic Publishers, Dordrecht, Neth., p. 341.

[48] Larcher, W., Wagner, J., Tammathaworn, A. (1990): Effects of superimposed temperature stress on in vivo chlorophyll fluorescence of Vigna unguiculata under saline stress. – J. Plant Physiol 136: 92-102

[49] Lazof, D., Bernstein, N., Laüchli, A. (1991): Growth and development of the Lactuca sativa shoot as affected by NaCl stress: consideration of leaf developmental stages. – Bot. Gaz. 152: 72-76.

[50] Le Houérou, H.N. (2000): Utilization of fodder trees and shrubs in the arid and semiarid zones of west Asia and north Africa. – Arid Soil Resear. Rehab. 14: 101-135.

[51] Lee, G., Carrow, V., Duncan, A. (2005): Growth and water relation responses to salinity stress in halophytic sea shore Paspalum ecotypes. – Sci. Hort. 104: 221-236.

[52] Longstreth, D.J., Nobel, P.S. (1979): Salinity effects on leaf anatomy. – Plant Physiology 63: 700-703.

[53] Lu, C., Zhang, J. (1998): Thermostability of photosystem II is increased in salt-stressed sorghum. – Aust. J. Plant Physiol. 25: 317-324.

[54] Lutts, S., Kinet, J.M., Bouharmont, J. (1996): NaCl-induced senes cence in leaves of rice (Oryza sativa,L.) cultivars differing in salinity resistance. – Ann. Bot. 78: 389-398.

[55] Lutts, S., Lefèvre, I., Delpe´re´e, C., Kivits, S., Dechamps, C., Robledo, A., Correal, E. (2004): Heavy metal accumulation in the halophyte species Mediterranean saltbush. – Journal of Environmental Quality 33: 1271-1279.

[56] Maas, E.V., Grieve, C.M. (1987): Sodium induced calcium deficiency in salt-stressed corn. – Plant Cell and Environment 10: 559-564.

[57] Maas, E.V., Hoffman, G.J. (1977): Crop salt tolerancecurrent assessment. – Journal of Irrigation Drainage Division ASCE 103: 115-134.

[58] Maggio, A., Reddy, M.P., Joly, R.J. (2000): Leaf gas exchange and solute accumulation in the halophyte Salvadora persica grown at moderate salinity. – Environ. Exp. Bot. 44: 31-38.

[59] Matoh, T., Watanabe, J., Takahashi, E. (1986): Effects of sodium and potassium salts on the growth of a halophyte Atriplex gmelini. – Soil Science and Plant Nutrition 32: 451-459.

[60] McArthur, E.D., Sanderson, S.C. (1984): Distribution, systematics, and evolution of Chenopodiaceae: an overview: – In: Tiedemann, A.R., McArthur, E.D., Stutz, H.C., Stevens, R., Johnson, K.L., comps. Proceedings, Symposium on the Biology of Atriplex and Related Chenopods. Gen. Tech. Rep. INT-172. 1983 May 4B6; Provo, UT. Ogden, UT: USDA Forest Service, Intermountain Forest and Range Experiment Station: 14B23.

[61] McKell, C.M. (1989): The biology and utilization of shrubs. – San Diego, CA: Academic Press, Inc. 656 p.

[62] McKell, C.M. (1994): Salinity tolerance in Atriplex species : Fodder shrubs of arid lands. – In: Pessarakli P, ed. Handbook of plant and crop stress. New York, Marcel Dekker, Inc., 497-503.

[63] Mer, G., Bochkarev, A., Gupta, R., Bochkareva, E., Frappier, L., Ingles, C.J., Edwards, A.M., Chazin, W.J. (2000): Structural basis for the recognition of DNA repair proteins UNG2, XPA, and RAD52 by replication factor RPA. – Cell 103: 449-456

[64] Metzner, R., Litwin, G., Weil, G. (1965): The relation of expectation and mood to psilocybin reactions: a questionnaire study. – Psychedelic Rev. 5: 3-39.
[65] Misra, V.N., Shinde, V., Mohanty, R.K., Pandey, L., Kharakwal, J. (1997): – Man and Environment 32: 35.
[66] Mozafar, A., Goodin, J.R., Oerlti, J.J. (1970): Sodium and potassium interactions in increasing the salt tolerance of Atriplex halimus L. II. Nan and K+ uptake characteristics. – Agron J 62: 478-481.
[67] Munns, R. (1993): Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. – Plant Cell and Environment 16: 15-24.
[68] Munns, R. (2002): Comparative physiology of salt and water stress. – Plant Cell Environ. 25: 239-250.
[69] Munns, R., Guo, J., Passiora, J.B., Cramer, G.R. (2000): Leaf water status controls day-time but not daily rates of leaf expansion in salt-treated barley. – Australian Journal of Plant Physiology 27: 949-957.
[70] Naidoo, G., Naidoo, Y. (1998): Salt tolerance in Sporobolus virginicus: the importance of ion relations and salt secretion. – Flora 193: 337-344.
[71] Naidoo, G., Mundree, S.G. (1993): Relationship between morphological and physiological responses to waterlogging and salinity in Sporobolus virginicus (L.) Kunth. – Oecologia 93: 360-366.
[72] Nedjimi, B., Daoud, Y., Touati, M. (2005): Culture in vitro of Algierian halophyte (Atriplex halimus subsp. schweinfurthii) in presence of NaCl. – Proc. of International Congress on Improvement of Vegetable Production, Algeria. 143-144 (in French).
[73] Netondo, G.W., Onyango, J.C., Beck, E. (2004): Sorghum and salinity: I. Response of growth, water relations, and ion accumulation to NaCl salinity. – Crop Sci. 44: 797-805.
[74] Neumann, P. (1997): Salinity resistance and plant growth revisited. – Plant Cell Environ. 20:1193-1198.
[75] Ortiz-Dorda, J., Martinez-Mora, C., Correal, E., Simon, B., Cenis, J.L. (2005): Genetic structure of Atriplex halimus populations in the mediterranean basin. – Ann. Bot. 95: 827-834.
[76] Osman, A.E., Ghassaeli, F. (1997): Effects of Storage conditions and presence of fruiting bracts on the germination of Atriplex halimus and Salsola Vermiculata. – Exp. Agri 33: 149-155.
[77] Osmond, C.B., Bjorkman, O., Anderson, D.J. (1980): Physiological processes in plant ecology. Toward a synthesis with Atriplex. – New York, Springer-Verlag.
[78] Ouerghi, Z., Cornie, G., Roudani, M., Ayadi, A., Brulfert, J. (2000): Effect of NaCl on the photosynthesis of two wheat species (Triticum durum and Triticum aestivum) differing in their sensitivity to salt stress. – Journal of Plant Physiology 15: 519-527.
[79] Papp, J.C., Ball, M.C., Terry, N. (1983): A comparative study of the effects of NaCl salinity on respiration, photosynthesis, and leaf extension growth in Beta vulgaris L. (sugar beet). – Plant Cell Environ 6: 675-677.
[80] Priebe, A., Jaeger, H.J. (1978): Ein¯uss von NaCl auf waschstum und ionengehalt unterschiedlich saltztolerater P¯anzen. – Angewandte Botanik 52: 531-541.
[81] Ramoliya, P.J., Patel, H.M., Pandey, A.N. (2004): Effect of salinization of soil on growth and macro and micro nutrient accumulation in seedlings of Acacia catechu (Mimosaceae). – Annals of Applied Biology 144: 321-332.
[82] Ramos, J., Lopez, M.J., Benlloch, M. (2004): Effect of NaCl and KCl salts on the growth and solute accumulation of the halophyte Atriplex nummularia. – Plant and Soil 259: 163-168.
[83] Reddy, M.P., Vora, A.B. (1986): Changes in pigment composition, Hill reaction activity and saccharides metabolism in Bajra (Pennisetum typhoides S & H) leaves under NaCl salinity. – Photosynthetica 20: 50-55.

[84] Redondo-Gómez, S., Wharmby, C., Castillo, J.M., Mateos-Naranjo, E., Luque, C.J., de Cires, A. (2006): Growth and photosynthetic responses to salinity in an extreme halophyte, Sarcocornia fruticosa. – Physiologia Plantarum 128: 116-124.

[85] Richardson, S.G., McKell, C.M. (1980): Water relations of Atriplex canescens as affected by the salinity and moisture percentages of processed oil shale. – Agronomy Journal 72: 946-950.

[86] Shani, J., Ahronson, Z., Sulman, F.G. (1972): Insulin-potentiating effect of saltbush (Atriplex halimus) ashes. – Isr. J. Med. Sci. 8: 757-758.

[87] Shannon, M.C., Grieve, C.M. (1999): Tolerance of vegetable crops to salinity. – Sci. Hortic. 78(1/4): 5-38.

[88] Sharma, P.K., Hall, D.O. (1991): Interaction of salt stress and photoinhibition on photosynthesis in barley and sorghum. – J. Plant Physiol. 138: 614-619.

[89] Sharma, P.K., Hall, D.O. (1992): Changes in carotenoid composition and photosynthesis in barley and sorghum . – J. Plant Physiol. 140: 661-666.

[90] Shen, Z., Denton, M., Mutti, N., Pappan, K., Kanost, M.R., Reese, J.C., Reeck, G.R. (2003): Polygalacturonase from Sitophilus oryzae: Possible horizontal transfer of a pectinase gene from fungi to weevils. 9pp. – Journal of Insect Science 3: 24.

[91] Short, D.C., Colmer, T.D. (1999): Salt tolerance in the halophyte Halosarcia pergranulata subsp. pergranulata. – Annals of Botany 83: 207-213.

[92] Soussi, M., Ocaña, A., Lluch, C. (1998): Effects of salt stress on growth, photosynthesis and nitrogen fixation in chick-pea (Cicer arietinum L.). – Journal of Experimental Botany 49: 1329-1337.

[93] Storey, R., Wyn Jones, R.G. (1979): Quaternary ammonium compounds in plants in relation to salt resistance. – Phytochemistry 16: 447-451.

[94] Sudhakar, G., Jyothi, B., Venkateswarlu, V. (1991) Metal pollution and its impact on algae in flowing waters in India. – Archives of Environmental Contamination and Toxicology 21: 556-566.

[95] Uchiyama, Y. (1987): Salt tolerance of Atriplex nummularia. – Technical Bulletin Tropical Agricultural Research Center Japan 22: 1-69.

[96] Ungar, I.A. (1982): Germination ecology of halophytes. – In: Sen DN, Rajpurohit KS, eds. Contributions to the ecology of halophytes. The Hague: Dr. W. Junk Publishers, 143-154.

[97] Ungar, I.A. (1996): Effect of salinity on seed germination, growth and ion accumulation of Atriplex patula (Chenopodiaceae). – American Journal of Botany 83: 604-607.

[98] van Kooten, O., Snel, J.F.H. (1990): The use of chlorophyll fluorescence nomenclature in plant stress physiology. – Photosynth. Res. 25: 147-150.

[99] Véry, A.A., Robinson, M.F., Mansfield, T.A., Sanders, D. (1998) Guard cell cation channels are involved in Na^+-induced stomatal closure in a halophyte. – Plant Journal 14: 509-521.

[100] Wang, L.W., Showalter, A.M. (2004): Cloning and salt-induced, ABA independent expression of choline mono-oxygenage in Atriplex prostrata. – Physiologia Plantarum 120: 405-412.

[101] Wilson, C., Lesch, S.M., Grieve, C.M. (2000): Growth stage modulates salinity tolerance of New Zealand spinach (Tetragonia tetragonioides, Pall.) and red orach (Atriplex hortensis L.). – Ann. Bot. 85: 501-509.

[102] Yeo, A.R. (1998): Molecular biology of salt tolerance in the context of whole-plant physiology. – J. Exp. Bot. 49: 915-929.

[103] Yeo, A.R., Capon, S.J.M., Flowers, T.J. (1985): The effect of salinity upon photosynthesis in rice (Oryza sativa L.): gas exchange by individual leaves in relation to their salt content. – Journal of Experimental Botany 36: 1240-1248.

[104] Zid, E., Boukharis, M. (1977): Quelques aspects de la tolerance de l'Atriplex halimus L. au chlourure de sodium. – Oecologia Plantarum 12: 351-362.

EFFECT OF HEAVY METALS ON SELF-PURIFICATION PROCESSES IN RIVERS

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Abstract. The inhibitory effects of heavy metals on self-purification processes in surface water were studied. Toxicities of the metals were evaluated on the grounds of the results of the Biochemical Oxygen Demand (BOD) test in river water. The BOD inhibitions depended on the concentrations of metals and on the incubation time. After one day's incubation, a full inhibition was found to be caused by Ag at all tested concentrations above 0.02 mg.l^{-1} for Hg, Cd, Pb, and Cu above 1 mg.l^{-1}, for Be above 2 mg.l^{-1}, and for Co, Ni, and Sn^{II} above 5 mg.l^{-1}. A strong inhibitory effect ranging from 50 to 80% was observed for Cd (from 0.2 to 0.7 mg.l^{-1}), Cu (from 0.4 to 1.0 mg.l^{-1}), Be (from 0.5 to 2.0 mg.l^{-1}), Zn (from 1.2 to 5.0 mg.l^{-1}), Sn^{II} (from 2.2 to 4.2 mg.l^{-1}), and Cr^{VI} (above 4 mg.l^{-1}). As^{III}, As^{V}, Mn^{II}, Se^{IV} and Se^{VI} did not cause any BOD inhibition to 2 mg.l^{-1} (As) res. 5 mg.l^{-1} (other metals). The inhibitory effect of metals decreased significantly or disappeared with increasing incubation time. Being observed with all metals with toxic effects except for Be, Sn^{II}, and Cr^{III}, the decrease was more intense at the beginning of the incubation period.

Keywords: *self-purification, biochemical oxygen demand, heavy metals, toxicity*

Introduction

Heavy metals are toxic to the mixed culture of microorganisms responsible for the decomposition of organic compounds in surface waters. As aerobic biological processes are an essential part of the self-purification process occurring in surface water, heavy metals diminish the ability of such waters to purify themselves.

