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Susceptibility of Two Sitophilus species (Coleoptera: Curculionidae) to Essential Oils from Foeniculum vulgare and Satureja hortensis

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Abstract. This study was conducted to determine the insecticidal activity of essential oils from Fennel, *Foeniculum vulgare* (Apiaceae), and Summer savory, *Satureja hortensis* (Lamiaceae), against two stored-product insects. Essential oils from two species of plants were obtained by Clevenger-type water distillation and their fumigant toxicities were tested against adults of the wheat weevil, *Sitophilus granarius* and rice weevil, *Sitophilus oryzae* (Curculionidae). The mortality was determined after 24 and 48 hrs from beginning of exposure. LC_{50} values of each essential oil were estimated for each insect species. Fumigation bioassays revealed that essential oils from two plants had strong insecticidal activity on experimental insects. LC_{50} values indicated that *S. granarius* was more susceptible than *S. oryzae* to essential oils at the exposure time 24 and 48 hrs. The mortality effect of *S. hortensis* oil was lower than *F. vulgare* oil. The LC_{50} values decreased with the duration of exposure to the essential oil concentrations. In all case, responses varied according to plant material, concentration, and exposure time. These results indicated that essential oils from *S. hortensis* and *F. vulgare* could be applicable to the management of stored product insects to decrease ecologically detrimental effects of utilization synthetic insecticides.

Key words: Essential oil, *Foeniculum vulgare*, *Satureja hortensis*, Fumigant toxicity, *Sitophilus granarius*, *Sitophilus oryzae*.

Introduction

To preserve the quantity and quality of stored-product foodstuff particularly cereals it is necessary to reduce the population of the insect pests such as *Sitophilus* species. The rice weevil, *Sitophilus oryzae* (L.) and the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) are two of the most widespread and destructive insect pests of stored cereals. These pests are internal feeders and cause considerable loss to cereals affecting the quantity as well as quality of the grains (KUCEROVA *et al.*, 2003; PARK *et al.*, 2003).

Synthetic pesticides have been considered the most effective and accessible means to control insect pests of stored products (HUANG & SUBRAMANYAM, 2005). These chemicals are associated with undesirable effects on the environment due to their slow biodegradation in the environment and some toxic residues in the products for mammalian health (BENHALIMA *et al.*, 2004; ISMAN, 2006; HALDER *et al.*, 2010). The adverse effects of synthetic pesticides have amplified the need for effective and biodegradable pesticides. Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment. Among various kinds of natural substances that have received particular attention as natural agents for insect management are essential oils from aromatic plants. Essential oils are renewable, non-persistent in the environment and relatively safe to natural enemies, non-target organisms and human beings (HALDER *et al.*, 2010). Essential oils are defined as any volatile oil(s) that have strong aromatic components and that give distinctive odour, flavor or scent to a plant. These are the by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites (KOUL *et al.*, 2008). Because of the intensity of plant-insect interactions, the plants there have well-developed defense mechanisms against pests and are excellent sources of new insecticidal substances. Their components and quality vary with geographical distribution, harvesting time, growing conditions and method of extraction (YANG *et al.*, 2005). Effects of essential oils on stored-product insect pests have been extensively reported (OGENDO *et al.*, 2008; PARK *et al.*, 2008; BENZI *et al.*, 2009; AYVAZ *et al.*, 2010; NYAMADOR *et al.*, 2010; TAGHIZADEH-SAROUKOLAI *et al.*, 2010). Iran is a country comprised largely of arid and semiarid areas, and contains many indigenous aromatic plants such as Fennel and Summer savory. Fennel, *Foeniculum vulgare* Gaertner is a species of flowering plant in the Apiaceae (Umbelliferae). The insecticidal activity of some essential oils from Apiaceae has been evaluated against a number of stored product insects. For example, SAHAF *et al.* (2007) found the strong insecticidal activity of essential oil from *Carum copticum* (Apiaceae) on *S. oryzae* and *Tribolium castaneum* (Tenebrionidae). The mortalities of the insect species reached 100% at concentrations higher than 185.2 µl/l air and 12 hrs exposure times. In another experiment, CHAUBEY (2008) studied fumigant activity of the essential oils from *Anethum graveolens* and *Cuminum cyminum* (Apiaceae) on *Callosobruchus chinensis* (Bruchidae). The 24-hrs LC_{50} values against the adults of the insect were 10.8 and 11.0 µl oils, respectively. Summer savory, *Satureja hortensis* L., is a species in the mint family (Lamiaceae). There are numerous investigations on the insecticidal activity of essential oils from Lamiaceae family (RAJENDRAN & SRIRANJINI 2008; TUNAZ *et al.*, 2009; AYVAZ *et al.*, 2010). Furthermore, our earlier studies indicated that the essential oil from *Agastache foeniculum* (Lamiaceae) had strong fumigant toxicity on the adults of *Oryzaephilus surinamensis* (Silvanidae) and *Lasioderma serricorne* (Anobiidae) (EBADOLLAHI *et al.*, 2010a). In the other study, we found that *Lavandula stoechas* (Lamiaceae) was very toxic against *L. serricorne* and *Rhyzopertha dominica* (Bostrichidae) (EBADOLLAHI *et al.*, 2010b).

Therefore, the main goal of the present study was to evaluate the insecticidal activities of essential oils from *F. vulgare* and *S. hortensis* grown in Iran in the control of two stored-grain insects, *S. granarius* and *S. oryzae*.

Material and methods

Plant materials and extraction of essential oils. The ripe seeds of *Foeniculum vulgare* and aerial parts from 1.5 cm of top of *Satureja hortensis* at flowering stage were harvested from plants grown in the experimental farm of the Department of Horticultural, University of Urmia, West Azerbaijan, Iran. These materials were air dried in the shade at room temperature (26-28 °C) for 20 days and stored in darkness until distillation. The essential oils were isolated from dried plant samples by hydrodistillation using a Clevenger apparatus. Conditions of extraction were: 50 g of air-dried sample, 1:10 plant material/water volume ratio, 3 hrs distillation. The essential oils were collected, dried over anhydrous sodium sulfate and stored at 4 °C until use.

Insect cultures and experimental conditions. *Sitophilus granarius* (L.) and *Sitophilus oryzae* (L.) were reared in a 1 L wide-mouthed glass jars containing wheat grains. Mouth of the jars was covered with a fine mesh cloth for ventilation and to prevent escape of the weevils. Parent adults were obtained from laboratory stock cultures maintained at the Entomology Department, University of Urmia, Iran. The cultures were maintained in an incubator at 27 ± 2 °C and 60 ± 5% RH (Relative Humidity). Insects used in all experiments were 1 to 7 day old adults. All experimental procedures were carried out under the same environmental conditions as the cultures.

Bioassays. The fumigant bioassays were conducted as described by NEGAHBAN *et al.* (2007), with slight modifications. In this study, tested insects were confined in the plastic tubes. Thus, there was no direct contact between the oil and the insects. Different concentrations were prepared to evaluate mortality of insects after an initial dose-setting experiment. Concentrations of *Foeniculum vulgare* oil tested on *S. granarius* were 10, 14.92, 22.32, 33.41 and 50 µl/ l air and on *S. oryzae* were 25, 31.13, 38.74, 48.21 and 60 µl/ l air. *S. granarius* were exposed to the essential oil of *Satureja hortensis* at 20, 26.85, 36.85, 36.05, 48.41 and 70 µl/ l air and *S. oryzae* at 30, 37.08, 45.83, 56.64 and 70 µl/ l air. Each concentration was applied to filter paper stripe (4 × 5 cm, Whatman N° 1) and the impregnated filter papers were then attached to the screw caps of glass jars with volumes of 1 L. Twenty insect adults were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with clothes mesh and each tube were placed at the bottom of glass jar. Caps of glass jars were screwed tightly. The jars were kept in the incubator and mortality was determined after 24 and 48 hrs from beginning of exposure. Each experiment was replicated for five times at each concentration. The control insects were maintained under the same conditions without any essential oil. When no leg or antennal movements were observed, insects were considered dead.

The experiments were arranged in a completely randomize design and the data were analyzed with ANOVA. The means were separated using the Duncan's test at the 5% level. The LC_{50} values with their fiducial limits were calculated by probit analysis using the SPSS version 16.0 software package.

Results and Discussion

All the treatments with the essential oils showed significant level of toxicity to the insects. *Foeniculum vulgare* essential oil showed strong fumigant activity against *S. oryzae* and *S. granarius* adults. Compare means showed that there were significant differences in the mortality of *S. oryzae* and *S. granarius* exposed to different concentrations of *F. vulgare* oil for 24 and 48 hrs ($P \le 0.05$, Duncan's test) (Fig. 1).

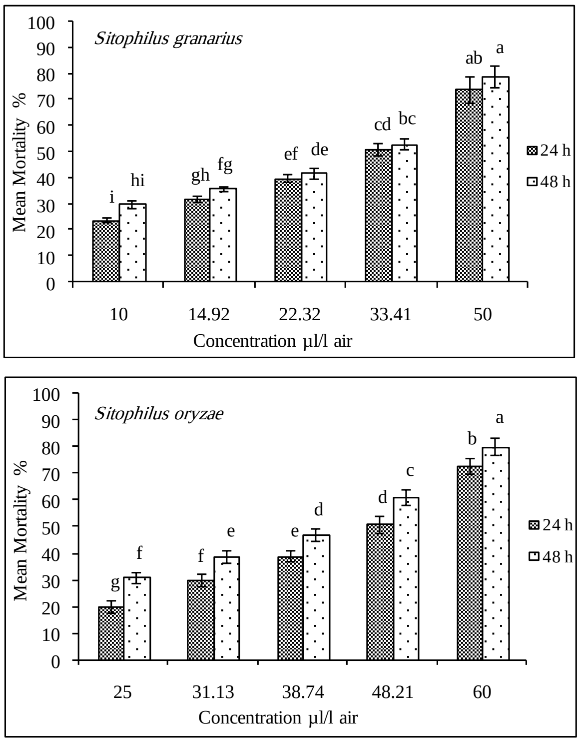

Fig. 1. Mean mortality (%) of *Sitophilus granarius* and *Sitophilus oryzae* exposed to different concentrations of the essential oil from *Foeniculum vulgare*. Different letters on top of columns indicate significant differences according to Duncan's test at $p \le 0.05$. Columns with the same letter are not significantly different. Vertical bars indicate standard error (±).

The graphs in Fig. 2 display that *Satureja hortensis* essential oil was very toxic on *S. oryzae* and *S. granarius* and there were significant differences in percentage mortality of insects exposed to different concentrations for 24 and 48 hrs ($P \le 0.05$, Duncan's test).