Our investigation focused on the evaluation of the toxic effect of heavy metals on the Biochemical Oxygen Demand (BOD) in surface water under the conditions of analytical BOD determination. The course of the BOD was evaluated during several days. The pollution with organic compounds made it possible to carry out the tests without a typical dilution of tested water with dilution water and without seeding or enrichment by other compounds except salts of metals.

Review of Literature

The toxic effects of heavy metals have been studied mostly with respect to the activated sludge [2, 10, 11].

The influence of metals on wastewater treatment evaluated by BOD or COD is, in comparison with the effect on BOD in surface water, considerably lower with respect to a higher concentration of biomass in the environment of a biological reactor. For example, concentrations not exerting any depression on the wastewater treatment efficiency are 0.1 mg.l^{-1} for Hg, 1 mg.l^{-1} for Cu, 2 mg.l^{-1} for Cd, Ni, Cr^{III}, and Cr^{VI}, 5 mg.l^{-1} for Pb, and 10 mg.l^{-1} for Zn and Sn [5].

Since the 1970s, several authors have investigated the inhibitory effect of metals on the BOD under diverse conditions and with the use of various substrates.

[7] chose incubation periods of 5 and 14 days, [9] measured the BOD course for 10 days, and [1, 6, 8] did so for 5 days. The first two investigators used sewage as the substrate, while [8] used a peptone solution, [6] a milk waste, and [1] a river water. [4] tested the influence of Cd on the BOD in solutions of glucose/glutamic acid and glucose/phenol. Sewage was usually used for seeding; only [8] used a culture of *A. hydrophila* strain and [1] tested unseeded samples. The results presented by various authors are often very different (*Table 1*), a fact that can be explained by different test conditions.

Table 1. *Inhibitory effects of selected heavy metals on BOD$_5$ (concentrations in mg.l^{-1})*

Metal	**Mowat (1976)**			**Stones (1991)**	**Schubert (1980)**			**Mittal *et al.* (2000)**			**Berkun (2005)**		
	BOD inhibition, %												
	20	**50**	**80**	**50**	**20**	**50**	**80**	**20**	**50**	**80**	**20**	**50**	**80**
Ag	0.025	0.18	>10	>0.25	0.01	0.05	1						
Cd	1.2	4.5	>20	> 5	1.0	8	>10						
Co	1.2	5.0	>20					0.5	15				
Cr^{III}	1.6	6.0	14	2.5							0.4	0.6	0.9
Cr^{VI}											0.08	0.14	0.2
Cu	0.2	3.0	18.5	2.5	1.0	3	>10	2.0			0.3	0.7	1.3
Hg	0.07	0.4	0.6	>0 .5	0.1	1	5			0.1	0.05	0.15	0.3
Ni	0.9	7	>20	> 5	1.4	2.6	5	0.5	1.0	10	0.3	0.5	0.8
Pb				5	>10			5.0					
Sn^{II}	12	24	>40										
Zn	6	>40		>25	2.6	5	10	5			0.3	0.8	2.8

Materials and Methods

The evaluation of the metal influence was based on the results of the BOD test [3].

The measurements were carried out in river water taken from the Svratka River about 1 km downstream from the Brno (Czech Republic) water reservoir. Suspended impurities were separated by filtration through gauze. The average water composition is given in *Table 2*.

Table 2. *Average composition of river water*

Basic components	**Heavy metals**		
pH	7.9	Al, mg.l^{-1}	0.06
Total residue, mg.l^{-1}	246	As, mg.l^{-1}	0.00
Loss on ignition, mg.l^{-1}	89	Cd, mg.l^{-1}	0.00
COD$_{Cr}$, mg.l^{-1}	5.4	Cr, mg.l^{-1}	<0.03
N-NH$_4$, mg.l^{-1}	0.47	Cu, mg.l^{-1}	<0.01
N-NO$_2$, mg.l^{-1}	0.05	Fe, mg.l^{-1}	0.06
N-NO$_3$, mg.l^{-1}	3.25	Mn, mg.l^{-1}	0.06
N$_{tot}$, mg.l^{-1}	3.77	Ni, mg.l^{-1}	<0.02
BOD$_5$, mg.l^{-1}	3.29 ± 1.00	Pb, mg.l^{-1}	<0.05
O$_2$, mg.l^{-1}	8.0 ± 0.7	Zn, mg.l^{-1}	0.06

The BOD tests were conducted immediately after the collection of samples and the temperature adjustment. Oxygen bottles with volumes of about 300 ml were filled with the samples and incubated at a temperature of 20 ± 1 °C in the thermostat. The concentration of dissolved oxygen was measured at the beginning and then after 1, 2, 3, 4, and 7 days of incubation by means of a WTW Oxi3000 microprocessor oximeter. The sensitivity of the oxygen determination was 0.01 mg.l – 1. Curves showing the dependence of the BOD on the incubation time and the inhibition of the BOD on the incubation time were plotted.

Three to four parallel blank tests (water without addition of metals) were carried out for each metal or group of metals. The standard deviations of the average BOD values after several representative incubation times are presented in *Table 3.*

Table 3. *Standard deviations of the average BOD values*

Incubation time, days	Average BOD, mg.l^{-1}	Std. deviation, %	Std. deviation, mg.l^{-1}
1	0.99	10 ± 8	0.10 ± 0.08
4	2.76	5 ± 3	0.14 ± 0.11
7	3.90	4 ± 3	0.16 ± 0.12

Metallic compounds were fed into river water as aqueous solutions prepared from chemicals specified in *Table 4* and distilled water. The accurate concentrations of metals in the stock solutions and in river water were determined by AAS. Three parallel tests with one to three different compounds of each metal were usually carried out. The average results are presented in this paper.

Table 4. *Overview of chemicals used to prepare the test solutions*

Metal	Compound
Ag	$AgNO_3$
As^{III}	$NaAsO_2$
As^{V}	$Na_2HAsO_4.7H_2O$
Be	$BeSO_4.4H_2O$
Cd	$CdCl_2.H_2O$, $Cd(NO_3)_2.4H_2O$, $CdSO_4.H_2O$
Co	$CoCl_2.6H_2O$
Cr^{III}	$Cr(NO_3)_3.9H_2O$, $KCr(SO_4)_2.12H_2O$
Cr^{VI}	$K_2Cr_2O_7$
Cu	$CuCl_2.2H_2O$, $Cu(NO_3)_2.3H_2O$, $CuSO_4.5H_2O$
Hg	$HgCl_2$
Ni	$NiCl_2.6H_2O$, $Ni(NO_3)_2.6H_2O$, $NiSO_4.6H_2O$
Pb	$PbCl_2$, $Pb(NO_3)_2$
Se^{IV}	Na_2SeO_3
Se^{VI}	$Na_2SeO_4.10H_2O$
Sn^{II}	$SnCl_2.2H_2O$
Zn	$ZnCl_2$, $ZnSO_4.7H_2O$
Ag	$AgNO_3$

Results

The results obtained after one day's incubation, representing a stage of a shock charge, are presented in *Table 5*. The interpolated results obtained after five days' incubation, representing a stage of bacterial adaptation, are summarised in the same table.

Table 5. *Inhibition effects of metals on the BOD*

Metal, mg.l^{-1}	0.02	0.1	0.2	0.4	1.0	2.0	5.0
	BOD inhibition, %						
Incubation time of 1 day							
Ag	89	100	100	100	100		
As^{III}			–14	–4	–2	–2	
As^{V}			–7	–7	1	19	
Be		41	49	49	55	74	
Cd		37	50	67	88	91	98
Co		13	13	13	47	63	83
Cr^{III}		–8	–4	11	17	24	33
Cr^{VI}		0	1	8	24	36	68
Cu		27	40	63	78	100	100
Hg^{II}	–5	3	9	32	100	100	
Mn^{II}			0	–4	1	–1	8
Ni		–7	2	19	42	69	87
Pb			0	0	88	100	100
Se^{IV}		–17	–11	–9	–12	–2	9
Se^{VI}		–16	–12	–14	–12	–8	–10
Sn^{II}		–5	5	1	25	45	92
Zn		0	17	32	48	57	73
Incubation time of 5 days							
Ag	15	82			83		
As^{III}			–14	–4	–3	–2	
As^{V}			–6	–3	–7	9	
Be		24	35	39	65	90	
Cd		2	3	14	22	33	46
Co		8	15	5	36	36	48
Cr^{III}		–6	0	16	20	28	40
Cr^{VI}		4	7	9	16	19	34
Cu		–2	18	35	46	63	100
Hg^{II}	–5	6	4	44	55	66	
Mn^{II}			–2	–5	–8	4	6
Ni		0	4	17	30	43	52
Pb		0	0	19	24	43	60
Se^{IV}		–5	–5	–2	–3	5	9
Se^{VI}		–7	–3	–5	–6	–2	1
Sn^{II}		3	9	5	32	45	83
Zn		–11	–1	5	15	34	45

□	no effect
░	inhibition effect
▓	strong inhibition effect
■	total inhibition – toxicity

Dependences of the BOD and of the BOD inhibition related to the blank samples on the incubation time are similar for all tested metals. Typical curves relating to Ni are exemplified in *Fig. 1* and *Fig. 2*.

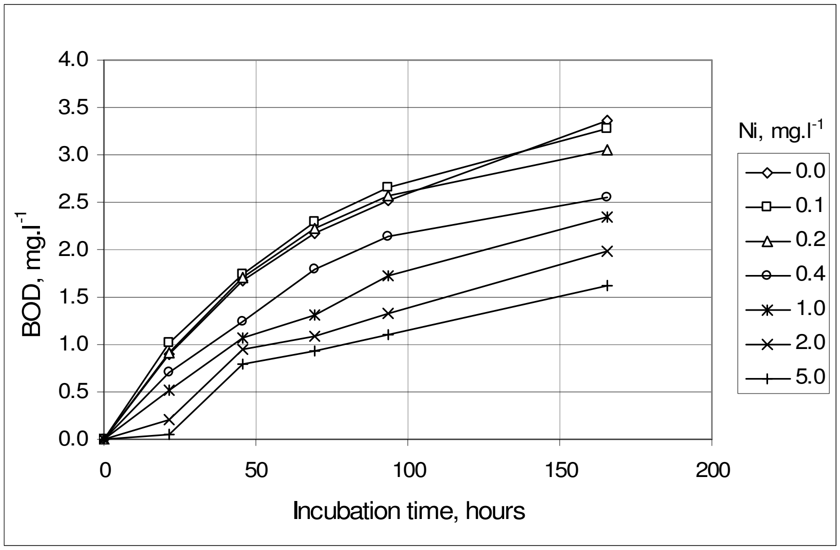

Figure 1. *Dependence of the BOD on the incubation time for Ni*

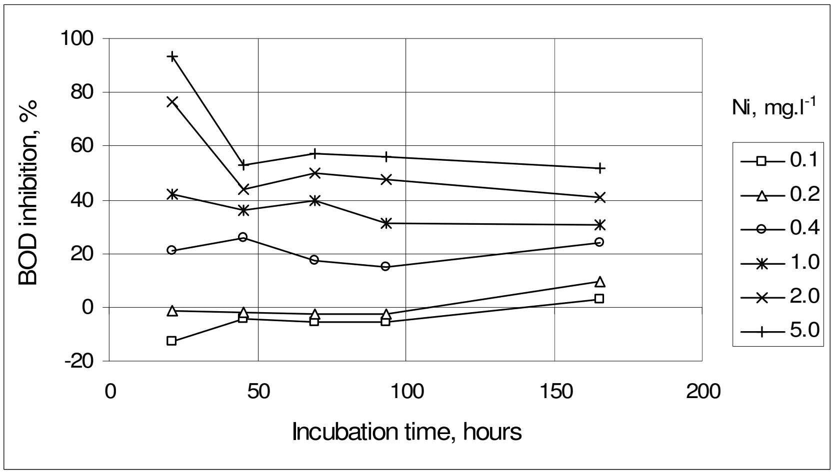

Figure 2. *Dependence of the BOD inhibition on the incubation time for Ni*

Discussion

The concentration of ammonia-nitrogen in river water was low: 0.47 mg.l^{-1} on average (*Table 2*). It was proved that the growth of nitrification bacteria was not significant and that the BOD in the tested samples was caused by the decomposition of organic matter by microorganisms.

The inhibition effects of metals on BOD values depended on their concentrations and on the incubation time. They decreased significantly or disappeared with the increasing incubation time (*Figs. 1* and *2*). The decrease was more intense at the beginning of the incubation time. It was observed with all metals with toxic effects except Be, Sn^{II}, and Cr^{III}. This phenomenon can be primarily explained by a partial adaptation of the mixed culture of microorganisms and by the fact that the more resistant organisms overgrew the sensitive ones. Physical and chemical processes (precipitation, complex formation, sorption, etc.) also cannot be excluded.

In the case of some heavy metals, after exceeding a certain concentration in the tested medium, the inhibition effect ranged between 80 and 100%, where the decomposition of organic matter is supposed to be fully inhibited (*Table 5*).

After one day's incubation, the full inhibition was found for Ag at concentrations above 0.02 $mg.l^{-1}$, for Hg, Cd, and Pb above 1 $mg.l^{-1}$, for Cu above 2 $mg.l^{-1}$, and for Co, Ni, and Sn^{II} above 5 $mg.l^{-1}$. A strong inhibition effect ranging between 50 and 80% was reported for Cd (from 0.2 to 0.7 $mg.l^{-1}$), Cu (from 0.4 to 1.0 $mg.l^{-1}$), Be (from 0.5 to 2.0 $mg.l^{-1}$), Zn (from 1.2 to 5.0 $mg.l^{-1}$), Sn^{II} (from 2.2 to 4.2 $mg.l^{-1}$), and Cr^{VI} (above 4 $mg.l^{-1}$). As^{III}, As^{V}, Mn^{II}, Se^{IV}, and Se^{VI} did not cause BOD inhibition in the concentrations up to 2 $mg.l^{-1}$ (for As) and 5 $mg.l^{-1}$ (for other metals).