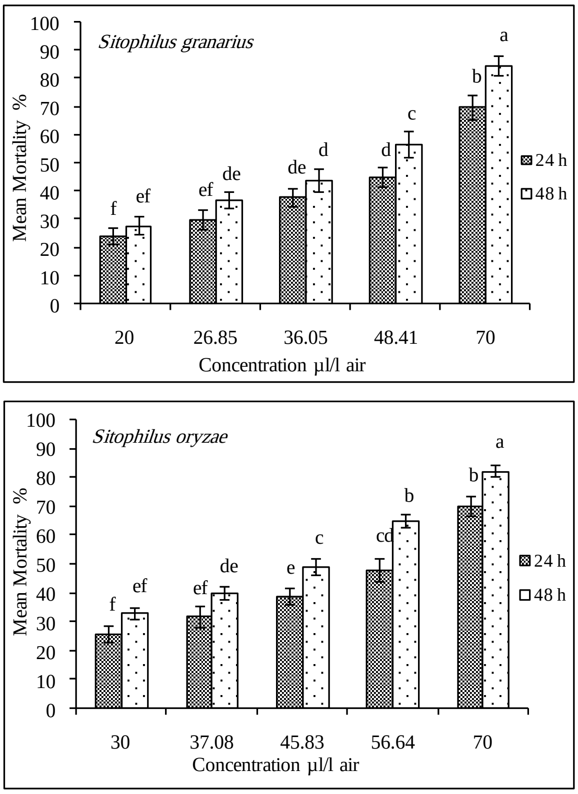

Fig. 2. Mean mortality (%) of *Sitophilus granarius* and *Sitophilus oryzae* exposed to different concentrations of the essential oil from *Satureja hortensis*. Different letters on top of columns indicate significant differences according to Duncan's test at $p \leq 0.05$. Columns with the same letter are not significantly different. Vertical bars indicate standard error (±).

Probit analysis showed that the concentration for the essential oil of *F. vulgare* to cause 50% mortality (LC_{50}) in *S. granarius* was 27.30 µl/l air (95% lower and upper fiducial limit (FL) = 23.62 – 32.38), whereas in *S. oryzae* was 44.16 µl/l air (95% FL= 41.03 – 48.21) after 24 hrs of treatment (Table 1). Therefore, *S. oryzae* was noted to be more tolerant than *S. granarius* to *F. vulgare* essential oil.

The LC_{50} values of the *S. hortensis* oil on *S. oryzae* and *S. granarius* were 52.96 µl/l air (95% FL= 48.49 – 59.34) and 46.89 µl/l air (5% FL= 41.38 – 55.27), respectively, for 24 hrs exposure time (Table 1).

The mortality effect of essential oil from *S. hortensis* was lower than *F. vulgare* essential oil. LC_{50} values for two insects were decreased after 48 hrs from commencement of exposure time and *S. granarius* was more susceptible than *S. oryzae* to two essential oils and times (Table 1). In all case, the insecticidal activity varied with insect species, concentrations of the oil and extension of exposure times.

Discussion

Essential oils from various plants have shown promise as sources for insecticides. Earlier attempts to explore the toxicity of plant derivatives against *Sitophilus granarius*

Table1. LC_{50} values of essential oils from *Foeniculum vulgare* and *Satureja hortensis* against the adults of *S. granarius* and *S. oryzae*.

Essential oil	Insect (N: 100)	Time (h)	LC_{50} (Min – Max) (µl/l air)	χ^2 (df= 3)	Sig *	Intercept	Slope
F. vulgare	*S. granarius*	24	27.30 (23.62 – 32.38)	3.60	0.31	2.82	1.82
		48	22.00 (19.02 – 25.41)	3.05	0.38	2.48	1.87
	S. oryzae	24	44.16 (41.03 – 48.21)	2.26	0.52	- 0.99	3.64
		48	37.52 (34.53 – 40.63)	3.14	0.37	- 0.28	3.36
S. hortensis	*S. granarius*	24	46.89 (41.38 – 55.27)	3.47	0.33	1.39	2.16
		48	37.62 (33.70 – 42.17)	4.27	0.23	1.17	2.43
	S. oryzae	24	52.96 (48.49 – 59.34)	3.72	0.30	- 0.17	3.00
		48	42.99 (39.51 – 46.400	2.23	0.53	- 0.63	3.45

N: Number of the tested insects for each time

* Since the significance level is greater than 0.150, no heterogeneity factor is used in the calculation of confidence limits

and *Sitophilus oryzae* have been made by essential oils. ASLAN *et al*. (2005) evaluated essential oil from the plant species *Micromeria fruticosa*, *Nepata racemosa* and *Origanum vulgare* (Lamiaceae) for their toxicities against the adults of *Lasioderma serricorne* (Anobiidae) and *S. granarius* and larvae (third instar) of *Ephestia kuehniella* (Pyralidae). In that study, although insecticidal activities against these pests were achieved with essential oils of all three plant species, the oil of *O. vulgare* was found to be the most effective against *S. granarius.* KORDALI *et al*. (2006) studied the toxicity of essential oils isolated from three *Artemisia* species (*A. absinthium*, *A. santonicum* and *A. spicigera*) to *S. granarius*. All of the essential oils tested were found to be toxic to adults of *S. granarius.* The oils showed about 80–90% mortality of granary weevil, *S. granarius* at a dose of 9 µl/l air after 48 hrs of exposure. KORDALI *et al*. (2008) tested insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* on *S. granarius* and *Tribolium confusum*. *Origanum acutidens* oil caused 68.3% and 36.7% mortality of *S. granarius* and *T. confusum* adults, respectively, after 96 hrs of exposure. Results showed that the oil was more toxic against *S. granarius* as compared with its toxicity against *T. confusum.* BENZI *et al*. (2009) investigated the biological activity of essential oils from leaves and fruits of pepper tree, *Schinus molle*, against S. *oryzae*. Their study showed repellent, fumigant activity, nutritional indices, and feeding deterrent action of pepper tree oils on *S. oryzae* adults. With respect to fumigant activity, neither of the essential oils was found to be toxic. These findings are parallel with the results of present study for sensibility of *S. granarius* and *S. oryzae* to essential oils isolated from plants.

Previous studies demonstrated that essential oils and extracts isolated from *F. vulgare* and *S. hortensis* have pesticides effects. For example, ASLAN *et al*. (2004) tested essential oils from *S. hortensis*, *Ocimum basilicum* and *Thymus vulgaris* (Lamiaceae) for their toxicities against the nymphs and adults of *Tetranychus urticae* (Acari: Tetranychidae) and adults of *Bemisia tabaci* (Aleyrodidae). Although desirable insecticidal and acaricidal activities against both of these pest species were achieved with essential oils of the three plant species, *S. hortensis* was found to be the most effective, compared with the other two species. In another experiment, *F. vulgare* fruit extract gave 67% and 100% mortality (contact action) in *Attagenus unicolor japonicus* larvae at 5.2 mg/cm^2, 21 and 28 days after treatment respectively (HAN *et al*., 2006). IBRAHIM *et al*. (2006) were disclosed effect of flower extract of *F. vulgare* on hatching, migration and mortality of the root-knot nematodes, *Meloidogyne incognita*. The acaricidal activities of components derived from *F. vulgare* seed oil were demonstrated against the stored product mite, *Tyrophagus putrescentiae* adults using direct contact application (LEE *et al*., 2006). IŞIK & GÖRÜR (2009) studied the aphidicidial activity of *F. vulgare* essential oil against cabbage aphid, *Brevicoryne brassicae* (Aphididae), under laboratory conditions. Applications of *F. vulgare* essential oil significantly reduced the reproduction potential of the cabbage aphid and resulted in higher mortality. These studies confirm results of present investigation related to insecticidal effects of *F. vulgare* and *S. hortensis*.

LEE *et al*., (2001b) suggested that the toxicity of essential oils to stored-product insects was influenced by the chemical composition of the oil. Previous studies indicated that Methyl Chavicol (= Estragole) and Limonene in the essential oil of *F. vulgare* (IBRAHIM *et al*., 2006; MIGUEL *et al*., 2010) and Carvacrol, Thymol, γ-Terpinene and p-Cymene in the *S. hortensis* essential oil (RAZZAGHI-ABYANEH *et al*., 2008; MIHAJILOV-KRSTEV *et al*., 2009) were the major components. LEE *et al*. (2001a) showed *p*-Cymene (LC_{50} = 25.0 µl/l air) was the most toxic fumigant on *S. oryzae*, followed by, α-Terpinene (LC_{50} = 71.2 µl/l air) and Carvacrol (LC_{50} = 79.4 µl/l air). PAPACHRISTOS & STAMOPOULOS (2004) investigated relationship between the chemical composition of the essential oils from *Lavandula hybrida*, *Rosmarinus officinalis* and *Eucalyptus globulus* and their insecticidal activity against *Acanthoscelides obtectus*. They found that p-Cymene, S(-)Limonene, R(+)Limonene, γ-Terpinene and α-Terpineol exhibited insecticidal activity against both male and female adults. LOPEZ *et al*., (2008) reported that Estragole is example of toxic fumigant compound in the essential oils from coriander (*Coriandrum sativum*), caraway (*Carum carvi*) and basil (*Ocimum basilicum*) that is active against insect pests. Therefore, the insecticidal activity of *F. vulgare* and *S. hortensis* essential oil could be related to these constituents. On the other hand, these results demonstrated that the essential oils isolated from different plants might have different toxicity, which can be attributed to their different chemical composition and different major or minor components.

Foeniculum vulgare and *Satureja hortensis* used as culinary and medicinal plants are considered to be less harmful than most conventional insecticides. Apart from a natural origin, the essential oils of *F. vulgare* and *S. hortensis*, like most of plant essential oils, can pose fewer or lesser risks to human health and the environment. However, further research is needed in order to evaluate the effectiveness of *F. vulgare* and *S. hortensis* essential oils, explore their mode of action and establish their utility as natural insecticidal agents.

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Studies on the Dung-inhabiting Beetles (Insecta: Coleoptera) Community of Western Anatolia, Turkey

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Abstract. In Bozdağlar Mountain of western Turkey, the diversity and composition of the dung-inhabiting beetles in two locations situated in different altitudes (600 m and 900 m) in 2004 and 2006 assemblages were sampled. A total of 5.709 individuals from 88 species belonging to the families Scarabaeidae, Aphodiidae, Geotrupidae, Carabidae, Hydrophilidae, Histeridae and Ptilidae of the order Coleoptera are recorded.

Key words: Ecology, dung-inhabiting beetles, Coleoptera, Bozdaglar Mountain, Turkey.

Introduction

The recycling of dung in this way improves soil texture and returns nutrients and water to the soil (BORNEMISSZA & WILLIAMS, 1970). The dung-inhabiting insects form a highly diverse community including specialized coprophagous and predatory species of beetles and flies, as well as an array of generalist consumers, which colonize feces during the different stages of decomposition (KOSKELA & HANSKI, 1977; HANSKI, 1987; 1990; 1991; PINERO & AVILA, 2004). Great numbers of beetles frequent in dung of herbivorous mammals. The majority beetle families being especially characteristic: Scarabaeidae, Aphodiidae, Hydrophilidae, Staphylinidae, Histeridae, Ptilidae and Silphidae in the order Coleoptera.

Dung-inhabiting beetles are a frequent topic of many ecological studies as well phenology, population dynamics; dispersal abilities etc. are well-known for many species. The dung insect communities are characterized by the dominance of scarab beetles which also called dung beetles (mainly Scarabaeidae and Aphodiidae). Dung beetles feed mainly on droppings of mammals. Doing this, they decompose dung, thus benefiting both to pasture and animal health. Dung beetles are important enough in manure and nutrient recycling. They compete with pestiferous flies and parasitic nematodes, enrich the soil by burying large quantities of nutrient-rich dung, and effectively mix and aerate soil through tunneling (BERTONE, 2004; BERTONE *et al.*, 2005). Almost all Scarabaeidae and Aphodiidae species are coprophagous. Among other dung-inhabiting beetles, adult Hydrophilidae are coprophagous. Ptiliidae feed on decaying vegetable material and on fungi under bark. Many of the predatory species are found associated with animal dung. The carnivores seem to be very dependent on the number of suitable prey in the droppings. In generally, species belonging to Carabidae, Staphylinidae and Histeridae families in dung are known as predator.

Up to now, there is no comprehensive data on dung inhabiting-beetles have been

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published in Turkey. In this paper, it has been presented the results of a study concerning fauna of the order Coleoptera from Bozdağlar Mountain, Manisa province of western Turkey. The results of this study also provide some ecological data of dung-inhabiting beetles.

Material and methods

Study Area

The seasonal activity of the rove beetles was studies in 2004 and 2006 in two locations (ca 3 ha each) situated in different altitudes (600 m a.s.l. and 900 m a.s.l.) near Dagmarmara village, Manisa province of western Turkey. The coordinates of the locations at 600 m and 900 m are 38°23′37″N, 27°49′09″E and 38°20′09″N, 27°50′47″E, respectively (Fig.1).

The location at 600 m a.s.l. is situated about 2 km north of Dagmarmara village within farm lands. There are pastures of various sizes situated among the plantations of *Pinus brutia* Ten., *Quercus* spp., *Castanea sativa* MILL. and the orchards of *Prunus avium* L., *Pyrus malus* L., *Cydonia vulgaris* PERS. on this locality. On the pastures, *Polypodium* sp., *Cistus creticus* L., *Trifolium bocconei* SAVI, *Medicago × varia* (MARTYN) ARCANG., *Rubus canescens* L. occur except of grasses. On the pasture where the samples were collected, a total of 30-40 cows and several horses feed all day long on the pasture and they are brought back to their shelters at night.

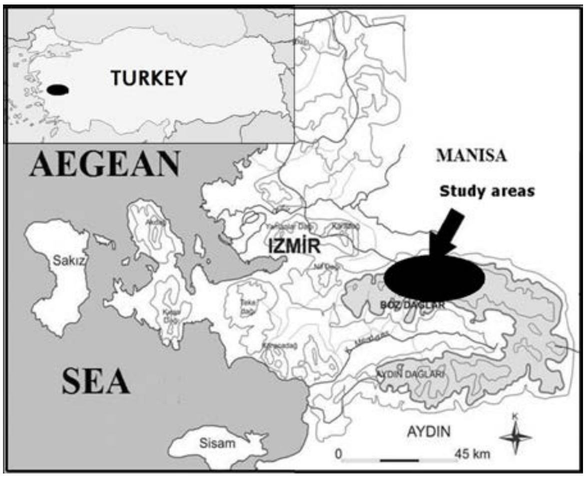

Fig. 1. Location of trapping study area and neighboring fields at Bozdağlar Mountain, western Turkey.

The location at 900 m a.s.l. is situated ca. 5 km southeast of the pasture at 600 m, out of the agricultural land and is therefore less impacted by human activities. The samples were collected on a large pasture surrounded by the forest of *Pinus nigra* (ARNOLD). Except of grasses, *Polypodium* sp., *Verbascum* sp., *Juniperus oxycedrus* L., *Pyrus amygdaliformis* VILL., *Rosa canina* L., *Cistus laurifolius* L. were common on the pasture. In this area, a total of 80-100 cows, without any other mammalians, feed on this pasture all day long without going back to their shelters at night from April up to November.

In generally, variation in humidity was different between two localities. The rainy season from November to May and from June to late October there is a rainless period at these localities. According to our observation, the location at 900 m is more humid than 600 m, with scarce rain even through summer period. Average temperature and average rainfall amount of Manisa province are provided in Fig. 2.

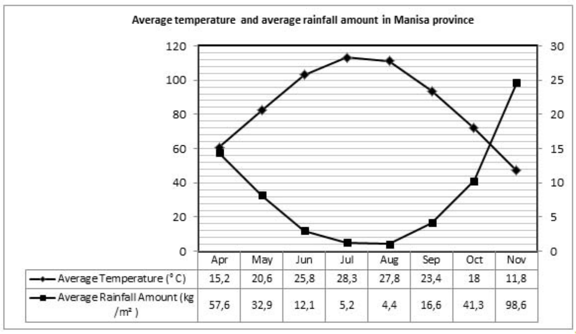

Fig. 2. Average temperature and average rainfall amount in Manisa province, Western Anatolia (MGM, 2011).

It has been classified insects into five trophic groups: predators, opportunistic predators, coprophages, opportunistic coprophages and omnivores. Predators (histerid and staphylinid beetles, especially *Philonthus* species) eat only live prey, whereas coprophages (scarabeid, aphodiid and hydrophylid beetles) eat (exclusively or principally) dung. Omnivores feed both on live prey and dung. Opportunists appear in dung as predators or coprophages, but are not restricted to excrement (carabid, tenebrionid, and some staphylinid and aphodiid beetles). Classification of species into trophic groups was based on direct observations in the study area and literature (KOSKELA & HANSKI, 1977; CAMBEFORT, 1991; PINERO & AVILA, 2004).

Sampling

For this study, both localities were visited in ca. 14-day intervals from mid-April to the mid November. During the winter, cows were not present on the pastures and the beetles were therefore not sampled in this period. Samples were collected randomly by a handle shovel, placed into a plastic jars and transported to the laboratory, where the insects were separated from the dung. Fifteen samples of ca. 50 g of dung were collected during each visit on the locality. The material referred to in this study is deposited in the Lodos Entomological Museum (LEMT), Department of Plant Protection, Aegean University (Izmir, Turkey) and author's private collections.

In the previous parts of these studies, species belonged to Histeridae (ANLAŞ et al., 2007), Hydrophilidae (ANLAŞ *et al.*, 2008), Scarabaeoidea (ANLAŞ *et al.*, 2011), Staphylinidae (ANLAŞ, in prep.) and Carabidae (ANLAŞ & TEZCAN, in prep.) have been evaluated and published.

Results and Discussion

In this study, totally 5.709 specimens representing 88 species belonging to 10 families (Scarabaeidae, Aphodiidae, Geotrupidae, Staphylinidae, Carabidae, Hydrophilidae, Histeridae, Tenebrionidae, Silphidae and Ptiliidae) of the order Coleoptera were collected in two locations situated in different altitudes (600 m and 900 m) in 2004 and 2006. The beetle families with the highest number of species were Staphylinidae (26 spp.) and Scarabaeidae (23 spp.), while Scarabaeidae (34.6 % of the beetles), Staphylinidae (22.7 %) and Aphodiidae (20.7 %) were the most abundant families (Table 1 and Fig. 3).

Table 1. Number of specimens collected of families at both altitudes during 2004 and 2006 for this study.

Family	Number of species	2004		2006		Sum	Ratios %
		600 m	900 m	600 m	900 m		
Scarabaeidae	23	427	626	320	601	1974	34.6
Aphodiidae	8	266	326	241	347	1.180	20.7
Geotrupidae	2	0	7	0	3	10	< 1
Carabidae	8	12	71	12	54	149	2.6
Staphylinidae	26	325	371	243	357	1.296	22.7
Hydrophilidae	5	188	453	115	173	929	16.3
Histeridae	12	17	66	9	46	138	2.4
Ptiliidae	2	5	2	11	0	18	< 1
Tenebrionidae	1	7	0	3	0	10	< 1
Silphidae	1	0	0	0	5	5	< 1
Total	**88**	**1.247**	**1922**	**954**	**1.586**	**5.709**	**100**
		3.169		**2.540**			

In the previous parts of this study, number of specimens of the recorded species collected at both altitudes during 2004 and 2006 and their dominance values were given before (see references).

The total number of specimens collected in the area located at 600 m was 2.201 and 38.6 % as percentage of the total catch; 3.508 in the area located at 900 m and 61.4 % as percentage of the total catch. In 2004, the number of specimens was 3.169 (55.5 %) and in 2006 it was 2.540 (44.5 %).

The main five species were with the following numbers as percentage of the total catch: *Sphaeridium scarabaeoides*, 12.75 %; *Aphodius fimetarius*, 10.65 %; *Onthophagus* *ruficapillus*, 10.40 %; *Onthophagus taurus*, 6.62 % and *Aleochara tristis*, 8.7 %. Results showed that five species of Coleoptera dominated in the 88 species captured in Table 2. Most of the recorded species generally widely distributed those with Euro-asiatic, cosmopolitan, Palaearctic, Holoarctric distribution. Some of them less-widely distributed species such as Mediterranean.