After five days' incubation, a full inhibition occurred with the additions of Ag (above 0.1 $mg.l^{-1}$), Be (above 1.5 $mg.l^{-1}$), and Sn^{II} and Cu (above 5.0 $mg.l^{-1}$). A strong inhibition was observed with the additions of Hg at about 1.0–2.0 $mg.l^{-1}$ and with additions of Pb and Ni at 2.5–5.0 $mg.l^{-1}$.

Differences were found between Cr^{III} and Cr^{VI}. The inhibition effect of Cr^{VI} on the BOD after one day's incubation was greater than that of Cr^{III}, while after a prolonged period of incubation the converse was true (*Table 5*). The compounds of As^{III} - As^{V} and Se^{IV} - Se^{VI} were not inhibiting in the tested concentration range and therefore their potentially variant influence could not be determined.

Negative values of inhibition observed in some tests mean a stimulation effect (*Table 5*). As these values did not exceed 20%, they were supposed to lie in the range of measurement deviations. The stimulating effect of zinc at concentrations of about 0.05 $mg.l^{-1}$ (about 10%) is in agreement with the statements made by [6].

They reported a stimulating effect of zinc typically less than 20% for concentrations from 0.1 to 1.0 $mg.l^{-1}$. The data are not yet sufficient to unambiguously prove the stimulating effect of Zn.

The increase in the inhibition effect with the increase in the concentration of a metal is not identical for all metals at a given incubation time. It implies that the row of metals sequenced according to their decreasing inhibition effect changes in some cases with the concentration of the particular metals and with the incubation time. The sequence of metals according to the concentration at which the inhibition effect exceeded 50% after one day's incubation was, for example, Ag – Cd – Cu – Be – Hg – Pb – Zn – Co – Ni – Sn^{II} – Cr^{VI}, while for the 80% inhibition effect it was Ag – Cd – Hg – Pb – Cu – Ni – Sn^{II} – Co. The sequence of metals according to the rising concentration at which a 50% inhibition was reached after five day's incubation was Ag – Be – Hg – Cu – Sn^{II} – Pb – Ni. Metals not included in the sequences did not cause 50% or 80% inhibition in the tested concentration range.

The BOD course of the blank samples suited the kinetics of the first order. The addition of a metallic salt caused a deformation of the BOD-curve. Lag-phases could be observed for the following heavy metals: one day for Ag (at 0.01 $mg.l^{-1}$), Pb (at 0.6 $mg.l^{-1}$), and Ni (at 2 $mg.l^{-1}$), and two days for Ag (at 0.4 $mg.l^{-1}$), Cd (at 2 $mg.l^{-1}$), and Sn^{II} (at 5 $mg.l^{-1}$). After the lag period, oxidation started rapidly but then decreased again so that the resulting oxidation curve had a sigmoidal shape (*Fig. 1*).

The inhibition effects of the different salts (sulphates, chlorides, nitrates; *Table 4*) of the tested metals were evaluated. Standard deviations of the data relating to a certain metal, concentration, and incubation time were calculated. The standard deviations decreased slightly with increasing metal concentrations and incubation time. However, the standard deviation was small: 3.2% on average and 10.7% maximum. Thus, it can be stated that in the cases of Cd, Cr^{III}, Cu, Ni, Pb, and Zn the use of different salts of the tested metals did not develop different BOD inhibitions.

In some cases, a comparison of our results with those found by other authors showed a good agreement for certain concentrations of metals but not for the whole concentration range. In exceptional cases, considerable differences were found in the whole concentration range.

Our results for five-day incubation time are comparable with those reported by [7]. The inhibitions by a great number of metals (Ag, Cd, Cu, Ni, Co, Cr^{III}) were very similar. Our experiments documented a somewhat lower toxicity of mercury, while greater differences were found for zinc and tin. While [7] reported 20% inhibition at concentrations of 6 $mg.l^{-1}$ Zn or 12 $mg.l^{-1}$ Sn^{II}, our experiments reported the same inhibition already at a concentration of about 1 $mg.l^{-1}$.

[4] reported a considerably smaller effect of cadmium in comparison with our and Mowat's results. For example, at the concentration of 6.1 $mg.l^{-1}$ Cd determined for two different substrates, the BOD inhibitions were only 14.9 and 26.5%. The differences can be explained by the use of easily degradable substrates by [4].

In comparison with our experiments, [6] reported inhibition effects that were similar for nickel and cobalt but higher for mercury after incubation for five days. These authors reported a quite anomalous behaviour for copper. They found a BOD inhibition ranging from 20 to 50% for concentrations of copper between 2 and 10 $mg.l^{-1}$. Above this concentration, the inhibitory effect decreased. In our experiments, the inhibitory effect of copper was determined at concentrations from 0.4 to 1.0 $mg.l^{-1}$ Cu. These results indicate among others also the influence of the substrate quality, which affects the composition of the mixed bacterial population.

Berkun's experimental conditions [1] were similar to ours. After five days' incubation, he reported similar concentrations causing 20% BOD inhibitions for Cu and Ni. However, in the cases of other metals, Berkun found greater inhibitory effects and less steep dependance of the BOD inhibition effect on metal concentration. He also found a greater inhibition effect of Cr^{VI} than of Cr^{III} after five days' incubation. The BOD of river water used in Berkun's experiments was higher than in ours (5.0 to 14.1 $mg.l^{-1}$). Other data on the river water composition ($N\text{-}NH_4$, metals, other toxic compounds, etc.) were not available.

In comparison with concentrations of metals in surface waters, the concentrations of metals that have an inhibitory effect on the BOD are considerably higher. This indicates that heavy metals in natural waters with no extreme pollution do not influence the rate of microbial decomposition of organic matter, thus having no impact on self-purification processes. But the accumulation of metals in biomass and the hazards for the end element of the food chain (humans) cannot be omitted.

Conclusions

- It was found that the inhibitory effects of heavy metals on the self-purification process started at much higher concentrations of metals than those typically found in surface water.
- The inhibitory effect of BOD depends on the concentration of metals and on the incubation time.

- The toxic effects of heavy metals typically decreased with the increasing incubation time. This effect was not observed with Be, Sn^{II}, or Cr^{III}.
- Metals that showed high toxicity (above 50% inhibition at concentrations below 1.0 $mg.l^{-1}$) after incubation for one day included Ag, Be, Cd, Cu, Hg, and Pb, while Co, Ni, and Zn caused an inhibition of about 40–50% at this concentration, and inhibitions by Sn^{II}, Cr^{III}, and Cr^{VI} were low.
- Ag and Be showed high inhibition effects even after five days' incubation. The effects of Hg and Cu were also considerable.
- As, Se, and Mn did not show any inhibitory effect up to concentrations of 5.0 $mg.l^{-1}$.
- A lag-phase appeared in the BOD-curves affected by Ag, Cd, Ni, Pb, and Sn^{II}.
- None of the tested heavy metals had an evidently stimulating effect.
- It was found that the inhibition effect depended considerably on the quality of the medium in which the test was performed. The toxicity measured in river water is mostly higher than that measured in wastewater. Thus, the study would seem to propose the use of surface water for the evaluation of the effects of toxic compounds on the self-purification processes.

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REFERENCES

[1] Berkun, M. (2005): Effects of Ni, Cr, Hg, Cu, Zn, Al on the dissolved oxygen balance of streams. – Chemosphere 59: 207-215.

[2] Gikas, P. (2008): Single and combined effects of nickel (Ni(II)) and cobalt (Co(II)) ions on activated aludge and on other aerobic microorganisms. – J. Hazard. Mater. 159(2,3): 187-203.

[3] ISO 10 707 (1994): Water quality. Evaluation in an aqueous medium of the "ultimate" aerobic biodegrability of organic compounds. Method by analysis of biochemical oxygen demand (closed bottle test).

[4] Kansal, S.K., Mehrotra, I. (1982): BOD Exertion in presence of cadmium ion. – Indian J. Environ. Hlth. 24(4): 285-291.

[5] Liebmann, H. (1960): Handbook of Freshwater- and Wastewater Biology (German). Vol. II. – VEB G. Fischer Verlag. Jena.

[6] Mittal, S.K., Ratra, R.K. (2000): Toxic effect of metal ions on biochemical oxygen demand. – Water Res. 34(1): 147-152.

[7] Mowat, A. (1976): Measurement of metal toxicity by biochemical oxygen demand. – J. Water Poll. Contr. Fed. 48(5): 853–865.

[8] Schubert, R.H.W. (1980): Research directed to limiting of the sewage I. Experim. Investig of the effect of degradable and toxic substances on the self-purification processes in the sewage. – Zentrabl. Bacteriol. Microbiol. Hyg. Abt., Orig. B 171: 497-511.

[9] Stones, T. (1981): A résumé of the kinetics of the BOD test. – Water Pollut. Control 80: 513-520.

[10] Vankova, S., Kupec, J., Hoffmann, J. (1999): Toxicity of chromium to activated sludge. – Ecotox. Environ. Safe. 42(1): 16-21.

[11] Xie, B., Kang, K.S., Nakamura, E., Itoh, K. (2002): The effect of heavy metals on the activated sludge process and its microbial community analysis using 16S ribosomal DNA. – Int. J. Environ. Pollut. 16(6): 571-588.

PROPOSING AN EFFICIENT INDICATOR OF GRAZER DISTRIBUTION ON HETEROGENEOUS HILL VEGETATION

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Abstract. Irish and UK uplands and peatlands are of international importance but are under threat from several factors, including heavy grazing pressure. Sheep preferentially graze patches of acid grassland with short dense swards, sometimes referred to as ‘grazing lawns’, and have been implicated in damage to uplands. The aim of this study was to increase our understanding of resource selection by grazers to further inform the design and implementation of conservation strategies. Grazing lawn frequency and habitat condition were mapped and GPS collars were used to track Scottish Blackface sheep on a hill farm in Ireland. Weighted compositional analysis (multivariate analysis of variance) was used to test for random use of different categories of grazing lawn frequency and habitat condition. Grazing lawn frequency was spatially uneven and habitat condition ranged from undamaged to very severely damaged areas. Typically, selection of differing categories of habitat condition was not significant ($P>0.05$), although the highest selection rank was consistently for the ‘moderate-undamaged’ category. Sheep most selected 1 ha grid squares containing numerous/extensive grazing lawns ($P<0.05$) day, night and year-round. As a simple, efficient indicator of hill use by sheep, which would be a valuable input in models predicting grazing impact on hill vegetation, the mapping of grazing lawn frequency is suggested.

Keywords: *agriculture; ecology; environment; resource management; resource selection*

Introduction

Irish and UK uplands and peatlands are of international importance due to the limited global distribution of the habitats, plant communities and fauna they support [9, 56]. Many of these habitats and associated species are protected under the EC Habitats (92/43/EEC) and Birds (79/409/EEC) Directives [21], and are included in biodiversity action plans [16, 37] and agri-environment scheme measures [14, 49]. Despite this, the quality of remaining upland and peatland continues to be under threat from heavy grazing pressure, burning, afforestation, peat extraction, undergrazing and/or land abandonment [22, 48, 56, 57].

Damage in this study refers to habitat conditions which fail to meet biological conservation objectives. While habitats are affected by natural phenomena such as topography [24] and wind-driven rain [11], management practices including grazing [55] play a key role in habitat condition and can be altered. Therefore, the focus of this study is on grazer behaviour as grazing is the predominant management practice on

Irish hills where heavy grazing pressure is a main threat [50]. This is also the case in other countries, particularly the UK [22, 48].

Damage assessment classifications have been devised for upland areas and include indicators of grazing-related damage [18, 44]. It is widely known that different grazing animals have different effects on vegetation and that different plant communities and associated soils have different carrying capacities [6, 29, 52]. Numerous researchers have studied the impacts of livestock grazing on upland systems (e.g. [8, 15, 23]) but it is believed that grazer selection of areas with differing condition status has yet to be quantified. It is known that sheep choose to use a small proportion of the total area available to them [61] and exhibit habitat and vegetation selection with a preference for acid grassland patches [12, 35, 61]. Acid grassland can have short, dense swards and often has abundant *Nardus stricta* (L.), [25] (characteristic plant species described). [45] referred to these conspicuous grassland patches as ‘grazing lawns’, probably because they are a product of grazing and resemble well maintained lawns.

Plant biomass and herbage intake rates are important factors in foraging efficiency and patch selection by grazers [12]. Damaged areas support a higher percentage cover of exposed soil and, consequently, a lower percentage cover of vegetation [18]. Conversely, grazing lawns have a dense canopy which promotes greater forage yields per bite compared with lightly grazed vegetation [45].

Surprisingly, very little published quantitative information is available on resource selection by sheep grazing heterogeneous hill vegetation [2]. Since then, valuable information on patch selection by sheep has been obtained using plot-based trials with artificial patches and field observations (e.g. [19, 28]), which complements previous trials by [12] for example. GPS tracking devices have recently been used to investigate habitat selection rankings in a complex hill farm environment by collecting a large amount of data round-the-clock with high location accuracy [61]. As management prescriptions on complex hill habitat assemblages probably need to vary between sites [35], GPS tracking, although useful for detailed studies on a number of sites, is unlikely to be carried out on a farm-to-farm basis. Therefore an alternative, more rapid method (that could be carried out at farm level) for estimating grazer distribution, to identify areas under greatest grazing pressure, would be a useful tool in conservation management. [61] investigated habitat selection rankings but acid grassland was considered to be under-represented and no account was taken of habitat condition. Therefore the objectives of this study are to investigate spatio-temporal use of grazing lawns (frequency classified for 1 ha grid squares) and patches with differing habitat condition status.