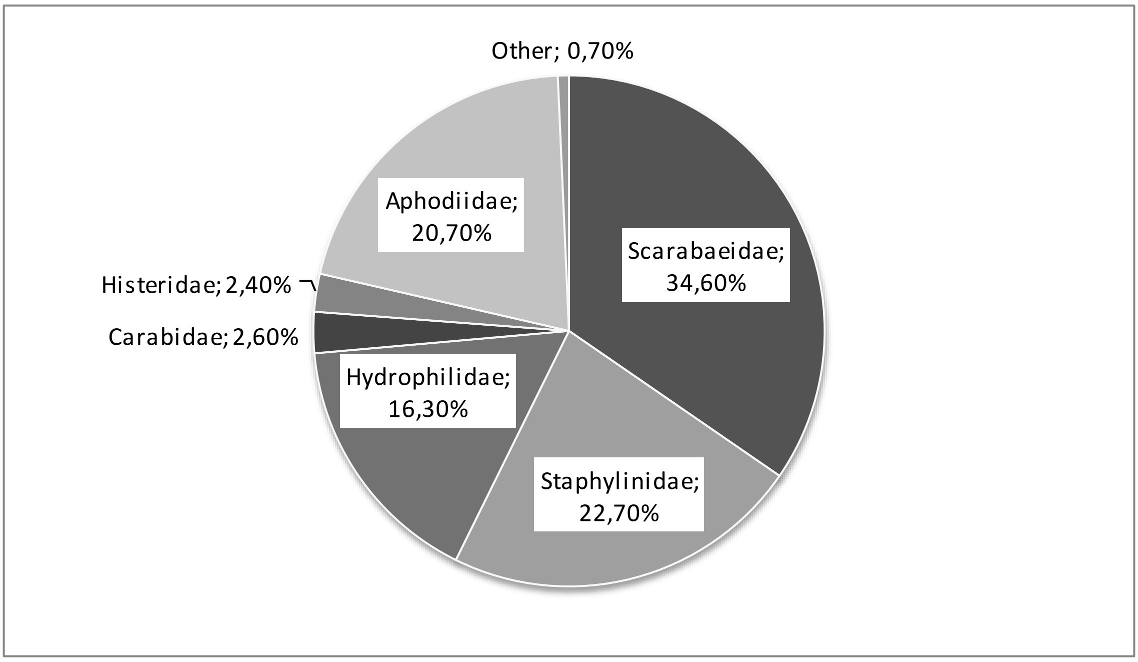

Fig. 3. Ratios of specimens collected of families at both altitudes during 2004 and 2006 for this study.

Table 2. Number of specimens and dominance value of first five species of Coleoptera dominated for this study.

First five dominate species	Family	Number of specimens	Dominance value %
Sphaeridium scarabaeoides (LINNAEUS, 1758)	Hydrophilidae	728	12. 75 %
Aphodius fimetarius (LINNAEUS, 1758)	Aphodiidae	608	10.65 %
Onthophagus ruficapillus BRULLÉ, 1832	Scarabaeidae:	594	10.40 %
Onthophagus taurus (SCHREBER, 1759)	Scarabaeidae	378	6.62 %
Aleochara tristis GRAVENHORST, 1806	Staphylinidae	377	6.60 %

Coprophagous beetles were more abundant in dung pats than predatory beetles (MENÉNDEZ & GUTIÉRREZ, 1997) as well as in our results. Most of the beetle species were coprophages (43 species, 4533 specimens), with more than the species richness than opportunistic coprophagous (2 species, 18 specimens) or predatory beetles (28 species, 473 specimens). In contrast, opportunistic predaceous beetles (8 species, 238 specimens) and omnivores (7 species, 446 specimens) were represented by the lowest number of species. In general, the assemblage was dominated by coprophages

(79.4 % of total abundance), omnivores (7.8 %) and predators (8.3 %), whereas opportunistic detritivores and opportunistic predators were scarce. However, the two sites differed in the abundance of coprophages, opportunistic predators and omnivores.

In this study; the Carabidae specimens which were collected, generally occurred in the whole period of sampling, but it was absent or showing low abundance in August. The peaks were recorded at the end of September and during October. From the staphylinids Oxytelinae species, which are not strongly restricted to dung and use other decaying substrates (SOWIG & WASSMER, 1994), were active mainly in spring period. Oxytelinae species, such as *Anotylus* species, are a saprophagous. *Aleochara tristis,* which are feed commonly on the eggs, larvae, and puparia of various scathophagous and necrophagous Diptera (KLIMAZEWSKI, 1984; LIPKOW, 1992), generally showed highest abundance in autumn months and Staphylininae species, mainly the predator genus *Philonthus*, occurred in the whole period of sampling from spring to autumn period. The scarabeid and aphodiid species of this study were absent or showing low abundance in the period ca. from July to August with peaks in spring and in autumn in both years and on both localities. The hydrophilid species *Sphaeridium scarabaeoides* occurred in the whole period of sampling both in 2004 and 2006, with the peaks in the second half of April, first half of July and in first half of September in both years and on both localities. Histerid species occurred during the whole period of sampling both in 2004 and 2006, with peaks in the second half of May, second half of September. it is generally less abundant in the summer period.

The results of this study from Turkey agree with data from Southern Europe, but differ from those from central and northern Europe especially in earlier onset of high abundance peaks and in low abundance or absence of the beetles during late summer. Comparison of studies of species composition and seasonal dynamics of many dung-inhabiting beetles shows that the precise pattern of the recorded species can differ according to geographic position of the studied localities (e. g. HANSKI & KOSKELA, 1978; LOBO, 1993; WASSMER, 1994; PALESTRINI *et al.*, 1995; PINERO & AVILA, 2004).

It is important to more extensively studies on the in dung-habiting beetles which are a significant biological control agent and recycling of dung, returns nutrients in terrestrial ecosystems. It is hoped that current data will be contributed other studies that will be carried out in other locations in Turkey.

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Comparison of Plant Diversity and Stand Characteristics in Alnus subcordata C.A.Mey and Taxodium distichum (L.) L.C. Rich

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Abstract. Stand characteristics and understory plant diversity were investigated in low-drained man-made stands of *Alnus subcordata* C.A.Mey and *Taxodium distichum* (L) L.C. Rich. The trees were planted with distances of 3 × 3 m and 4 × 4 m in northern Iran. In these stands, herbaceous and woody species were counted in plots of 20 × 20 m. Then, indexes of richness, *H/* diversity, *J/* equitability and Jaccard similarity (*JI*), tree growths, cover crown percentage, and litter layer thickness of each stand were assessed following 17 years after planting. The results revealed that the greatest diameter at breast height (D.B.H) and stem height were observed in *Alnus* 4 × 4 m. By contrast, crown cover percentage and litter thicknesses were greater in *Taxodium* stands. Species richness, *H/* diversity and *J/* equitability indexes, Jaccard similarity (*JI*) of *Alnus* stands were greater than those of *Taxodium* stands. In reality, small and light canopy of *Alnus* is the main reason that the solar radiation can penetrate easily to forest ground and affect understory plant diversity. *Alnus* as a native tree species, due to greater growth attributes and higher diversity indices in their stands are proposed for plantations in such low-drained sites of northern Iran.

Keywords*:* *Alnus subcordata* C.A.May, Plant diversity, Plantations, *Taxodium distichum* (L) L.C. Rich.

Introduction

In recent years, concern for the extinction of species and populations due to human activities has stimulated a number of observational and experimental studies on the relationships between species richness and ecosystem functioning (SINGH *et al.*, 2005). Maintaining of biodiversity in young stands established after clear-cutting is a challenge to foresters and wildlife biologists. Biodiversity is often used to compare the forest ecosystems ecological status and evaluate the forest communities and ecosystems (ESMAILZADEH & HOSSEINI, 2008).

Owing to the dominant position of trees in forests and their impact on various ecological gradients, the identity and composition of tree species can be expected to influence plant biodiversity, *i.e.* understory vegetation diversity and composition (BARBIER *et al.,* 2008). Plant species diversity in the forest understory extensively has been studied because the understory is a major component of forest ecosystems and plays an important role in many ecological functions and processes (POORBABAEI & POORRAHMATI, 2009). High species diversity in ecosystems led to high food chain and more complex network environment (EMBERLIN, 1983). In this regard, plantation by woody species and prevention of inappropriate harvesting plays an important role in maintaining diversity of forest ecosystems (CARNUS *et al.*, 2006).

Plantations compared with natural stands, particularly by changes of light and nutrient can affect habitat conditions and plant species composition (LEGARE *et al.*, 2001). Light is commonly considered to be the major limiting factor of forest vegetation cover and (or) richness (BARBIER *et al.*, 2008). GILLIAM & TURRILL (1993) showed that understory layer is more limited by light availability when canopy is closed; and MESSIER *et al.*, (1998) found that shade-intolerant tree species, such as aspen, white birch and jack pine, transmit further light than shade-tolerant tree species like balsam fir and white spruce. Indeed, in full light conditions, when the canopy is removed, or on poor site, nutrient availability become more critical to the understory and the better adapted species should be more competitive and productive (LEGARE *et al.*, 2002). Temporal changes in species diversity in a managed forest show the effects of forest management on small-scale colonization and extinction. Overall, plantations with non-native species as well as coniferous species in most areas differently affects vegetation and produce the requisite approve to ecologically sustainable forest management requires understanding the effects of forest management on plant and plant species diversity (NAGAIKE *et al.*, 2003). However, high species diversity of understory plants has been reported within plantations in surrounding natural forests (YIRDAW, 2001, NAGAIKE, 2002, NAGAIKE *et al.*, 2006). Also some assumptions are often made, e.g., that hardwoods are more favorable to biodiversity than conifers (BARBIER *et al.*, 2008). The aim of plantations has changed from a single objective (wood production) studies on biodiversity in plantations have been increasing (HARTLEY, 2002, NAGAIKE, 2002). Therefore, studies of species diversity in managed forests are required to predict and manage the transition of species diversity in such forests.

North of Iran has been covered by a deciduous temperate commercial forest on the northern slopes of the Alborz Mountains overlooking the Caspian Sea. Between mountain region and Caspian Sea, there are low-drained plain areas whereas during last decades have been involved in agriculture, reforestation or deforestation activities. Development in plantations of native species such as Caucasian alder (*Alnus subcordata* C.A.May) and exotic woody species, like bald cypress (*Taxodium distichum* (L) L.C. Rich), has occurred in these areas in recent decades. The purpose of this study is to examine plant species diversity and similarity of understory species in these plantations with 3 × 3 m and 4 × 4 m planting distances and determine how some stand characteristics can effect on plant diversity. The results of this study can also declare which tree species is more suitable for plantation in these sites.

Material and methods

Site Characteristics

Site study is located in southern coast of the Caspian Sea, 10 Km from Amol city, north of Iran (34°36′N″, 19°52′E″, 10 m above sea level. Rainfall with wetter months occurs between September and March, and a dry season from April to August. The climate is temperate on based Demarton climate classification, with a mean annual temperature of 16.9 °C and mean annual precipitation of 883 mm for along with the 1990 to 2008 years. The soil of plantations is poor drainage and has a silty-loam texture with pH 7.6–8.1.

The species of *Alnus subcordata* C.A. Mey (Caucasian alder) and *Taxodium distichum* (L.) L.C. Rich. (Bald cypress) were planted in this area in 1992, where previously covered by natural stands dominated by *Carpinus betulus* and *Parrotia perssica.* In reality, the plantations were 17 years in research time. *Alnus subcordata* and *Taxodium distichum* were planted with two planting distances of 3 × 3 m and 4 × 4 m. Not any thinning operations were made in these plantations.