Methods

Study area

The study area comprised of 216.9 ha of upland and peatland at the Teagasc Hill Sheep Farm in Co. Mayo in the west of Ireland (53°37’ N, 09°41’ W). The dominant habitats were blanket bog and wet heath, with fragmented patches of acid grassland. Habitat distribution and sheep selection rankings of habitats are described in full by [61]. The study area, which was within the catchment area of the Erriff River, was part of the Mweelrea/Sheeffry/Erriff Complex candidate Special Area for Conservation and proposed Natural Heritage Area. The study area was on the south-southeasterly slopes of Ben Gorm and ranged in altitude from 15-275 m OD, with the highest, steepest slopes in the northwest corner. The site was Class 5 for agricultural land use [26]. Soils were mainly organic, consisting of peats, lithosols, humic/peaty podzols and gleys. Peat depths ranged between 30 and 525 cm [58]. Pegs marked a 100x100 m grid.

A maritime temperate climate prevailed with the nearest synoptic meteorological station located in Belmullet, Co. Mayo, approximately 70 km distant. Based on the most recent 30-year averages (1961-1990) recorded at Belmullet, the mean daily temperature was 14.0 °C in July and 5.7 °C in January, and the annual mean daily duration of bright sunshine was 3.5 h [46] undated. The mean annual rainfall 1993-2005 recorded on-site was 2086.4 mm (L. O'Malley *pers. comm.*). The minimum and maximum hours of daylight at the study area were calculated as 7 h 27 min and 17 h 4 min, respectively.

Scottish Blackface sheep grazed the study area at stocking rates of 0.4 ewes/ha in spring (March-May), 0.9 ewes/ha in summer (June-August) and autumn (September-November) and 0.8 ewes/ha in winter (December-February). These calculations were based on 2004-2005 averages, omitted lambs and included hoggets at a ratio of 3 hoggets:2 ewes. The land was grazed for 348 days in 2004 and 351 days in 2005 and supplementary feed was not given in the study area. Ewes lambed in early April with a productivity of 1.0 lamb/ewe (based on mean data 2004-2006). Approximately 80 females were retained annually as replacements. (L. O'Malley *pers. comm.*).

Tracking ewes

Four Scottish Blackface ewes ('core ewes') plus seven substitutes, all two years old, were selected at random at the start of the study. This age group was selected because it had experience of the study area and was likely to survive for the duration of the study. Substitute ewes were tracked only when core ewes were unavailable pre-lambing either because they were of low body condition or twin-bearing. Four ewes were tracked in each of nine season-based tracking periods between February 2004 and April 2006 producing 36 ranges in total. A 'range' is the collection of location data for an individual in any one tracking period. Simultaneous flock observations for 58% of ranges/64% of individuals [61] indicated no unusual social behaviour by collared ewes. However, one core ewe was a member of a social group that chose to occupy a fenced exclosure which was under a different grazing regime to that of the study area (removed from analyses as explained below).

GPS collars (GPS_2200R, Lotek Wireless, Ontario, Canada) weighing 720 g were used to track ewes. Collars were programmed to record locations at 10-min intervals using scheduling software (GPSHOST, Lotek Engineering, Ontario, Canada) and current satellite almanac files from Lotek. Locations were stored onboard the collar and retrieved after 5 weeks. This was the maximum time taken for recordings to cease, either through battery pack expiry or data storage capacity (5028 differential locations) being reached [43].

Location data were downloaded to a PC and corrected to increase accuracy using post-differential correction software (N4, V.1.2138, Lotek Engineering, Ontario, Canada) and files from the nearest active base station 54 km distant (NUI Galway Base Station, Ordnance Survey Ireland, www.osi.ie). Post-differentially corrected GPS data have an accuracy of approximately 7 m radius [41]. Locations with a position dilution of precision value of more than 10 were excluded to further increase accuracy without excessive loss of GPS data [13, 42].

Habitat condition and grazing lawn frequency surveys

Habitat condition of the study area was originally mapped in 1999 [5] and ground-truthed for change in 2005. Assessment was made following the method described by [18] (now the National Parks and Wildlife Service) which uses six condition categories; undamaged, moderate-undamaged, moderately damaged, moderately-severely damaged, severely damaged and very severely damaged. These six categories are combined into three groups; undamaged, moderately damaged and severely damaged. Habitat condition indicators include but are not exclusive to grazing-related damage and are defined for each habitat type. Indicators include vegetation cover and growth, particularly the cover and condition of *Calluna vulgaris* (L.) Huds and the cover of *Nardus stricta* (L.), species richness, sward height, exposed soil and evidence of burning.

Grazing lawn frequencies were allocated for each complete or part 100x100 m grid square in 2005. Categories of grazing lawn patches (~4-7 m^2 or the equivalent area) were; (i) none, (ii) few (1-5 patches), (iii) several (6-10) and (iv) numerous/extensive (>10). In the instance of a part grid square, on the boundary of the study area, it was classified as numerous/extensive if acid grassland occupied $\geq$0.3 of its area.

The habitat condition and grazing lawn frequency maps were digitised using geographical information system software (ArcGIS Desktop, V.9.1, ESRI Inc., Redlands, CA, USA).

Data analysis

Data handling

Typically in wildlife tracking studies, animals are caught, tagged and released within their home range. However in this study, the flock was brought in from the study area, collars were fitted to selected ewes in an adjacent yard and then sheep were released from the yard and left to make their own way back to their chosen areas. To explore habitats selected by sheep and filter out data directly influenced by handling, the first 3 days after release were excluded. This 3-day period was chosen objectively by identifying core areas as 95% cores from inflections on cluster polygon incremental analysis plots [40] and scrutinising location data against 95% polygons to identify the time taken for sheep to leave the yard, reach a 95% polygon and stay there for longer than an overnight stop en route (taken as 11 h 51 min, the annual mean non-daylight hours). The longest time taken by an individual was 2 days 23 h, rounded to 3 days.

Spatial and temporal autocorrelation of GPS data

Locations from the same individual are not independent data, therefore tests for resource selection were based on summary statistics of resource use from the individual [38, 39] using compositional analysis. This allows comparison of both multiple individuals and resource categories in the same test [1]. Of the 36 ranges from nine tracking periods, 11 individuals were tracked and consequently 11 ranges (one per individual) were potentially suitable for compositional analysis. Range selection was made objectively, regardless of season, based on Minimum Convex Polygon (MCP) incremental analysis which is indicative of range stability [39], i.e. ranges where ewes were most settled were selected. Two individuals were omitted because; (a) dynamic interaction analyses [39] showed only two of 11 ranges were non-independent (Jacob's index was 0.769 based on geometric mean distances between same-time locations) so only one range (the most stable) of the pair was used and (b) one individual chose to occupy a fenced exclosure outside the study area that was under a different management regime, affecting resource classification. The final sample size was nine because (a) data that breach analysis assumptions of data independence or misrepresent typical habitat availability to the flock were rigorously omitted and (b) the cost of the tracking collars and replacement battery packs for each sampling period was a limiting factor. Sample sizes for seasonal tests differ and are explained below.

Ranges of individuals tracked in more than one season were suitable for separate seasonal tests. However, ranges were also included in seasonal analyses where the same individual was tracked for the same season for more than one sampling year. This may breach test assumptions of data independence but these ranges are included to minimise omission of data as independence could not be tested. Six of the original 36 ranges were omitted because the same individual repeatedly occupied an area outside the study area, and two further ranges were omitted as dynamic interaction analyses indicated that data (from three pairs of ranges consisting of three individuals and three ranges) were not independent (Jacob's index >0.5). Therefore, 28 ranges were suitable for seasonal tests.

Datasets

Seven datasets were produced in this study (*Table 1*). The 'Complete' dataset contained all post-processed data for the nine independent ewes. This dataset was then subdivided to form two additional datasets, 'Diurnal' and 'Nocturnal', based on mean sunrise and sunset times for each range. Seasonal datasets were produced that contained all post-processed data, excluding three days post-release, for 28 ranges divided between the four seasons.

Table 1. *Composition of datasets*

Dataset	No. of ranges	No. of individuals	No. of years sampled	Total no. of locations
Complete	9	9	3	21 250
Diurnal	9	9	3	11 073
Nocturnal	9	9	3	10 177
Spring	9	8	3	14 353
Summer	7	4	2	20 967
Autumn	6	3	2	17 359
Winter	6	3	2	15 070

Range and resource analysis

The tracked ewe location data, habitat condition map and grazing lawn frequency map were imported into range analysis software (Ranges7, Anatrack Ltd, Dorset, UK). MCPs were produced which link the outermost locations and are widely used as a broad estimate of animal ranges (e.g. [1]). The boundary of the study area, i.e. the area available to animals, was determined by a stockproof fence in this instance. Habitat condition and grazing lawn frequency analyses were performed using Ranges7 to estimate (i) the resource content of the study area, (ii) resource content of ranges (i.e. MCPs) and (iii) resource use at location.

Statistical analysis

Selection of habitat condition and grazing lawn frequency categories was examined using weighted compositional analysis (Compos Analysis V.6.2+, Smith Ecology Ltd, Abergavenny, UK). Proportions of resource use were compared with those available, using Wilks' lambda (Λ) test (MANOVA). Analyses were carried out at two selection levels based on selection levels identified by [36]:

(i) broad, comparing proportions of resources present within ewe ranges with those available in the study area accessible to sheep, and

(ii) detailed, comparing proportions of resources used at location with those available within individual ewe ranges.

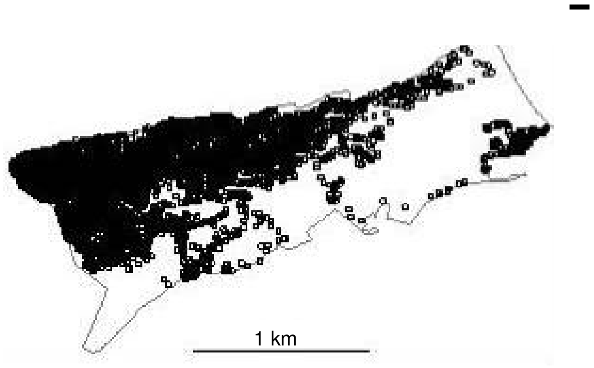
It is unlikely that resource use and available percentage data follow a multivariate normal distribution, hence randomisation tests were used to evaluate the significance of Λ and t values [1].

Results

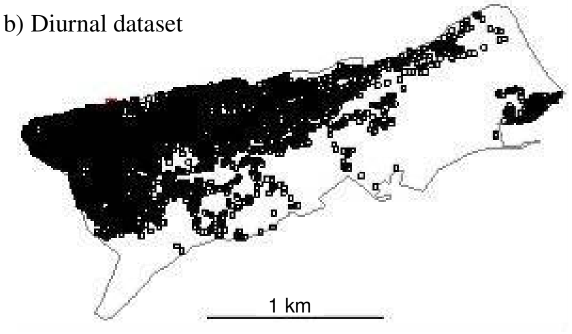
Ewe locations

Most occupation during day, night and year-round was in the northwest quarter of the study area (*Figs 1, 2*). Nocturnal locations were distinctly more clustered than diurnal locations (*Fig. 1b, c*). While seasonal distribution patterns could not be compared in this instance because the individuals tracked and number of individuals and ranges differ (*Table 1*), *Figure 2* indicates a tendency towards seasonal variation.

a) Complete dataset with diurnal and nocturnal locations combined



b) Diurnal dataset



c) Nocturnal dataset

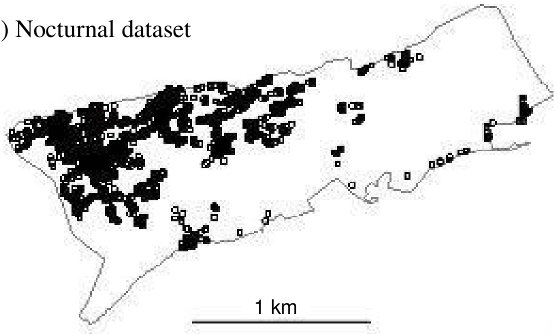
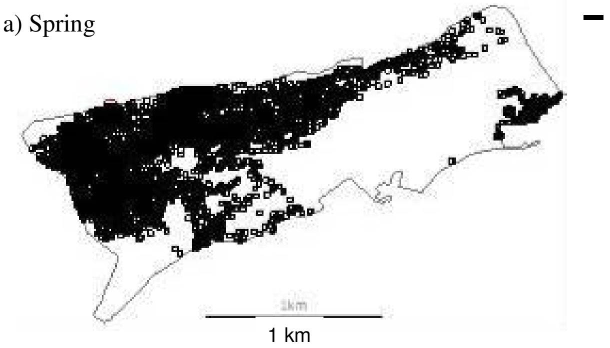
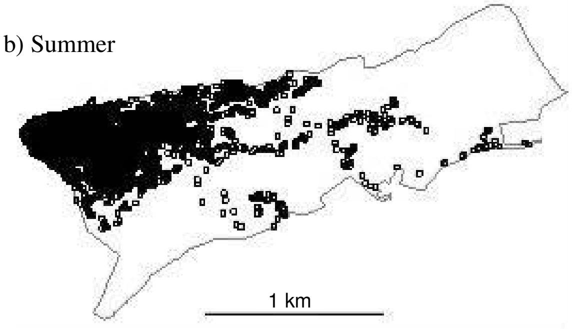


***Figure 1.** Location data of nine ewes tracked between February 2004 and April 2006 using GPS collars.*

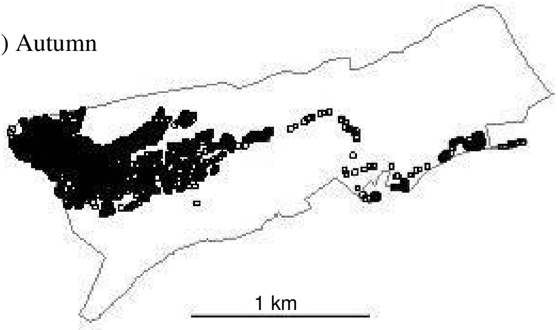
a) Spring



b) Summer



c) Autumn



d) Winter

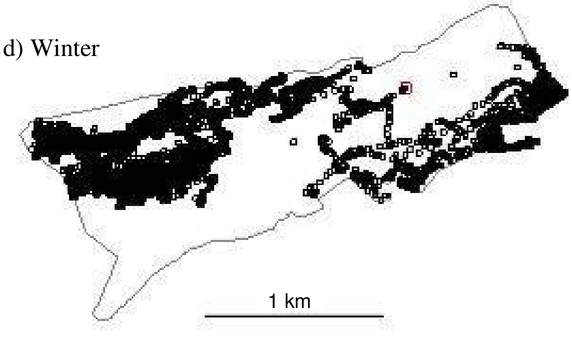


Figure 2. *Seasonal location data of ewes tracked between February 2004 and April 2006 using GPS collars.*

Grazing lawn frequency

The dominant categories of grazing lawn frequency available across the study area were few (36.8%) and numerous/extensive (34.3%), (*Table 2a*). Distribution of grazing lawns was clearly uneven with the most numerous/extensive occurring in the northwest corner (*Fig. 3*). Use was significantly non-random ($P<0.05$) with numerous/extensive patches consistently selected most at broad and detailed selection levels, day and night, and across all four seasons (*Table 3*). There was slight variation in sequences of the subsequent three ranks between broad and detailed selection levels (*Table 3*), day and night (*Table 3a, b*), summer and the other three seasons at the broad level (*Table 3c*), and between autumn and the other three seasons at the detailed level (*Table 3d*). 'None' was consistently selected least at the broad level for all seven datasets (*Table 3a, c*) but mostly this was not the case at the detailed level (*Table 3b, d*).

Table 2. *Availability of grazing lawns and habitat conditions in the 216.9 ha study area.*

Category	% available	Group
a) Grazing lawn frequency		
None	11.4	n/a
Few (0-5)	36.8	n/a
Several (6-10)	17.4	n/a
Numerous (>10)/extensive	34.3	n/a
b) Habitat conditions		
Undamaged (U)	29.5	Undamaged (U)
Moderate-undamaged (MU)	22.3	Moderately damaged (M)
Moderately damaged (M)	21.0	Moderately damaged (M)
Moderate-severely damaged (MS)	5.5	Moderately damaged (M)
Severely damaged (S)	2.0	Severely damaged (S)
Very severely damaged (VS)	19.8	Severely damaged (S)

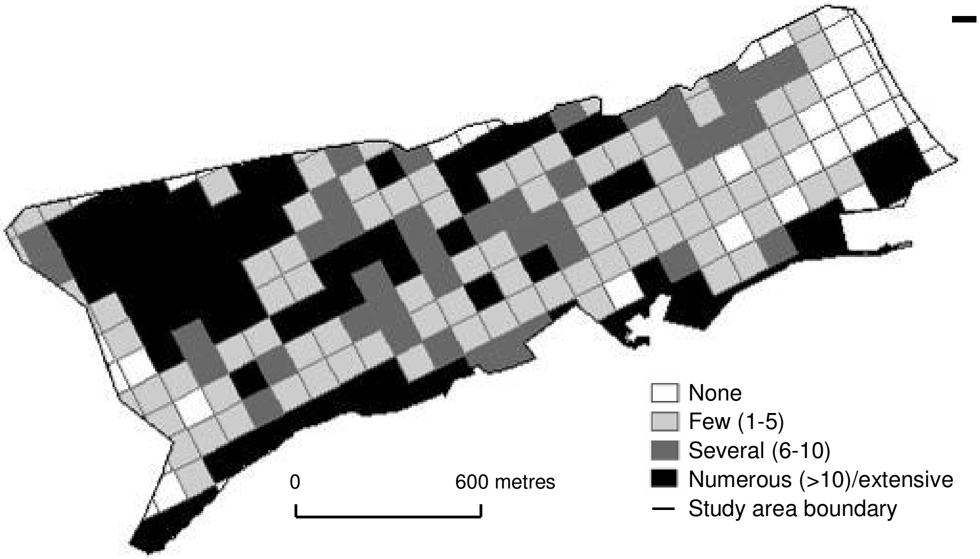


Figure 3. *Distribution of grazing lawns by grid square.*

Table 3. *Tests for random use, by Scottish Blackface hill sheep, of grid squares consisting of varying grazing lawn frequencies. The grazing lawn frequency ranking is shown in parentheses when Λ is not significant and '>>>' denotes a significant difference between two consecutively ranked frequency categories.*

Dataset	**Randomness test** **Λ**	 ***P***	**Grazing lawn frequency rankings (most>least selected)**	**% of total locations**
a) Nine individuals, broad selection level (MCP[a] vs. study area)				
Complete	0.293	0.015 *	Numerous >>> several > few >>> none	100.0
Diurnal	0.270	0.005 **	Numerous >>> several > few >>> none	100.0
Nocturnal	0.280	0.003 **	Numerous >>> few > several > none	100.0
b) Nine individuals, detailed selection level (locations vs. MCP)				
Complete	0.111	0.005 **	Numerous > none > several > few	100.0
Diurnal	0.138	0.027 *	Numerous > none > several > few	100.0
Nocturnal	0.187	0.006 **	Numerous >>> several > none > few	100.0
c) Seasonal use, broad selection level (MCP vs. study area)				
Spring	0.237	0.006 **	Numerous >>> few > several >>> none	100.0
Summer	0.021	0.042 *	Numerous >>> several > few >>> none	100.0
Autumn	0.189	0.127	(Numerous > few > several >>> none)	100.0
Winter	0.362	0.285	(Numerous > few > several >>> none)	100.0
d) Seasonal use, detailed selection level (locations vs. MCP)				
Spring	0.149	0.036 *	Numerous > none > several > few	100.0
Summer	0.185	0.116	(Numerous > none > several > few)	100.0
Autumn	0.059	0.026 *	Numerous > several > few > none	100.0
Winter	0.151	0.185	(Numerous > none > several > few)	100.0

Habitat condition

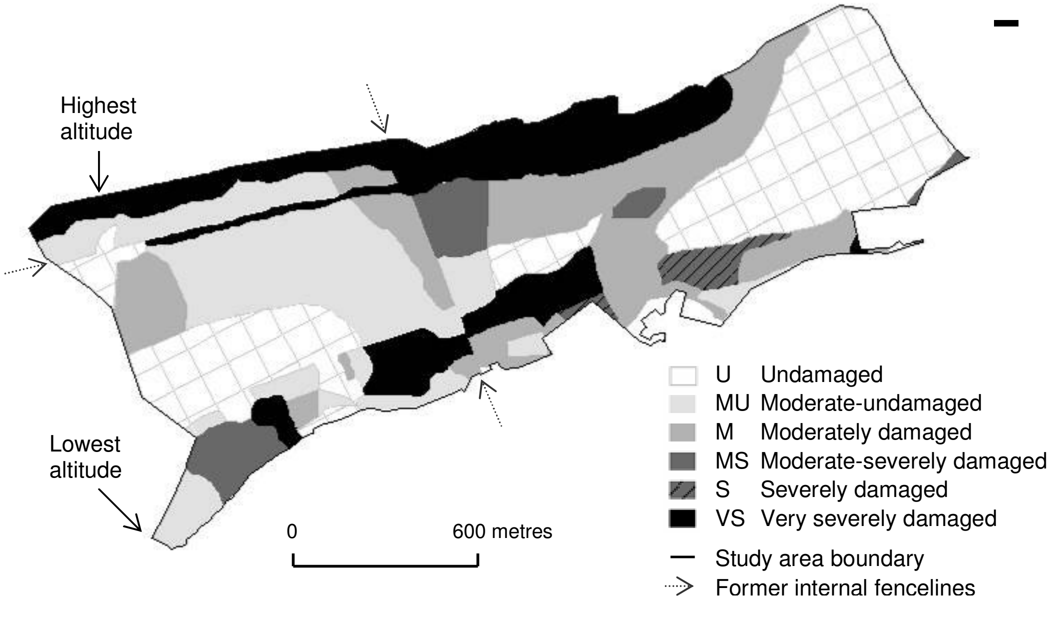


Figure 4. *Habitat condition map of the study area.*

All six categories of habitat condition, from undamaged to very severely damaged, occurred across the study area (*Table 2b, Fig. 4*). The most severe damage was associated mainly with blanket bog on the mountain ridge (northern boundary) and also, in places, at low altitude (*Fig. 4*). Two former internal fencelines were evident on the habitat condition map. An initial visual comparison between the sheep occupation and habitat condition figures suggested that the most abundant habitat condition in the northwest quarter of the study area, where sheep occupation tended to be concentrated, was moderately damaged.

Use of habitat condition categories was random for 20 of 28 tests which suggests that selection by ewes was not significant ($P>0.05$), (*Table 4, 5*). Nevertheless, rank sequences are still thought to be meaningful [61]. The moderate-undamaged category was consistently selected most at the broad and detailed levels, day and night, and across all seasons with the exception of autumn at the detailed level where it was the second-most selected category (*Table 4a, b; Table 5a, b*). Very severely damaged areas were second-most selected with nine ewes at the broad level and in summer at both levels (*Table 4a; Table 5a, b*), selected least for the remaining three seasons at the detailed level (*Table 5b*), of intermediate ranks at the detailed level with nine ewes (*Table 4b*) and of various ranks for seasons at the broad level (*Table 5a*). Undamaged areas were selected considerably more within ewe ranges by day than at night when they were selected least (*Table 4b*).

Table 4. *Tests for random use of habitat condition categories by nine individual Scottish Blackface hill sheep based on three datasets. The habitat condition ranking is shown in parentheses when Λ is not significant and '>>>' denotes a significant difference between two consecutively ranked condition categories.*

Dataset	**Randomness test** **Λ**	 ***P***	**Habitat condition rankings (most>least selected)**[a]	**% of total locations**
a) Broad selection level (MCP[b] vs. study area) with all six categories				
Complete	0.266	0.172	(MU > VS > M > MS > U > S)	100.0
Diurnal	0.156	0.033 *	MU > VS > M > MS > U > S	100.0
Nocturnal	0.217	0.120	(MU > VS > M > MS > S > U)	100.0
b) Detailed selection level (locations vs. MCP) with all six categories				
Complete	0.000	0.065	(MU > M > VS > U > MS > S)	100.0
Diurnal	0.005	0.023 *	MU > U > M > VS > MS > S	100.0
Nocturnal	0.000	0.032 *	MU > M > VS > MS > U > S	100.0
c) Broad selection level (MCP[b] vs. study area) with three condition groups				
Complete	0.535	0.104	(M>S>>>U)	100.0
Diurnal	0.547	0.114	(M>S>>>U)	100.0
Nocturnal	0.391	0.045 *	M>S>>>U	100.0
d) Detailed selection level (locations vs. MCP) with three condition groups				
Complete	0.312	0.030 *	M>S>U	100.0
Diurnal	0.456	0.105	(M>U>S)	100.0
Nocturnal	0.650	0.672	(M>U>S)	100.0

[a] U = Undamaged area, MU = Moderate-Undamaged area, M = Moderately damaged area, MS = Moderate-Severely damaged area, S = Severely damaged area, VS = Very Severely damaged area.
[b] MCP = Minimum Convex Polygon, used to estimate ewe ranges.

Severely damaged and moderate-severely damaged categories were omitted from seasonal analyses at the detailed level (*Table 5b*) because low use prohibited analyses from running, hence tests were repeated with data for all six categories combined into three condition groups (*Table 2*). Consistent with findings using six categories where moderate-undamaged was generally most selected, the moderately damaged group was selected most at both selection levels, day, night and year-round except in summer at the broad selection level when severely damaged areas were most selected (*Table 4c, d; Table 5c, d*).

Table 5. *Seasonal tests for random use of habitat condition categories by Scottish Blackface hill sheep. The habitat condition ranking is shown in parentheses when Λ is not significant and '>>>' denotes a significant difference between two consecutively ranked condition categories.*

Dataset	**Randomness test** **Λ**	 ***P***	**Habitat condition rankings (most>least selected)**[a]	**% of total locations**
a) Broad selection level (MCP[b] vs. study area) with all six categories				
Spring	0.105	0.066	(MU > M > VS > U > MS > S)	100.0
Summer	0.004	0.067	(MU > VS >>> U > M > S > MS)	100.0
Autumn	0.000	0.031 *	MU >>> U > M > VS > S >>> MS	100.0
Winter	0.047	0.318	(MU > M >>> U > MS > VS > S)	100.0
b) Detailed selection level (locations vs. MCP) with all six categories				
Spring	0.296	0.179	(MU > M > U > VS)	100.0
Summer	0.255	0.164	(MU > VS > M > U)	100.0
Autumn	0.198	0.283	(U > MU > M > VS)	100.0
Winter	0.434	0.448	(MU > M > U > VS)	100.0
c) Broad selection level (MCP vs. study area) with three condition groups				
Spring	0.431	0.013 *	M>>>S>U	100.0
Summer	0.404	0.095	(S>M>>>U)	100.0
Autumn	0.244	0.082	(M>>>U>S)	100.0
Winter	0.442	0.028 *	M>U>S	100.0
d) Detailed selection level (locations vs. MCP) with three condition groups				
Spring	0.346	0.151	(M>U>S)	100.0
Summer	0.565	0.286	(M>S>U)	100.0
Autumn	0.198	0.070	(M>U>S)	100.0
Winter	0.304	0.168	(M>U>S)	100.0

[a] U = Undamaged area, MU = Moderate-Undamaged area, M = Moderately damaged area, MS = Moderate-Severely damaged area, S = Severely damaged area, VS = Very Severely damaged area.
[b] MCP = Minimum Convex Polygon, used to estimate ewe ranges.