Research method

In each stand, 6 plots 20 × 20 m were selected in regular distances from each other (KENT & COKER, 1992) and in each plot; diameters of trees at 1.3 m height (D.B.H.)

and tree heights were measured. Crown covers percentage of each stand as an expression of the light conditions for the ground vegetation (HARDTLE *et al.*, 2003). Litter thickness was measured in four selected points of plot corners and its average was calculated (POORBABAEI & POORRAHMATI, 2009). In each plot of 20 m × 20 m abundance sociability of herbaceous and woody species was visually estimated following modified Braun Blanquet scale (KENT & COKER, 1992).

Biodiversity indexes were used to evaluate plant diversity. Species diversity and richness in each plot were quantified using three indexes: the Shannon–Wiener diversity index (*H/*), Equitability (*J/*), and richness, r = the number of species per unit area - data in percent was use in indexes, *H/* and *J/* (BARBOUR *et al.*, 1999). The *H/* and *J/* values were calculated from the frequency of occurrence of each species per plot (i.e., 1–6) using the following formula based on MAGURRAN (2004):

$$J^{/} = \frac{H^{/}}{\ln m} \qquad H^{/} = -\sum_{i=1}^{m} pi \ln pi \; ,$$

where *pi* is the frequency of occurrence of each species relative to the total frequency of occurrence of all species in each plot, and m is the number of species in each plot.

Jaccard similarity index *(JI)* was selected for clarify of similarity species between each tree stand (with specified plantation density) with another stand (with specified plantation density) (LUDWING & RENOLDS, 1988):

$$JI = \frac{a}{a + b + c} \; ,$$

where *a* is the species in common in two stands, *b* is the only number of species identified in first stand and c is the only number of species identified in second stand.

For all the analyses, SPSS v.11.5 software was used. Normality of the data distribution was checked by Kolmogorov–Smirnov test, and Levene's test was used to examine the equality of the variances. One-way analyses (ANOVA) of variance were used to compare stands with normal distribution data (herbaceous diversity; stands characteristic; litter thickness; crown cover percentage). H Kruskal-Wallis was used to compare nonparametric data of the stands (woody diversity). Tukey-HSD (stand characteristic) and Duncan (herbaceous diversity; litter thickness; crown cover percentage) tests were used to separate the averages of the dependent variables which were significantly affected by the stands. In nonparametric data, Mann-Whitney U tests were used to separate the averages of the dependent variables which were significantly affected by stands (woody diversity).

Results

Stand characteristics, crown cover percentage and litter thickness

Results showed that diameter in breast height (D.B.H) in stands of 4 × 4 m planting distance was the highest and in stands of 3 × 3 m planting distance was the lowest ($P < 0.01$, Tukey-HSD) (Table 1). In both planting distances *Alnus* had greater diameter in breast height (D.B.H) and height compared to *Taxodium* (Table 1). In both planting spacing *Taxodium* plantations had the higher crown cover and litter thickness in comparison with *Alnus* plantations (Table 1).

Herbaceous and Woody species diversity

In herbaceous layer, *H/* diversity index and richness index were significantly higher in *Alnus* stands than *Taxodium* stands ($P < 0.01$, Duncan), but *J/* equitability index did not differ in stands (Table 2). In woody layer, diversity index *H/*, richness indexes and equitability index *J/* were significantly higher in *Alnus* stands ($P < 0.05$, H Kruskal-Wallis) than *Taxodium* stands (Table 3).

Floristic composition and similarity (JI)

There were 19, 21, 10 and 12 herbaceous species in *Anus* 3×3, *Alnus* 4×4, *Taxodium* 3×3 and *Taxodium* 4×4 plantations, respectively (cumulative numbers) (Table 4). Number of woody species was 14, 13, 7 and 7 in the same order previous forests (Table 5). Both in herbaceous layer and woody layer the higher similarity *(JI)* was found between *Alnus* 3×3 m and *Alnus* 4×4 m, and between *Taxodium* 3×3 m and *Taxodium* 4×4 m. *Alnus* 3×3 m and *Alnus* 4×4 m had higher similarity *(JI)* than *Taxodium* 3×3 m and *Taxodium* 4×4 m (Table 6 and 7).

Table 1. The mean of D.B.H. and height of trees, litter thickness and crown cover percentage (± sd) in the plantations

Plantation	D.B.H (cm)	Height (m)	Litter thickness (cm)	Crown cover (%)
Alnus 3×3 m	22.79 ±0.39 a	17.38±0.74 a	0.26 ± 0.07 c	43.16 ± 8.30 b
Alnus 4×4 m	27.21±1.12 b	19.52±0.44 a	0.18 ± 0.06 c	48.66 ± 7.66 b
Taxodium 3×3 m	19.68±1.29 c	10.72±0.79 b	7.41 ± 0.65 a	91.66 ± 4.57 a
Taxodium 4×4 m	24.57±0.67 ab	12.34±0.39 b	5.16 ± 0.47 b	83.50 ± 6.55 a
P Sig.	**	**	***	***

** $P < 0.05$ (ANOVA) *** $P < 0.01$ (ANOVA)

Table 2. Herbaceous species diversity indexes in the plantations

Stand	*Diversity* Index ($H^/$)	*Richness* Index	*Equitability* Index ($J^/$)
Alnus 3×3 m	1.66 a (0.14)	11.50 a (0.56)	0.68 (0.05)
Alnus 4×4 m	1.62 a (0.09)	11.33 a (0.49)	0.66 (0.02)
Taxodium 3×3 m	1.10 b (0.14)	5.16 b (0.60)	0.68 (0.07)
Taxodium 4×4 m	1.20 ab (0.07)	6.16 b (0.40)	0.66 (0.04)
F	5.71	41.32	0.077
P Sig.	0.005**	0.000**	0.972 ns

ns treatment effect not significant.** $P < 0.01$ (ANOVA) * $P < 0.05$ (ANOVA)

Table 3. Woody species diversity indexes in the plantations

Stand	*Diversity* Index ($H^/$)	*Richness* Index	*Equitability* Index ($J^/$)
Alnus 3×3 m	1.25 a (0.11)	5.00 a (0.36)	0.77 a (0.04)
Alnus 4×4 m	0.91 a (0.11)	4.50 a (0.56)	0.62 ab (0.06)
Taxodium 3×3 m	0.55 b (0.13)	3.16 b (0.16)	0.46 b (0.08)
Taxodium 4×4 m	0.49 b (0.10)	3.00 b (0.25)	0.43 b (0.09)
F	9.12	7.14	4.62
P Sig.	0.001**	0.002**	0.013*

** $P < 0.01$ (H Kruskal-Wallis), * $P < 0.05$ (H Kruskal-Wallis)

Discussion

Stand characteristics, crown cover percentage and litter thickness

Light is commonly considered to be the major limiting factor of forest vegetation cover and (or) richness (BARBIER *et al.,* 2008). In full light conditions, when the canopy is removed, or on poor site, nutrient availability may become more critical to the understory, the better adapted species should be more competitive and productive (LEGARE *et al.,* 2002). Thus, in the present study *Taxodium* stands that have more closed canopy than *Alnus*, light can play important role in diversity indexes. As a result, higher crown cover percentage in *Taxodium* plantations causes the increase of litter thickness. There are several researches for conifer stands have been well documented where canopy layer values were high; the cover of vascular plants and ground layer vegetation was reduced through shading (SAKURA *et al.,* 1985, HILL, 1986, SCHOONMAKER & MCKEE, 1988, FAHY & GORMALLY, 1998).

Table 4. List of herbaceous species in each stand

Herbaceous species	*Alnus* 3×3 m	*Alnus* 4×4 m	*Taxodium* 3×3 m	*Taxodium* 4×4 m
Calystegia sepium	×	×		
Cardamine impatiens	×	×		
Carex remota	×	×	×	×
Carex sylvatica	×	×	×	×
Conyza canedensis				
Cyperus sp.	×	×	×	×
Equisetum maximum	×	×	×	×
Galium aparine	×	×		
Humulus lupulus				
Iris sp.	×	×		
Juncus sp.	×	×		
Lamium album	×	×		
Mentha aquatic		×		
Mentha piperita				
Oplismenus undolatifolius				
Oxalis acetosella			×	×
Plantago major				
Poa sp.	×	×		×
Primula hecterochroma	×	×		
Pteris cretica		×		×
Ruscus hyrcanus	×	×	×	×
Sambacus ebulus	×	×		
Smilax excels	×	×	×	×
Solanum dulcamara	×	×	×	×
Stelaria media	×	×		
Urtica dioica	×	×	×	×
Viola alba	×	×	×	×
	19	21	10	12

Table 5. List of woody species in each stand

Woody species	*Alnus* 3×3 m	*Alnus* 4×4 m	*Taxodium* 3×3 m	*Taxodium* 4×4 m
Acer velutinum	×	×		
Alnus glutinosa	×	×		
Alnus subcordata	×	×		
Cornus australis			×	×
Crataegus monogyna	×	×	×	×
Diospyrus lotus	×			
Ficus carica	×	×	×	×
Gleditschia caspica	×	×	×	×
Mespilus germanica	×	×		
Morus alba	×	×	×	×
Petrocaria fraxinifoilia				×
Populus caspica	×	×		
Populus deltoids				
Prunus divaricate	×	×		
Quercus castaneifolia	×	×	×	
Salix aegyptiaca	×	×		
Taxodium distichum			×	×
Ulmus carpinifolia	×	×		
	14	13	7	7

Table 6. Jaccard's similarity index *(JI)* (in percent) of woody species in the plantations

Stand	*Alnus* 3×3 m	*Alnus* 4×4 m	*Taxodium* 3×3 m	*Taxodium* 4×4 m
Alnus 3×3 m		0.92	0.31	0.23
Alnus 4×4 m			0.33	0.50
Taxodium 3×3 m				0.75

Table 7. Jaccard's similarity index *(JI)* (in percent) of herbaceous species in the plantations

Stand	*Alnus* 3×3 m	*Alnus* 4×4 m	*Taxodium* 3×3 m	*Taxodium* 4×4 m
Alnus 3×3 m		0.90	0.45	0.47
Alnus 4×4 m			0.40	0.50
Taxodium 3×3 m				0.83

Litter also has physical effects on understory vegetation: seed under litter are deprived of light and seeds on it cannot root easily (BARBIER *et al.,* 2008). In our study broad-leaved and conifer plantations had lower and higher crown cover percentage respectively. Also, the litter thickness in *Taxodium* stands was higher than that in

Alnus stands. PIOTTO *et al.,* (2003) declared high overstorey transmission led to increase the litter decomposition. In this relation, WESENBEECK *et al.,* (2003) underlined that high crown cover in *Pinus patula* causes to decreased in diversity and alter the composition of native species. LEUSCHNER (1999) came to similar conclusions in an analysis of beech stands in central and north-west Germany, during which he also established a close negative correlation between the number of species and the density of fine roots from the tree layer in the humus horizons. PARITSIS & AIZEN (2008) suggest that plantations with more open canopy could favor plant diversity by increasing individual abundance and species richness. NAGAIKE *et al.,* (2006) observed young plantations (just before canopy closure and just after weeding) had the highest species diversity, richness, and light levels of the three ages of plantation. Greater species diversity and richness during the initial phase of vegetation development, before canopy closure (NIEPPOLA, 1992, HANNERZ & HAENELL, 1997) is caused primarily by colonization of many ruderal species (HALPERN, 1989, MILLER *et al.*, 1995).