Discussion

Sheep distribution

Sheep occupation being concentrated in the northwest quarter of the study area is probably attributable directly and indirectly to topography with the highest elevation, steep slopes, shelter-providing bowl-like features, shallower and better-drained soils and the most extensive patches of relatively better forage quality and availability than elsewhere in the area [4, 17, 57, 61].

Nocturnal GPS location data were more clustered than diurnal locations which was expected as sheep are well known to be active mostly during daylight hours and to rest at night [20, 59, 61]. Whilst sheep of other breeds have been reported to usually congregate in the same places at night [3], Scottish Blackface individuals do not return to the same place every night [31]. This is consistent with this study's findings of multiple nocturnal rest sites for nine tracked individuals (*Fig. 1c*). This probably does not have implications for management because, although occupying different nocturnal rest sites implies dispersal was greater than if sheep returned to the same sites, individuals have been found to occupy just 9-20% of this study area [61].

Grazing lawn frequency

Grid squares with numerous/extensive grazing lawns were associated with a range of features, i.e. a large bowl-like feature and adjacent hillock in the northwest corner, the riverbank on the western half of the southern boundary, farm tracks and earth banks along the eastern half of the southern boundary, and scattered hillocks, rock outcrops and/or sloping ground with relatively well-drained soils. Absence of grazing lawns was typically associated with areas of waterlogged, deep, quaking peat on relatively level ground, and occasionally with very steep north-facing slopes inaccessible to sheep on the northern boundary.

The most extensive grazing lawns were found in the northwest of the study area which correlates with sheep occupation patterns. Numerous/extensive patches were consistently selected most, probably because acid grassland habitats are preferred most by sheep on hills [12, 60, 61] and are a product of heavy grazing [25, 45].

Sheep most selected grid squares containing numerous/extensive grazing lawns even in winter which is inconsistent with possible expectations from reviewing literature as resources on favoured patches deplete after the growing season [35] and grazing lawns on this study site were previously found to be dominated by *Molinia caerulea* [51] which dies back in early autumn. Selection in winter of grid squares with numerous/extensive grazing lawns is probably explained by Scottish Blackface sheep being known to return to home ranges that are learned from dams [32] and to graze in close proximity to grassland patches [12]. Sheep were probably utilising alternative habitats as grid squares that contained numerous/extensive grazing lawns usually supported and may have been dominated by different habitats, particularly wet heath.

Habitat condition

Wind-driven rain and its effect on soil erosion is reported to be most severe close to the top of a hill [11], and this probably contributed to much of the damage associated with the mountain ridge along the northern boundary of the study site. The thin strip of severely damaged habitats running parallel to this in the northwest quarter marked a former fenceline (that was removed in 2001) along which poaching and sheep paths were observed, where sheep traversed the hill between shelter-providing bowl-like features dominated by acid grassland. Two severely damaged compartments on the southern boundary were associated with deep peat, relatively level ground, waterlogging most of the year, former peat extraction in places and routes habitually taken when sheep were gathered and released from the yard.

Grazers can facilitate damage to upland habitats, conversely, they increase diversity in plant species composition and structure and the fauna this supports [55]. For this reason, and because habitat condition assessment took flora diversity into account, the northwest area most occupied by sheep was predominantly classified as undamaged to moderately damaged. While increasing diversity has ecological benefits, acid grassland patches replace wet heath [27, 56, 57] which, unlike acid grassland, is listed under

Annex I of the Habitats Directive. Some acid grassland is desirable but the spread of grassland patches is likely and poses a threat to heathland communities [12].

Use of areas with differing categories of habitat condition was mostly random, which indicates there was not a direct correlation between sheep occupation and habitat condition. This is partly attributable to other factors of erosion that are not grazing-related and partly because the carrying capacity of different plant communities and topography vary. It is speculated that the main reasons are diet selection as discussed below and that the mapping accuracy of vegetation condition (following the guidelines) is much lower than that of the GPS location data. Additionally, a limitation of this study is that it is based on detailed information from only a small number of individuals, causing low power in statistical tests. However, this study justifies further research with larger sample sizes.

Findings that support possible expectations that use of damaged areas by sheep would be low include the selection ranking being lowest for very severely damaged areas in all seasons except summer and highest for moderate-undamaged areas in all seasons. Conversely, very severely damaged areas had the second-highest selection rank in several tests, and most tests for habitat condition selection were not significant ($P>0.05$), suggesting that sheep did not avoid damaged areas.

Very severely damaged areas had the second-highest selection rank in summer at both selection levels and the severely damaged condition group was most selected at the broad selection level in summer. This is consistent with the notion that hill sheep like the ‘bare bite’, i.e. sheep graze bare areas despite the availability of lush vegetation patches. This could be explained by selective grazing for preferred species during the growing season as [30] reported high proportions of *Narthecium ossifragum* (L.) Huds and *Eriophorum spp.* (L.) in the diets of Scottish Blackface sheep grazing blanket bog, and these species were dominant on areas of exposed peat (i.e. severely damaged areas) in this study.

Implications for conservation

Based on the finding that sheep consistently selected grid squares containing numerous/extensive grazing lawns, an efficient indicator of hill use by sheep would be to map acid grassland frequency following the simple method introduced in this study. This could have a secondary purpose of identifying areas of wet or dry heath most at risk of grazing-related damage, as most heather damage is known to occur within 5 m of grassland patch edges [12]. Grazing lawns contribute to the biodiversity value of a site [53] but are a threat to heathland communities that have been identified as being more important for conservation [21]. Determining correct stock numbers and management practices are crucial to prevent an increase in the number or extent of grazing lawns and to meet conservation objectives. Limits of acceptable change should be set for the proportion of grazing lawns and the extent of grazing lawns should be monitored.

The full spectrum of habitat conditions from undamaged to very severely damaged areas occurring on one site, combined with uneven use by grazers with unlimited access, supports the widely held belief that a single stocking rate for upland and peatland sites is inadequate. Stocking rate calculations need to be based on relative proportions of habitats available and habitat condition, as recognised by [49], and habitat selection exhibited by grazers [61]. In light of grazer distribution not corresponding directly to habitat conditions, stocking rates calculated at site level are probably still not enough to arrest and reverse grazing-related damage because areas of different categories of habitat condition require different action.

Various recommendations have been made to arrest damage to vegetation and promote recovery. A reduction in sheep numbers is often recommended [3, 7, 34]. Additional recommendations include sheep exclusion from severely damaged areas until vegetation has recovered [8, 54], cutting thrice-yearly of undamaged, but arguably undergrazed, *Molinia caerulea* (L.) Moench-dominant areas to promote recovery by *Calluna vulgaris* (L.) Hull [47] and mixed grazing of cattle or goats with sheep [10, 33]. However, cutting would not be a practical option on a site such as that studied with deep peats and steep slopes prohibiting tractor access, and grazing cattle would need to be restricted to periods when water levels are low to minimise poaching by heavier animals. Benefits from allowing vegetation to recover would include improved ecological, landscape and livestock production quality.

To conclude, a comprehensive understanding of resource use by grazers is fundamental in designing conservation strategies and management planning should consider habitat condition and grazer distribution as previously recognised. This study presents an efficient method for realising this objective, based on hill sheep selection of grid squares containing higher frequencies of grazing lawns. Use of very severely damaged areas was greatest in summer during the plant growing season and therefore inhibiting vegetation recovery. Temporary exclusion of livestock or reducing stocking rates during this season would facilitate an improvement in habitat condition. However, management recommendations cannot be inferred beyond the study area without further research on a number of sampling sites and with different habitat condition assemblages. The methods presented, including the rapid mapping of grazing lawn frequency introduced in this study and easily collecting vast, accurate data using GPS devices, are recommended for use in such studies in the future.

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REFERENCES

[1] Aebischer, N.J., Robertson, P.A., Kenward, R.E. (1993): Compositional analysis of habitat use from animal radio-tracking data. – Ecology 74: 1313-1325.

[2] Armstrong, H.M., Gordon, I.J., Hutchings, N.J., Illius, A.W., Milne, J.A., Sibbald, A.R. (1997): A model of the grazing of hill vegetation by sheep in the UK. II. The prediction of offtake by sheep. – Journal of Applied Ecology 34: 186-207.

[3] Bakker, J.P., de Bie, S., Dallinga, J.H., Tjaden, P., de Vries, Y. (1983): Sheep-grazing as a management tool for heathland conservation and regeneration in the Netherlands. – Journal of Applied Ecology 20: 541-560.

[4] Berggren-Thomas, B., Hohenboken, W.D. (1986): The effects of sire-breed, forage availability and weather on the grazing behavior of crossbred ewes. – Applied Animal Behaviour Science 15: 217-228.

[5] Bleasdale, A., Heffron, J. (1999): A Vegetation Condition Assessment of the Teagasc Hill Sheep Farm, Erriff, Co. Mayo. – Dúchas and Department of Agriculture and Food, Dublin.

[6] Bleasdale, A., Sheehy Skeffington, M. (1992): The influence of agricultural practices on plant communities in Connemara. – In: Feehan, J. (ed.) Environment and Development in Ireland, University College Dublin, Dublin.

[7] Bleasdale, A., Sheehy Skeffington, M. (1995): The upland vegetation of north-east Connemara in relation to sheep grazing. – In: Jeffrey, D.W., Jones, M.B., McAdam, J.H. (eds.) Irish Grasslands: Their Biology and Management, Royal Irish Academy, Dublin.

[8] Britton, A.J., Pearce, I.S.K., Jones, B. (2005): Impacts of grazing on montane heath vegetation in Wales and implications for the restoration of montane areas. – Biological Conservation 125: 515-524.

[9] Cadbury, C.J. (1987): Moorland birds - Britain's international responsibility. – In: Cadbury, C.J., Everett, M. (eds.) RSPB Conservation Review 1987, Royal Society for the Protection of Birds, Sandy.

[10] Celaya, R., Oliván, M., Ferreira, L.M.M., Martínez, A., García, U., Osoro, K. (2007): Comparison of grazing behaviour, dietary overlap and performance in non-lactating domestic ruminants grazing on marginal heathland areas. – Livestock Science 106: 271-281.

[11] Choi, E.C.C. (2002): Modelling of wind-driven rain and its soil detachment effect on hill slopes. – Journal of Wind Engineering and Industrial Aerodynamics 90: 1081-1097.

[12] Clarke, J.L., Welch, D., Gordon, I.J. (1995): The influence of vegetation pattern on the grazing of heather moorland by red deer and sheep. I. The location of animals on grass/heather mosaics. – Journal of Applied Ecology 32: 166-176.

[13] D'Eon, R.G., Delparte, D. (2005): Effects of radio-collar position and orientation on GPS radio-collar performance, and the implications of PDOP in data screening. – Journal of Applied Ecology 42: 383-388.

[14] Defra (2007): Environmental protection. <www.defra.gov.uk/environment/index.htm> [Last accessed November 2007].

[15] Dennis, P., Skartveit, J., McCracken, D.I., Pakeman, R.J., Beaton, K., Kunaver, A., Evans, D.M. (2008): The effects of livestock grazing on foliar arthropods associated with bird diet in upland grasslands of Scotland. – Journal of Applied Ecology 45: 279-287.

[16] Department of Arts Heritage Gaeltacht and the Islands (2002): National Biodiversity Plan. – Government of Ireland, Dublin.

[17] Doyle, G.J. (1982): The vegetation, ecology and productivity of Atlantic blanket bog in Mayo and Galway, western Ireland. – Journal of Life Sciences Royal Dublin Society 3: 147-164.

[18] Dúchas the Heritage Service, the Department of Agriculture and Food (1999): A Manual for the Production of Grazing Impact Assessments in Upland and Peatland Habitats. – Dúchas the Heritage Service & the Department of Agriculture and Food, Dublin.

[19] Dumont, B., Boissy, A. (2000): Grazing behaviour of sheep in a situation of conflict between feeding and social motivations. – Behavioural Processes 49: 131-138.

[20] England, G.J. (1954): Observations on the grazing behaviour of different breeds of sheep at Pantyrhaud Farm, Carmarthenshire. – British Journal of Animal Behaviour 2: 56-60.

[21] European Commission (2007): EU Nature Legislation. <http://ec.europa.eu/environment/nature/legislation/index_en.htm> [Last accessed November 2007].

[22] European Environmental Advisory Councils (1999): Annex: Agriculture and environment in eight European countries. <http://www.eeac-net.org/workgroups/pdf/WGAgriculture_Agenda200_Proposals_1999_Annex.pdf> [Last accessed May 2007].

[23] Evans, D.M., Redpath, S.M., Elston, D.A., Evans, S.A., Mitchell, R.J., Dennis, P. (2006): To graze or not to graze? Sheep, voles, forestry and nature conservation in the British uplands. – Journal of Applied Ecology 43: 499-505.

[24] Fenton, E.W. (1937): The influence of sheep on the vegetation of hill grazings in Scotland. – Journal of Ecology 25: 424-430.

[25] Fossitt, J.A. (2000): A Guide to Habitats in Ireland. – The Heritage Council, Kilkenny.

[26] Gardiner, M.J., Radford, T. (1980): Soil Associations of Ireland and their Land Use Potential. Soil Survey Bulletin No. 36 –An Foras Talúntais, Dublin.

[27] Gillmor, D.A., Walsh, J.A. (1993): County-level variations in agricultural adjustment in Ireland in the 1980s. – Geographical Viewpoint 21: 25-44.

[28] Ginane, C., Dumont, B. (2006): Generalization of conditioned food aversions in grazing sheep and its implications for food categorization. – Behavioural Processes 73: 178-186.

[29] Grant, S.A., Bolton, G.R., Torvell, L. (1985): The responses of blanket bog vegetation to controlled grazing by hill sheep. – Journal of Applied Ecology 22: 739-751.

[30] Grant, S.A., Torvell, L., Smith, H.K., Suckling, D.E., Forbes, T.D.A., Hodgson, J. (1987): Comparative studies of diet selection by sheep and cattle: blanket bog and heather moor. – Journal of Ecology 75: 947-960.

[31] Hewson, R., Verkaik, A.J. (1981): Body condition and ranging behaviour of Blackface hill sheep in relation to lamb survival. – Journal of Applied Ecology 18: 401-415.

[32] Hewson, R., Wilson, C.J. (1979): Home range and movements of Scottish Blackface sheep in Lochaber, north-west Scotland. – Journal of Applied Ecology 16: 743-751.

[33] Hodgson, J., Forbes, T.D.A., Armstrong, R.H., Beattie, M.M., Hunter, E.A. (1991): Comparative studies of the ingestive behaviour and herbage intake of sheep and cattle grazing indigenous hill plant communities. – Journal of Applied Ecology 28: 205-227.

[34] Hulme, P.D., Merrell, B.G., Torvell, L., Fisher, J.M., Small, J.L., Pakeman, R.J. (2002): Rehabilitation of degraded *Calluna vulgaris* (L.) Hull-dominated wet heath by controlled sheep grazing. – Biological Conservation 107: 351-363.

[35] Hunter, R.F. (1962): Hill sheep and their pasture: a study of sheep-grazing in south-east Scotland. – Journal of Ecology 50: 651-680.

[36] Johnson, D.H. (1980): The comparison of usage and availability measurements for evaluating resource preference. – Ecology 61: 65-71.

[37] Joint Nature Conservation Committee (2007): UK Biodiversity Action Plan. <www.ukbap.org.uk> [Last accessed December 2007].

[38] Kenward, R.E. (1992): Quantity versus quality: programmed collection and analysis of radio-tracking data. – In: Priede, I.G., Swift, S.M. (eds.) Wildlife Telemetry: Remote Monitoring and Tracking of Animals, Ellis Horwood, Chichester.

[39] Kenward, R.E. (2001): A Manual for Wildlife Radio Tagging. – Academic Press, London.

[40] Kenward, R.E., Clarke, R.T., Hodder, K.H., Walls, S.S. (2001): Density and linkage estimators of home range: nearest-neighbor clustering defines multinuclear cores. – Ecology 82: 1905-1920.

[41] Lotek Wireless (2000): N4 Differential Post-processing Software Users' Manual. – Lotek Wireless, Ontario.

[42] Lotek Wireless (2001): Small Animal GPS Location System: User's Manual: GPS 2000 Series. – Lotek Wireless, Ontario.

[43] Lotek Wireless (2004): Product sheet: GPS_2200 collar for livestock. <www.lotek.com/gps_2200l.pdf> [Last accessed April 2007].

[44] MacDonald, A., Stevens, P., Armstrong, H., Immirzi, P., Reynolds, P. (1998): A Guide to Upland Habitats: Surveying Land Management Impacts. Volume 1, Background Information and Guidance for Surveyors. – Scottish Natural Heritage, Perth.

[45] McNaughton, S.J. (1984): Grazing lawns: animals in herds, plant form, and coevolution. – American Naturalist 124: 863-886.

[46] Met Éireann (undated): Climate of Ireland. <www.met.ie/climate/climate-of-ireland.asp> [Last accessed April 2007].

[47] Milligan, A.L., Putwain, P.D., Cox, E.S., Ghorbani, J., Le Duc, M.G., Marrs, R.H. (2004): Developing an integrated land management strategy for the restoration of moorland vegetation on *Molinia caerulea*-dominated vegetation for conservation purposes in upland Britain. – Biological Conservation 119: 371-385.

[48] Moravec, J., Zemeckis, R. (2007): Cross Compliance and Land Abandonment. Institute for European Environmental Policy, Brussels.

[49] National Parks and Wildlife Service (2005): Farm Plan Scheme for Designated Areas and Commonages: Specifications Document. – The National Parks and Wildlife Service, Dublin.

[50] National Parks and Wildlife Service (2008): The Status of EU Protected Habitats and Species in Ireland. Report available online: <www.npws.ie/en/PublicationsLiterature/HabitatsDirectivereport07>.

[51] O'Dowd, D. (2005): A study of the vegetation of sheep rests at Teagasc Hill Sheep farm, Leenaun, with particular reference to *Nardus stricta*. – Unpublished BSc Thesis, National University of Ireland, Galway.

[52] O'Toole, M.A. (1985): History, distribution and stocking intensities of hill sheep in Ireland. – Journal of Life Sciences Royal Dublin Society 5: 87-92.

[53] Pearce-Higgins, J.W., Grant, M.C. (2006): Relationships between bird abundance and the composition and structure of moorland vegetation. – Bird Study 53: 112-125.

[54] Rawes, M. (1983): Changes in two high altitude blanket bogs after the cessation of sheep grazing. – Journal of Ecology 71: 219-235.

[55] Rowell, T.A., Clarke, M.J. (1988): Grazing. – In: Rowell, T.A. (ed.) The Peatland Management Handbook: Research and Survey in Nature Conservation No. 14, Nature Conservancy Council, Peterborough.

[56] Thompson, D.B.A., MacDonald, A.J., Marsden, J.H., Galbraith, C.A. (1995): Upland heather moorland in Great Britain: a review of international importance, vegetation change and some objectives for nature conservation. – Biological Conservation 71: 163-178.

[57] van Eck, H., Govers, A., Lemaire, A., Schaminée (1984): Irish Bogs: a Case for Planning. – Katholieke Universiteit, Nijmegen.

[58] Walsh, M., Mulqueen, J., Grennan, E., Rogers, M., Marren, N., Guinan, L., Nixon, D., Collins, J.F. (2000): Evaluation of the Impact of Livestock on the Hill Environment. – End of Project Reports: Sheep Series No. 10. Project No. 4043. Teagasc Agriculture and Food Development Authority, Sheep Research Centre, Athenry.

[59] Warren, J.T., Mysterud, I. (1991): Summer habitat use and activity patterns of domestic sheep on coniferous forest range in southern Norway. – Journal of Range Management 44: 2-6.

[60] Welch, D. (1984): Studies in the grazing of heather moorland in north-east Scotland. I. Site descriptions and patterns of utilization. – Journal of Applied Ecology 21: 179-195.

[61] Williams, B. (2008): Resource selection by Scottish Blackface sheep on a mosaic of upland and peatland habitats: implications for conservation management. – Unpublished PhD thesis, National University of Ireland, Galway.

VORACITY OF COCCINELLID SPECIES ON DIFFERENT PHENOLOGICAL STAGES OF THE OLIVE PEST *SAISSETIA OLEAE* (HOMOPTERA, COCCIDAE)

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Abstract. Coccinellidae are well known predators in agroecosystems. In olive groves they may exert control against scales, such as the black scale, *Saissetia oleae* (Olivier, 1791). Laboratory studies on the consumption of three phenological stages (eggs, first and second instar nymphs) of *S. oleae* by four coccinellid species (*Chilocorus bipustulatus*, *Scymnus (Pullus) subvillosus*, *Scymnus (Mimopullus) mediterraneus* and *Scymnus (Scymnus) interruptus*) were carried out. *C. bipustulatus* presented a significantly high consumption of eggs, first and second instar nymphs compared with the other species. All coccinellids consumed eggs and first instar nymphs; however the second instar nymphs were only consumed by *S. interruptus* and *C. bipustulatus*. In a second experiment, larval stages of *C. bipustulatus* were reared on different phenological stages of *S. oleae*. Coccinellid larvae fed with eggs or first instar nymphs completed their life cycle, contrarily to those that were fed with second instar nymphs. The apparent voracity of *C. bipustulatus* on the different phenological stages of *S. oleae* is an aspect that suggests the possible use of this coccinellid species in biological control programs against this pest in olive groves.

Keywords: Predation, *Chilocorus bipustulatus*, *Pullus* (*Mimopullus) mediterraneus*, *Scymnus (Pullus) subvillosus*, *Scymnus (Scymnus) interruptus*, black scale.

Introduction

In the Mediterranean countries, the black scale, *Saissetia oleae* (Olivier, 1791), is an important pest in the olive grove and natural enemies belonging to the Coccinellidae family are known to be associated with this pest [1, 2, 3, 7]

Voracity studies intend to investigate the potential of a natural enemy to consume a specific prey. This elucidation about the predatory characteristics of coccinellids is the basis for developing management strategies to successfully combat black scale infestations. Successful natural control of pests depends on the fact that a predator kills or consumes a sufficient number of pest individuals to maintain its density at a low level [13]. Such information would be useful for determining which developmental stage of *S. oleae* is the most predated and will facilitate further laboratory rearing of these insects, which is a prime objective in a biological control program [12].

Coccinellid species like *Chilocorus bipustulatus* (L.), *Scymnus (Pullus) subvillosus* (Goeze), *Scymnus (Mimopullus) mediterraneus* Iablokoff-Khnzorian and *Scymnus* *interruptus* (Goeze) are common in the olive grove of the Mediterranean region and both adults and larvae are predaceous stages [1, 3]. Field studies showed that the most predated phenological stages of *S. oleae* by coccinellids were potentially the first and second instar nymphs (Santos *et al.*, unpublished data). Moreover, *S. oleae* eggs have been used to feed different coccinellid species in laboratory cultures [1, 2, 3]. Therefore, it is important to gain insight about the effective predated stages of *S. oleae* by coccinellids. This information will help on further studies concerning the natural control of *S. oleae* by coccinellids in field and also the maintenance of laboratory cultures of coccinellids reared on *S. oleae*.

The objective of this study was to investigate, under controlled conditions, the ability of selected coccinellid species to use different phenological stages of *S. oleae* as food item.

Material and Methods

To study the consumption of different phenological stages of *S. oleae* by coccinellid species, two experiments were carried out: (1) screening of coccinellids voracity using adult stages, and (2) assessment of feeding rates, along the life-cycle of the most voracious coccinellid species, using eggs, first instar and second instar nymphs of *S. oleae* as food items.

To perform the first experiment, specimens belonging to the four most common coccinellid species namely, *S. mediterraneus*, *S. subvillosus*, *S. interruptus* and *C. bipustulatus*, were captured by the beating technique in an organic olive grove - Valbom-dos-Figos (41º 33’ 4’’ N, 7º 8’ 43’’ W) - located near Mirandela, Trás-os-Montes (north-east of Portugal). The three different phenological stages of *S. oleae* (eggs, first and second instar nymphs) were collected with the leaf.

In the laboratory, 30 coccinellid specimens of each species were placed in individual Petri dishes (9 cm diameter x 2 cm height) with moistened filter paper covering the bottom of the box and starved during 24 hours.

Each species was separated in three groups of ten specimens. Each group was fed daily with a different phenological stage of *S. oleae* during five days: group 1 was fed with 100 eggs/day, group 2 with 10 first instar nymphs/day and the third group with 10 second instar nymphs/day. During five days, specimens were allowed to forage for 24 h after which the food was renewed and the total number of prey eggs, N1 nymphs or N2 nymphs was recorded.

Based on the results of the first experiment, *C. bipustulatus* was selected to perform the second experiment where the consumption of eggs and first and second instar nymphs of *S. oleae* by larval stages and adults of *C. bipustulatus* was studied.

C. bipustulatus adult specimens were captured in the same grove mentioned above. In the laboratory, they were coupled in Petri dishes (9 cm diameter x 2 cm height) with moistened filter paper covering the bottom of the box and fed with *S. oleae* eggs. Newly laid eggs were transferred individually to a clean Petri dish with a soft brush and daily observed till hatching. Forty-five recently emerged L_1 larvae were divided in three groups with fifteen specimens. Group 1 was supplied with 100 eggs/day, group 2 with 10 first instar nymphs/day and group 3 with 10 second instar nymphs/day. Each Petri dish was examined daily to record consumption. Moulting marked the differentiation between larval stages. Freshly emerged adults were used to make the same set of experiments as in larval stages.

All experiments were conducted at a temperature of 25±2° C and under a 16L: 8D h regime.

Data analysis

Univariate statistical analyses were performed using the Statistica Statistical package, version 7.0 [15]. Data were evaluated for normality and homogeneity of variances with Kolmogorov-Smirnov test and Bartlett's test, respectively. One-way ANOVA was used to compare the consumption of *S. oleae* phenological stages by each coccinellid species. A significant level of 0.05 was used for all statistical tests.

Results and Discussion

In the first experiment, the food intake by the four coccinellid species tested was maintained constant during the five days, except in the case of the consumption of the first instar nymphs by *S. interruptus* that varied significantly (*Table 1*). *C. bipustulatus* showed a significantly high consumption for all phenological stages of *S. oleae* with a mean total consumed eggs, first instar and second instar nymphs respectively of 381.3 ± 15.3 (mean ± standard error of the mean - SE, n = 10), 8.6 ± 0.7 and 15.3 ± 1.9, when compared with the other species tested (*Fig. 1*). *S. interruptus* consumed a mean total of 160.1 ± 6.9 eggs, 5.2 ± 0.7 first-instar nymphs and 0.3 ± 0.2 second-instar nymphs and *S. subvillosus* consumed 150.4 ± 20.5 eggs and 4.5 ± 1.2 first-instar nymphs. On the other hand, *S. mediterraneus* showed a significantly low consumption of eggs and the lowest consumption of the first instar nymphs, with a mean total of respectively 52.9 ± 4.5 and 2.8 ± 0.4, relatively with the other species. Second instar nymphs were not consumed by *S. mediterraneus* and *S. subvillosus*.