Herbaceous and Woody species diversity

In herbaceous and woody layers, H' index diversity was highest in *Alnus* stands. These results may be for high budget light in crown (DEAL, 1997) and rich soil N *Alnus* stands (Eshter *et al.,* 2006). The higher diversity in plantations was mainly due to invasive species. Such patterns imply increased species diversity after severe human disturbance (POORBABAEI & POORRAHMATI, 2009). On the other hands, NAGAIKE *et al.,* (2006) in their investigation revealed that plantations 40 years of age had significantly lower species diversity and richness than young plantations, possibly as a result of declining light levels after canopy closure (BARBIER *et al.,* 2008). In herbaceous layer, there was not any significant difference between *Alnus* 3×3 and *Alnus* 4×4 m also in *Taxodium* stand. In this relation, our work seems to be consistent with that of LEGARE *et al.,* (2002), who describe the absence of significant variation of herb layer related to forest composition could be explained by the fact that light availability at the forest ground floor level and at 50 cm above the forest floor is not significantly different among the different stands.

Species richness is one measure of biodiversity and it is very important for ecosystem functioning, stability and integrity (COROI *et al.,* 2004). It is widely accepted that broad-leaved forests have higher plant species richness than conifer stands (BARBIER *et al.,* 2008). Also COROI *et al.* (2004) reveled that plant species richness in the broad-leaved riparian stands was almost double that of the alien conifer plantations. Such a result was found in present study, particular in *Alnus* stands. WELCH & SCOTT (1997) reported a strong decline of shade-tolerant understory plant species in a 20-year old pine plantation in England and they predicted that colonization would be the main factor limiting further development of understory plant diversity in the plantation. This trend may be one of the reasons of decline in understory plant species in *Taxodium* stands investigated in our study whereas the diversity indices in these stands was lower than those in *Alnus* stands. MICHELSEN *et al.,* (1996) found that most of the understory herbs in Ethiopian highland plantations of *Pinus*, *Eucalyptus* and other alien tree taxa were widespread. Incoherence reasons for this result and result of present study can be affirmed that such study was conducted in lowland, while the study of MICHELSEN *et al.* (1996) it was conducted in highland. High plant species richness in alder stands also has been reported in coastal Oregon (FRANKLIN & PECHANEC, 1968, DEAL, 1997).

Floristic composition and similarity (JI)

There were 19, 21, 10 and 12 herbaceous species in *Anus* 3×3 m, *Alnus* 4×4 m, *Taxodium* 3×3 and *Taxodium* 4×4 plantations, respectively. Also number of woody species was 14, 13, 7 and 7 in the same order above mentioned stands. Low number of herbaceous and woody species in plantations compared to other studies may be owing to low drainage soil (VANN &

MEGONIGAL, 2002). This trend was found in POORBABAEI & POORRAHMATI (2009) study, who reported low number of herbaceous and woody species on poor drainage site.

All plantation stands of the present study exhibited low similarity *(JI)* of woody species composition. A similar result, i.e. low similarity among different plantation species was reported by PANDE *et al.*, (1998). SENBETA *et al.*, (2002), who found that the intensity of light reaching the forest floor may differ in accordance with the intensity of crown cover, and this may influence understory plants colonization.

Conclusions

From this investigation it can be concluded that in comparison with *Taxodium*, *Alnus* stands have higher growth attribute, less crown cover percentage and litter thickness, higher diversity indices, and can maintain plant diversity and facilitate succession process better than *Taxodium* stands. These are because of small and light canopy of *Alnus* trees, whereas light penetrates easily to forest ground, or may be for higher concentrations of total nitrogen and ammonium in its litter (ESHTER *et al.*, 2006, GLAESSENS *et al.*, 2010). On the base of the results of this investigation, it seems that *Alnus subcordata* as a fast-growing species can be proposed for plantations in plain areas of northern Iran where the site is a low-drained.

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Macrophyte-Based Assessment of the Ecological Status of Lakes in Bulgaria

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Abstract. The aquatic macrophytes of lakes, situated in the whole territory of Bulgaria, were monitored during 2009. Six lake groups were established using differences in characteristics reflecting altitude, high calcium content and salinity, and altered hydromorphology and/or artificial origin. Abundance and species composition were assessed at each lake according to the requirements of the EU Water Framework Directive, using the assessment procedure of macrophyte-based assessment system, proposed by the Bavarian Environment Agency. The procedure included calculation of the 'ecological quality ratio' (EQR) for each of 78 water bodies, based on transect monitoring data. For 31 of these lakes, a macrophyte assessment system was applied, for the remaining 47 lakes macrophyte quantity was insufficient or depopulation was assessed. Ecological status classification of lakes is based on the calculation of a Reference Index value. The Reference Index quantifies the deviation of species composition and abundance from reference conditions and classifies sites as one of the five possible ecological quality classes specified in the Directive. The EQR indicating Good and High (Maximum) Ecological Status/Potential for macrophytes was achieved in 12 of the 31 lakes which fulfilled the criteria for assessment. The water quality parameters in lake types were discussed.

Key words: aquatic macrophytes, lakes, ecological status, Water Framework Directive

Introduction

The study summarized the implementation and adaptation of macrophyte-based assessment system in lakes in Bulgaria for assessment of ecological status, including eutrophication impacts, based on species composition and abundance of aquatic macrophytes.

For this purpose Reference Index (SCHAUMBURG *et al.*, 2007) was chosen to be applied and adapted in Bulgaria, while classifies water bodies by regional approach and reflects different kinds of ecological stresses. Reference Index (RI) methodology has been established to determine the ecological status/potential via integrative way as required by the Water Framework Directive (EUROPEAN UNION, 2000). The method is based on the occurrence and abundance of submerged species assigned to type specific species groups (A, B and C). The RI system has been generally applied in practice and implemented by governmental institution (e.g. Bavarian Water Management Agency). Additionally set of 14 water parameters was elaborated and was tested within lake groups.

Basic study of aquatic vegetation in Bulgaria had been accomplished 30 years ago by KOCHEV & JORDANOV (1981). In

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recent years the inventory of about 9000 wetlands with their biodiversity was conducted (MICHEV & STOYNEVA, 2007). The assessment of 80 water bodies was made on the basis of four main metrics (phytoplankton biovolume; Algae Groups Index; transparency, chlorophyll a) and three additional metrics (% Cyanobacteria; intensity of algal "bloom" and presence of toxic species) by CHESHMEDJIEV *et al.* (2010). Trophic state index for scaling the eutrophication along the Bulgarian Black Sea coastal zone was applied by MONCHEVA *et al.* (2002). High-mountain lakes in Rila Mountain were studied by Kalchev et al. (2004), particularly the variables biomass and size structure of bacterio- phyto- and zooplankton, and occurrence of three fish species, and multivariate statistical methods were tested for pollution assessment from SIMEONOVA *et al.* (2010).

The study aimed at assessment of ecologcal status/potential of lakes on the Bulgarian territory, representative for all lake types. For the first time dataset for 78 water bodies was collated for the calculation of the 'ecological quality ratio' (EQR) based on macrophytes as a component of the "Macrophytes and Phytobenthos" biological quality element, as demanded by the Directive.

Material and methods

Aquatic vegetation was studied in the period June – September 2009 at 78 water bodies on the Bulgarian territory, sub-ecoregion 12-1 (Danube); sub-ecoregion 12-2 (Black Sea) and ecoregion 7 (Eastern Balkans).

All submerged, floating-leafed, helophyte and amphiphyte species (Charophytes, Bryophytes and Tracheophytes) were recorded at each lake. At each lake, 1–15 sites were investigated according to the lake size. Sampling was carried out following the recommendations of SCHAUMBURG *et al.* (2007). Nomenclature accepted in GROLLE & LONG (2000) for liverworts and HILL *et al.* (2006) for mosses was followed. Taxonomy of vascular plants followed Flora Europaea (TUTIN *et al.*, 1968–1980; 1993). The abundance of each species was noted according KOHLER (1978). Calculated Reference index was transformed in EQR, where value „1" reflects the best possible ecological status in terms of the Water Framework Directive (WFD), i.e. high ecological status/potential (ecological status class 1); value of "0" stands for poor ecological status/potential. The unreliable status class 5 which reflects the highest degree of degradation of a water system is designated in case of a proven macrophyte depopulation (e.g. due to mowing, clearing, high input of nutrients, introduction of herbivorous fish, etc.). Index limits for attribution of the ecological status class were related to river types.

In order to describe the environmental conditions affecting aquatic vegetation, thirteen parameters of anthropogenic pressure and water quality were used. *In situ* were measured acidity (pH), electrical conductivity (C in $\mu S\ cm^{-1}$), dissolved oxygen ($mg\ L^{-1}$), Secchi disc reading (SD), and turbidity (FNU). Chemical analysis of river water was performed just after sampling on spectrophotometer NOVA 60 (MERCK) following adopted standards: ammonium nitrogen - ISO 7150/1, nitrite and nitrate nitrogen - EN 26777 and ISO 7890-1, total nitrogen (TN) - EN ISO 11905-1, phosphate phosphorus - EN ISO 6878, total phosphorus (TP) - EN ISO 6878, biochemical oxygen demand - EN 1899-1,2, and chemical oxygen demand - ISO 15705.

Results and Discussion

Lake types and pressure parameters

The main descriptors of the biocoenotic lake types for the assessment with macrophytes according to the WFD are the following: ecological region; geology by calcium content; stratification. Based on them and additional descriptors (altitude, salinity and altered hydromorphology and/or artificial origin), the reported seventeen lake types for Bulgaria (CHESHMEDJIEV et al., 2010) were assigned to the following six groups: high mountain glacial lakes; mountain and semi-mountain lakes & reservoirs; carst and other natural lakes; lowland and riparian lakes and swamps (wetlands); lowland reservoirs and transitional waters (Table 1). The last lake group included six water bodies with varying salinity (from 0.2 at Shablensko to 63.4‰ at Atanasovsko Lake). Mean depth and water parameters measured values varied significantly both between the lake groups and among water bodies in the same group (Table 1).