Table 1. *Consumption by coccinellids species (n = 10) of three phenological stages of Saissetia oleae (mean ± standard error of the mean) during five days of experiment.*

Coccinellid species	Food item	Days					$F_{4,45}$	*P*
		1	2	3	4	5		
Chilocorus bipustulatus	eggs	81.6±3.75	76.3±3.04	77.9±3.51	75.4±3.04	70.1±6.27	1.04	n.s.
	N1	2.6±0.40	1.8±0.44	1.5±0.27	1.2±0.25	1.5±0.34	2.37	n.s.
	N2	4.1±0.71	3.5±0.50	2.9±0.28	2.6±0.50	2.2±0.36	2.34	n.s.
Scymnus (Mimopullus) mediterraneus	eggs	10.5±1.89 ^{ab}	12.9±1.38 ^{ab}	13.9±1.55 ^{a}	7.5±1.30 ^{b}	8.1±1.49 ^{ab}	3.40	*
	N1	0.9±0.18	0.8±0.25	0.5±0.17	0.4±0.22	0.2±0.13	2.20	n.s.
	N2	0	0	0	0	0	-	-
Scymnus (Pullus) subvillosus	eggs	40.7±6.49	32.8±5.36	27.9±5.57	27±4.32	22±3.39	1.90	n.s.
	N1	1.5±0.31	0.9±0.28	0.7±0.33	0.7±0.37	0.7±0.40	1.04	n.s.
	N2	0	0	0	0	0	-	-
Scymnus (Scymnus) interruptus	eggs	30.7±5.34	36.6±3.63	33±3.16	34.6±3.56	25.2±3.91	1.21	n.s.
	N1	2±0.37 ^{a}	1.6±0.40 ^{ab}	0.8±0.20 ^{bc}	0.6±0.22 ^{bc}	0.2±0.13 ^{c}	6.85	***
	N2	0.2±0.13	0	0	0.1±0.10	0	1.44	n.s.

Means sharing the same letter within rows are not significantly different at p>0.05. ns- non significant.

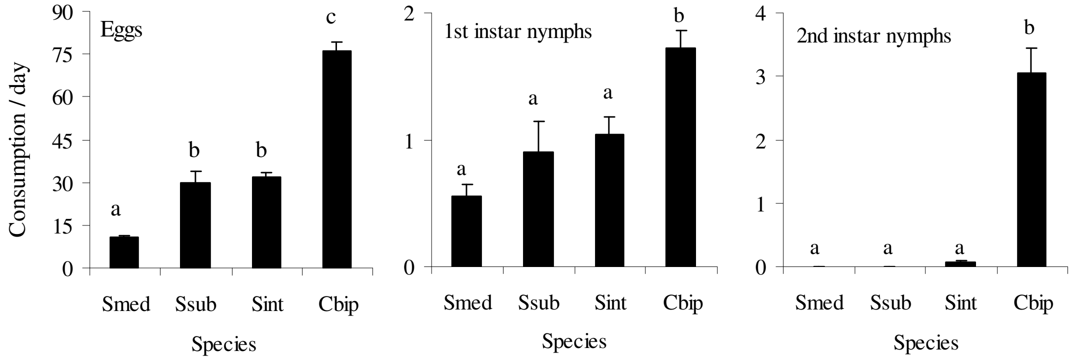

Figure 1. *Consumption (mean ± standard error of the mean) of the three phenological stages of Saissetia oleae by the four coccinellid species tested: Smed - Scymnus mediterraneus, Ssub - Scymnus subvillosus, Sint - Scymnus interruptus, Cbip - Chilocorus bipustulatus. n=10. Different axis scales were used. Bars sharing the same letter are not significantly different at P>0.05.*

In laboratory studies [1] showed that it was possible to obtained consecutive generation of *C. bipustulatus* fed with *S. oleae* (phenological stage was not specified) but unsuccessful attempts were obtained for *S. subvillosus*. Also, [2] showed that *S. mediterraneus* completed its life cycle consuming a large amount of eggs of *S. oleae*.

The consumption of the different phenological stages of *S. oleae* depends mostly on the characteristics of the tegument of the prey [5], the relation between the sizes of predator and prey and also the nutritional quality of the prey [11]. Easily penetrated covers are more likely to be suppressed than thick covers [5]. In this way, it was probable that first instar nymphs are softer and easier to handle by smaller coccinellid species (e. g. *S. mediterraneus*) than the second instar nymphs. On the other hand, because of their large size, adult *C. bipustulatus* can easily chew through the covers of greater nymphs that provide higher energetic gain than smaller nymphs [10]. Thus, the consumption of each phenological stage of *S. oleae* can be considered as a trade-off between its energetic value and the morphological characteristic of the integument that makes predation by coccinellids difficult.

The consumption of *S. oleae* eggs by the majority of coccinellid species will be difficult under field conditions due to the hard integument of the egg-bearing females. Eventually, only *C. bipustulatus* can predate egg-bearing females because it possesses highly modified mandibles that are unique to the genus - they are acutely angled with a single tooth at their apex, which can be inserted between the scale and the substrate [5]. Other coccinellid species consume preferentially first and second instar nymphs of *S. oleae* that are abundant and accessible stages in summer and autumn in olive groves [8; Santos *et al.*, unpublished data]. Nonetheless, due to its high nutritive value, eggs can be used as food for rearing laboratory cultures since they were successfully fed by all coccinellid species.

In the second experiment, the consumption of *S. oleae* eggs and first instar nymphs by the successive larval stages of *C. bipustulatus* increased with their development. The general pattern of food consumption by larval stages showed a gradual increase immediately after each moult but in third and fourth larval stages, a small decline occurred as larvae approached the ecdysis (*Fig. 2*). No differences were found between the developmental period of specimens fed with eggs and those fed with first nymphal nymphs. [9] observed a similar pattern in all larval stages of *Chilocorus nigritus* (F.) reared on the cyanophyllum scale *Abgrallaspis cyanophylli* (Signoret). A total mean of 976 ± 11.22 eggs of *S. oleae* were consumed by each larva of *C. bipustulatus*. First instar larvae consumed 3.6%, second instars 9.5%, third instars 27.3% and fourth instars 59.6% of the total eggs intake. Considering the intake of first instar nymphs of *S. oleae*, a mean total number of 86 ± 6.1 was consumed by each larva of *C. bipustulatus*, where first instar larvae consumed 10.3%, second instar 12.2%, third instar 26.5% and fourth instar 51.0% of total intake. Compared with *C. nigritus*, the consumption by first instar larvae of *C. bipustulatus* was generally high and the fourth very low - 3 and 73% respectively [9].

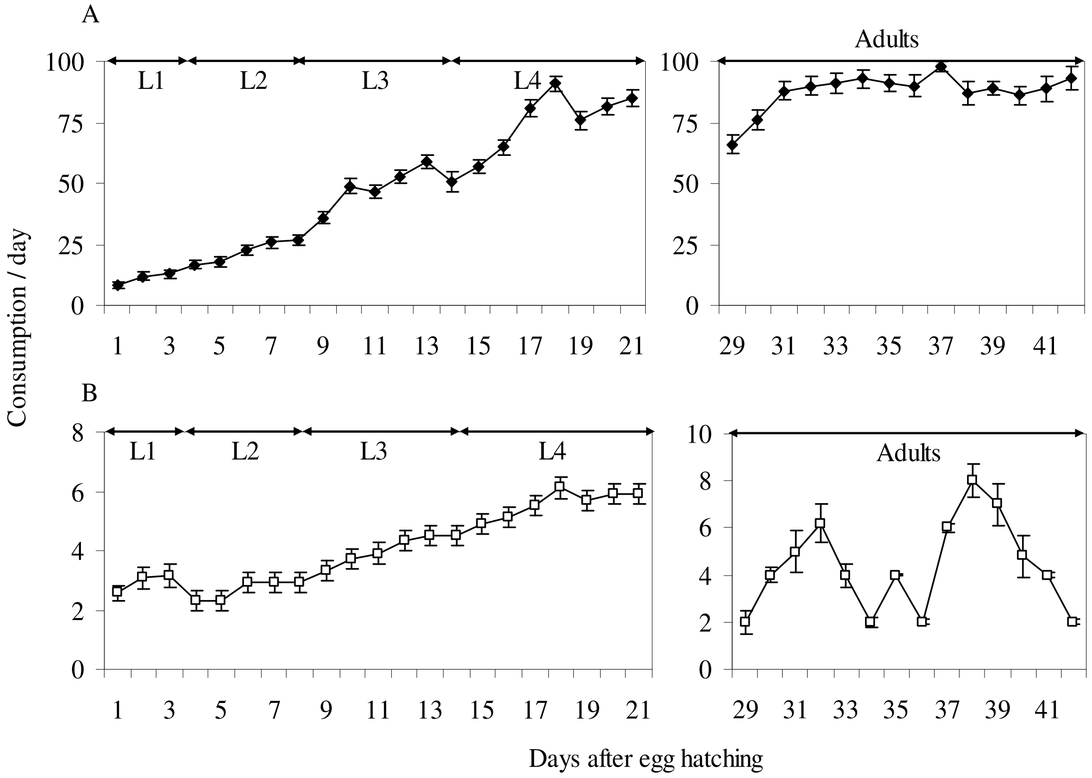

Figure 2. *Consumption of eggs (A) and first instar nymphs (B) (mean ± SE) of Saissetia oleae by Chilocorus bipustulatus larvae and adult stages. Different axis scales were used. n=15.*

The egg consumption by adults increased in the first six days after eclosion and then it remained constant till the end of the experiment *(Figure 2).* According [9], adult food intake in coccidophagous coccinellids is known to be low after eclosion, increasing gradually to a peak at the end of the first week. Each adult consumed a mean of 1227 ± 51.98 eggs after 14 days of the experiment. The consumption of the first instar nymphs increased in the first four days and a total mean of 61 ± 11.2 first instar nymphs of *S. oleae* was consumed by each adult specimen after 14 days of experiment.

None of the second instar nymphs supplied were totally consumed by the first larval specimens of *C. bipustulatus*, which only developed to the second instar and consequently, the life cycle was not completed. As a result, alternative preys should be essential items in the first larval stages of this coccinellid species to successfully complete its life cycle. Despite their polyphagy, coccinellid adults tend to feed more certain types of food [6] and the beneficial effect that food has on individual predators leads to increased rates of growth, development and fertility, and decreased rates of mortality [4].

This study provides a better understanding of the consumption of different phenological stages of *S. oleae* by coccinellid species and the capacity of both larvae and adult stages of *C. bipustulatus* to feed on this pest and complete their life-cycles. The apparent voracity of *C. bipustulatus* on the different phenological stages of *S. oleae* is an attribute that should make it an important predator contributing for the natural biological control of the pest in the olive grove. Moreover, the larva is the most voracious stages of coccinellids [14], requiring great amounts of food to grow up rapidly. This aspect supports the likely use of this coccinellid species in biological control programs against *S. oleae*. In this context, the mass release of larval specimens, particularly the most resistant third instar larvae, can be done in order to maximize the predaceous action of *C. bipustulatus* in the olive grove.

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REFERENCES

[1] Argyriou, L.C., Katsoyannos, P. (1977): Coccinellidae species found in the olive-groves of Greece. – Annales de L´Institut Phytopathologique Benaki 11: 331-345.

[2] Ba M’hamed, T., Chemseddine, M. (2001): Assessment of temperature effects on the development and fecundity of *Pullus mediterraneus* (Col., Coccinellidae) and consumption of *Saissetia oleae* eggs (Hom., Coccoida). – Journal of Applied Entomology. 125: 527-531.

[3] Ba M’hamed, T., Chemseddine, M. (2002): Selective toxicity of some pesticides to *Pullus mediterraneus* Fabr. (Coleoptera: Coccinellidae), a predator of *Saissetia oleae* Bern. (Homoptera: Coccoidea). – Agricultural and Forest Entomology 4: 173-178.

[4] Begon, M., Harper, J.L., Townsend, C.R. (1996): Ecology- Individuals, Populations and Communities. 3rd Edition. – Blackwell Science, Oxford.

[5] Honda, J.Y., Luck, R.F. (1995): Scale morphology effects on feeding behavior and biological control potential of *Rhyzobius lophanthae* (Coleoptera: Coccinellidae). – Annals of the Entomological Society of America 88: 441-450.

[6] Iperti, G. (1999): Biodiversity of predaceous coccinellidae in relation to bioindication and economic importance. – Agriculture, Ecosystems and Environment 74: 323-342.

[7] Magro, A., Hemptinne, J.L. (1999): The pool of Coccinellids (Coleoptera: Coccinellidae) to control Coccids (Homoptera: Coccoidea) in Portuguese citrus groves. – Boletín de Sanidad Vegetal “Plagas” 25: 311-320.

[8] Pereira, J.A.C. (2004): Bioecologia da cochonilha negra, *Saissetia oleae* (Olivier), na oliveira, em Trás-os-Montes. PhD Thesis. Universidade de Trás-os Montes e Alto Douro, Vila Real.

[9] Ponsonby, D.J., Copland, M.J.W. (2000): Maximum feeding potential of larvae and adults of the scale insect predator, *Chilocorus nigritus* with a new method of estimating food intake. – Biocontrol 45: 295-310.

[10] Provost, C., Lucas, E., Coderre, D., Chouinard, G. (2006): Prey selection by the lady beetle *Harmonia axyridis*: the influence of prey mobility and prey species. – Journal of Insect Behavior 19: 265-277.

[11] Roger, C., Coderre, D., Boivin, G. (2000): Differential prey utilization by the generalist predator *Coleomegilla maculata lengi* according to prey size and species. – Entomologia Exprimentalis et Applicata 94: 3-13.

[12] Sahayaraj, K., Paulraj, M.G. (2001): Rearing and life table of reduviid predator *Rhynocoris marginatus* Fab. (Het., Reduviidae) on *Spodoptera litura* Fab. (Lep., Noctuidae) larvae. – Journal of Applied Entomology 125: 321-325.

[13] Sengonca, C., Al-Zyoud, F., Blaeser, P. (2005): Prey consumption by larval and adult stages of the entomophagous ladybird *Serangium parcesetosum* Sicard (Col., Coccinellidae) on the cotton whitefly, *Bemisia tabaci* (Genn.) (Hom., Aleyrodidae), at two different temperatures. – Journal of Pest Science 78: 179-186.

[14] Stathas, G.J. (2000): *Rhyzobius lophanthae* prey consumption and fecundity. – Phytoparasitica 28: 1-9.

[15] StatSoft, Inc. (2004): STATISTICA (data analysis software system), version 7. www.statsoft.com.