Ecological status/potential

The total number of aquatic macrophyte species observed in a single lake ranged between 1 and 27. Twenty-six were registered indicator species, while number of accompanying species was 44 (Table 2). A number of indicator species were specifically occurring in one or two lakes only, such as *Lemna trisulca*, *Nymphaea alba* and *Stratiotes aloides* (Srebarna lake), *Nuphar lutea* (Shabla lake), *Potamogeton pusillus* (Ovchi kladenets), *Myriophyllum verticillatum* and *Elodea canadensis* (Batak reservoir, Choklyovo swamp), *Spirodela polyrrhiza* (Pchelina reservoir, Srebarna lake), *Zannichellia palustris* (Kovachitsa and Aleksandrovo reservoirs). Of the more common indicator species (*Myriophyllum spicatum*, *Ceratophyllum demersum*, *Potamogeton pectinatus*, etc.), most species showed high abundance.

Ecological classification was restricted to 31 lakes with sufficient macrophyte abundance. Extreme eutrophication leading to depopulation of submerged macrophytes is integrated in the RI by assigning sites with very low or missing vegetation to bad status (unreliable), if natural reasons for low macrophyte abundance such as coarse substrate, changeable water level, presence of herbivorous fish (*Ctenopharyngodon idella*), etc. can be excluded. While natural reasons for low macrophyte abundance can not be excluded at the rest 47 assessed lakes, the assessment of their ecological status/potential based on macrophytes was not possible and further researches are recommended.

Only three reservoirs from the vast majority of lakes in Bulgaria classified as 'heavily modified and artificial water bodies' had 'maximum ecological potential' (Table 2). The major part of the lakes assessed was in moderate ecological status/potential based on macrophytes.

Conclusions

Based on abiotic typology parameters and the database of Bulgarian lakes, six types of macrophyte-based lake classification have been identified: high mountain glacial lakes; mountain and semi-mountain lakes & reservoirs; karst and other natural lakes; lowland and riparian lakes and swamps (wetlands); lowland reservoirs and transitional waters.

The method for the macrophyte-based assessment of the ecological status of lakes relies on the Reference Index which comprises indicators of the taxonomic composition and macrophyte abundance of water bodies.

Existing monitoring data can be a starting point to assessment of possible trends in lakes in Bulgaria.

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Table 1. *The morphometric characteristic and water quality parameters in lake types.*

	High mountain glacial (n = 5)			Mountain and semi-mountain lakes & reservoirs (n = 40)			Carst and other natural lakes (n = 3)		Lowland and riparian lakes and swamps (wetlands) (n = 2)		Lowland reservoirs (n = 22)			Transitional waters (n = 6)		
Lake features	Min	Max	Med	Min	Max	Med	Min	Max	Min	Max	Min	Max	Med	Min	Max	Med
Max depth (m)	7	16.05	16	3.7	110	19	8.5	13	5	7	1.5	42	15.75	0.25	7.5	1.45
Water quality indicators																
pH	7.19	7.8	7.47	6.65	9.5	8.35	8.04	9.11	7.72	9.08	7.95	9.29	8.67	8.42	8.89	8.48
Conductivity (µS cm^{-1})	9.7	19.8	15	29	863	275	202	564	558	593	201	1044	423.5	584	105003	1537
Dissolved oxigen (mg L $^{-1}$)	7.2	8.71	7.9	4.4	11.1	7.86	8	13.1	8.15	9.9	4.9	18.4	8.6	1.14	8.64	5.88
COD (mg L $^{-1}$)	3	6.8	6.35	<4	43.8	12.3	10.4	44.5	20	61	5.5	62	20.15	0.005	71	33.15
BOD_5 (mg L $^{-1}$)	<1	1		<1	12.4	2.275	2.5	12.7	7.1	17	1	15.8	5.4	1.3	7.5	4.85
PO4-P (mg L $^{-1}$)			<0.01	<0.01	0.223	0.026	<0.01	0.029	<0.01	0.021	<0.01	0.044	0.023	0.005	0.317	0.045
TP (mg L $^{-1}$)	<0.01	0.002		<0.01	0.334	0.0305	0.017	0.198	0.108	0.147	<0.01	0.218	0.057	0.055	0.364	0.1105
NH4-N (mg L $^{-1}$)	<0.01	0.032	0.022	<0.01	0.664	0.04	0.019	0.029	0.045	0.108	0.01	0.002	0.08	0.042	0.17	0.074
NO2-N (mg L $^{-1}$)	<0.002	0.003	0.003	<0.002	0.039	0.005	0.002	0.041	0.029	0.058	1.55	0.067	0.81	0.007	0.025	0.0155
NO3-N (mg L $^{-1}$)			<0.20	<0.2	0.89	0.16	0.23	0.63	<0.2	0.38	<0.2	0.0085	0.25	<0.2	0.6	0.4
TN (mg L $^{-1}$)			<0.5	<0.5	2	1.2	<0.5	1.5	1.1	1.3	<0.5	4.1	1.2	0.5	3	0.7
SD (m)	6.5	16.05	11.275	0.4	7.5	2.3	1.1	3.5	0.9	1.2	0.3	5	0.8	0.25	1.95	0.9
Turbidity (FNU)	<1	1		<1	28	3	1	7	12	20	2	61	10	2	83	7

Table 2. *Species composition and assessed ecological status/potential at studied 78 lakes during 2009.*

Lake	BG Type	Latitude	Longitude	Height a.s.l. [m]	RI	ES/EP	Indicator Species	Accompanying species
High mountain glacial								
Redzhepsko lake (Rila mountain)	L1	42.04461	23.30064	2344				
Bezbog lake	L1	42.31586	23.09073	2240				*Drepanocladus sendtneri* (Schimp. ex H.Müll.) Warnst.
								Racomitrium microcarpon (Hedw.) Brid.
Chernoto lake	L1	41.58508	22.58254	2375				
Dolno Georgiysko lake	L1	41.44465	23.22531	2294				
Beli Iskar reservoir	L1	42.08145	23.3408	1875				
Mountain and semi-mountain lakes & reservoirs								
Yarlovtsi reservoir	L2	42.80352	22.54049	798	0	moderate	*Elodea nuttallii* (Planch.) H.St.John	*Alisma plantago-aquatica* L.
							Lemna minor L.	*Bidens cernua* L.
							Myriophyllum spicatum L.	*Bidens tripartita* L.
							Potamogeton natans L.	*Juncus effusus* L.
								Lycopus europaeus L.
								Lythrum salicaria L.
								Utricularia vulgaris L.
Srechenska bara reservoir	L2	43.12223	23.12162	458				
Ognyanovo reservoir	L2	42.61465	23.74166	619	-4.5	moderate	*Ceratophyllum demersum* L.	*Phragmites australis* (Cav.) Trin. ex Steud.
							Myriophyllum spicatum L.	*Potamogeton trichoides* Cham. & Schltdl.
							Potamogeton nodosus Poir	*Typha latifolia* L.

							Potamogeton pectinatus L.	
Bebresh reservoir	L2	42.84675	23.78154	522				
Hristo Smirnenski reservoir (Gabrovo)	L2	42.81748	25.26554	533				
Yovkovtsi reservoir	L2	42.92175	25.79592	335	-47	moderate	*Ceratophyllum demersum* L. *Myriophyllum spicatum* L. *Potamogeton nodosus* Poir *Potamogeton pectinatus* L.	*Alisma gramineum* Lej. *Eleocharis palustris* (L.) Roem. & Schult. *Juncus effusus* L. *Lycopus europaeus* L. *Lythrum salicaria* L. *Mentha aquatica* L. *Mentha pulegium* L. *Mentha spicata* L. *Najas graminea* Delile *Potamogeton trichoides* Cham. & Schltdl. *Typha angustifolia* L.
Batak reservoir	L3	42.01152	24.12086	1103	-42	moderate	*Ceratophyllum demersum* L. *Elodea canadensis* Michx. *Myriophyllum verticillatum* L. *Myriophyllum spicatum* L. *Potamogeton natans* L. *Potamogeton crispus* L. *Potamogeton nodosus* Poir	*Najas minor* All.
Toshkov chark reservoir	L3	41.48544	24.1088	1419				
Studena reservoir	L3	42.51934	23.15144	848				
Vacha reservoir	L11	41.5604	24.26194	529				

Krichim reservoir	L11	41.5933	24.2757	419				
Koprinka reservoir	L11	42.4349	25.2938	386				
Zhrebchevo reservoir	L11	42.3701	25.175	259				
Kardzhali reservoir	L11	41.37915	25.20391	317				
Studen kladenets reservoir (wall)	L11	41.3714	25.3818	218	-50	moderate	*Myriophyllum spicatum* L.	*Najas minor* All.
Ivaylovgrad reservoir (wall)	L11	41.35057	26.06425	118	-50	moderate	*Myriophyllum spicatum* L.	
Iskar reservoir	L11	42.45753	23.5592	821				
Stamboliyski reservoir	L11	43.12031	25.16564	183	52	maximum	*Najas marina* L.	*Alisma gramineum* Lej.
							Potamogeton gramineus L.	
							Potamogeton nodosus Poir	
Eleshnitsa reservoir	L12	43.00333	27.46606	58				
Saedinenie reservoir	L12	43.32393	26.59885	172	10	good	*Lemna minor* L.	*Bidens tripartita* L.
							Potamogeton perfoliatus L.	*Lycopus europaeus* L.
							Potamogeton trichoides Cham. & Schltdl.	*Mentha aquatica* L.
								Mentha pulegium L.
								Phragmites australis (Cav.) Trin. ex Steud.
								Polygonum hydropiper L.
								Typha angustifolia L.
Yasna polyana reservoir	L12	27.344157	42.142399	84			*Myriophyllum spicatum* L.	
Daskal Atanasovo reservoir	L12	42.20484	25.55089	117	-50	moderate	*Myriophyllum spicatum* L.	*Phragmites australis* (Cav.) Trin. ex Steud.

							Scirpus lacustris L.	*Polygonum hydropiper* L. *Scirpus litoralis* Schrad. *Typha angustifolia* L.
Ovchi kladenets reservoir	L12	42.14084	26.14477	134	36	good	*Ceratophyllum demersum* L. *Lemna minor* L. *Myriophyllum spicatum* L. *Potamogeton crispus* L. *Potamogeton natans* L. *Potamogeton pusillus* L.	*Polygonum* sp. *Phragmites australis* (Cav.) Trin. ex Steud. *Scirpus litoralis* Schrad. *Typha angustifolia* L. *Veronica beccabunga* L.
Ovcharitsa reservoir	L12	42.14597	26.08234	134			*Myriophyllum spicatum* L.	*Cyperus longus* L. *Phragmites australis* (Cav.) Trin. ex Steud. *Scirpus litoralis* Schrad. *Typha angustifolia* L.
Kula reservoir (wall)	L12	43.91435	22.52832	202	-44	moderate	*Ceratophyllum demersum* L. *Chara* sp. *Elodea nuttallii* (Planch.) H.St.John *Myriophyllum spicatum* L. *Najas marina* L. *Potamogeton gramineus* L. *Potamogeton nodosus* Poir *Potamogeton trichoides* Cham. & Schltdl.	*Alisma gramineum* Lej. *Alisma lanceolatum* With. *Typha angustifolia* L.
Poletkovtsi reservoir	L12	43.85505	22.5138	203			*Myriophyllum spicatum* L. *Najas marina* L.	

							Potamogeton crispus L.	
Pancharevo reservoir	L12	42.58853	23.42169	608			*Ceratophyllum demersum* L. *Myriophyllum spicatum* L.	*Alisma plantago-aquatica* L. *Bidens tripartita* L. *Eleocharis acicularis* (L.) Roem. & Schult. *Eleocharis palustris* (L.) Roem. & Schult. *Typha angustifolia* L. *Typha latifolia* L. *Typha laxmannii* Lepech.
Sopot reservoir (wall)	L12	43.01123	24.42663	369	78	maximum	*Potamogeton gramineus* L. *Potamogeton pectinatus* L.	*Alisma gramineum* Lej. *Polygonum amphibium* L.
Yastrebino reservoir	L12	43.1547	26.27278	345				
Krapets reservoir	L12	43.05647	24.88699	406	36	maximum	*Chara* sp. *Elodea nuttallii* (Planch.) H.St.John *Myriophyllum spicatum* L. *Potamogeton gramineus* L. *Potamogeton pectinatus* L.	*Alisma gramineum* Lej.
Beli Lom reservoir	L12	43.40926	26.6836	280	-12.9	good	*Elodea nuttallii* (Planch.) H.St.John *Myriophyllum spicatum* L. *Potamogeton perfoliatus* L.	
Lomtsi reservoir	L12	43.44894	26.34388	214				
Kavatsite reservoir	L12	43.33687	26.25256	210			*Myriophyllum spicatum* L. *Potamogeton perfoliatus* L.	*Lycopus europaeus* L. *Lythrum salicaria* L. *Polygonum amphibium* L.

Boyka reservoir	L12	43.33102	26.00642	242			*Myriophyllum spicatum* L.	
Baniska reservoir	L12	43.43017	25.90641	166				
Belmeken reservoir	L13	42.10224	23.48575	1896				
Asenovets reservoir	L13	42.43055	26.1537	404				
Borovitsa reservoir	L13	41.45278	25.08367	483				
Dyakovo reservoir	L13	42.34589	23.08281	666			*Myriophyllum spicatum* L.	
Stoykovtsi resrvoir	L13	41.58508	22.58254	617				
Carst and other natural lakes								
Pchelina reservoir	L4	42.50905	22.82951	637	-52	poor	*Ceratophyllum demersum* L.	*Bidens cernua* L.
							Elodea nuttallii (Planch.) H.St.John	*Bidens tripartita* L.
							Lemna minor L.	*Polygonum amphibium* L.
							Myriophyllum spicatum L.	*Sparganium erectum* L.
							Najas marina L.	*Typha latifolia* L.
							Potamogeton pectinatus L.	
							Spirodela polyrrhiza (L.) Schleid.	
Choklyovo swamp	L4	42.39673	22.83257	875	46	high	*Ceratophyllum demersum* L.	*Lycopus europaeus* L.
							Chara sp.	*Mentha aquatica* L.
							Elodea canadensis Michx.	*Phragmites australis* (Cav.) Trin. ex Steud.
							Lemna minor L.	*Ricciocarpos natans* (L.) Corda
							Myriophyllum verticillatum L.	*Sparganium erectum* L.
							Potamogeton natans L.	*Typha latifolia* L.
							Potamogeton trichoides Cham. & Schltdl.	*Utricularia minor* L.

Rabisha reservoir	L4	43.73215	22.58757	295			*Utricularia vulgaris* L. *Myriophyllum spicatum* L.	
Lowland and riparian lakes and swamps (wetlands)								
Srebarna lake	L5	44.11258	27.07499	10	2	good	*Ceratophyllum demersum* L. *Lemna minor* L. *Lemna trisulca* L. *Nymphaea alba* L. *Potamogeton crispus* L. *Spirodela polyrrhiza* (L.) Schleid. *Stratiotes aloides* L. *Utricularia vulgaris* L.	*Alisma plantago-aquatica* L. *Berula erecta* (Huds.) Coville *Bidens tripartita* L. *Calystegia sepium* (L.) R.Br. *Epilobium hirsutum* L. *Hydrocharis morsus-ranae* L. *Lycopus europaeus* L. *Lythrum salicaria* L. *Mentha aquatica* L. *Phragmites australis* (Cav.) Trin. ex Steud. *Polygonum amphibium* L. *Polygonum hydropiper* L. *Rumex hydrolapathum* Huds. *Scirpus lacustris* L. *Solanum dulcamara* L. *Thelypteris palustris* Schott *Typha angustifolia* L. *Typha latifolia* L. *Vallisneria spiralis* L.
Bistraka lake/reservoir	L6	41.58375	23.04298	317				
Lowland reservoirs								
Tsonevo reservoir	L14	43.02502	27.41758	52	-50	moderate	*Butomus umbellatus* L. *Ceratophyllum demersum* L. *Myriophyllum spicatum* L.	*Alisma gramineum* Lej. *Bidens tripartita* L. *Najas minor* All.

							Najas marina L. *Potamogeton pectinatus* L. *Potamogeton perfoliatus* L.	*Polygonum hydropiper* L. *Typha angustifolia* L.
Ogosta reservoir (wall)	L14	43.38837	23.21495	191			*Myriophyllum spicatum* L. *Potamogeton nodosus* Poir	*Polygonum hydropiper* L.
Gorni Dabnik (wall)	L14	43.3524	24.33945	171	2	good	*Butomus umbellatus* L. *Ceratophyllum demersum* L. *Myriophyllum spicatum* L. *Najas marina* L. *Potamogeton crispus* L. *Potamogeton gramineus* L. *Potamogeton pectinatus* L. *Potamogeton perfoliatus* L. *Potamogeton trichoides* Cham. & Schltdl.	*Alisma lanceolatum* With. *Bidens tripartita* L. *Echinochloa crus-galli* (L.) P.Beauv. *Lycopus europaeus* L. *Najas minor* All. *Polygonum hydropiper* L.
Pyasachnik reservoir	L15	42.24129	24.35034	286				
Aheloy reservoir	L16	42.4238	27.3056	142				
Poroy reservoir	L16	42.4312	27.3724	28				
Drenovets reservoir	L16	43.69652	22.91433	177				
Hristo Smirnenski reservoir	L16	43.61538	23.01136	151				
Rasovo reservoir	L16	43.71423	23.24729	131				
Kovachitsa reservoir	L16	43.79377	23.34953	114			*Myriophyllum spicatum* L. *Zannichellia palustris* L.	*Alisma gramineum* Lej. *Lycopus europaeus* L. *Typha latifolia* L.
Dabnika reservoir	L16	43.20752	23.59096	346	-90	poor	*Elodea nuttallii* (Planch.)	

							H.St.John *Myriophyllum spicatum* L. *Potamogeton nodosus* Poir *Potamogeton pectinatus* L.	
Tricladentsi reservoir	L16	43.43251	23.63823	159				
Barzina reservoir	L16	43.57552	23.73506	84	0	good	*Myriophyllum spicatum* L. *Potamogeton crispus* L.	*Echinochloa crus-galli* (L.) P.Beauv. *Polygonum hydropiper* L.
Asparuhov val reservoir	L16	43.44538	23.37546	96				
Devets reservoir (wall)	L16	43.30598	23.95645	201	-68	moderate	*Ceratophyllum demersum* L. *Elodea nuttallii* (Planch.) H.St.John *Myriophyllum spicatum* L. *Potamogeton nodosus* Poir *Potamogeton pectinatus* L. *Potamogeton trichoides* Cham. & Schltdl.	*Alisma plantago-aquatica* L. *Epilobium hirsutum* L. *Lycopus europaeus* L. *Lythrum salicaria* L. *Typha angustifolia* L.
Enitsa reservoir (wall)	L16	43.36668	24.01623	171				
Krushovitsa reservoir (wall)	L16	43.3582	24.40803	115	-64	moderate	*Najas marina* L. *Potamogeton crispus* L. *Potamogeton nodosus* Poir *Potamogeton pectinatus* L.	
Telish resrvoir (wall)	L16	43.31923	24.23747	226				
Valchovets reservoir (wall)	L16	43.47443	24.49603	89	-62	moderate	*Ceratophyllum demersum* L. *Myriophyllum spicatum* L.	*Echinochloa crus-galli* (L.) P.Beauv. *Lycopus europaeus* L.

							Najas marina L.	*Najas graminea* Delile *Najas minor* All. *Polygonum hydropiper* L.
Aleksandrovo reservoir (wall)	L16	43.26968	24.92104	123	0	good	*Najas marina* L. *Potamogeton pectinatus* L. *Potamogeton trichoides* Cham. & Schltdl. *Zannichellia palustris* L.	*Alisma gramineum* Lej.
Kamenets reservoir	L16	43.35551	25.01291	95	0	good	*Butomus umbellatus* L. *Myriophyllum spicatum* L.	*Alisma gramineum* Lej. *Polygonum hydropiper* L. *Typha angustifolia* L. *Typha laxmannii* Lepech.
Antimovo reservoir	L16	43.98737	26.69923	98	-62	moderate	*Ceratophyllum demersum* L. *Elodea nuttallii* (Planch.) H.St.John *Lemna minor* L. *Myriophyllum spicatum* L. *Sparganium erectum* L.	*Typha angustifolia* L. *Typha latifolia* L.
Transitional waters								
Durankulak swamp	L7	43.67898	28.53981	4	-44	moderate	*Butomus umbellatus* L. *Ceratophyllum demersum* L. *Lemna minor* L. *Myriophyllum spicatum* L.	*Berula erecta* (Huds.) Coville *Calystegia sepium* (L.) R.Br. *Lycopus europaeus* L. *Mentha aquatica* L. *Phragmites australis* (Cav.) Trin. ex Steud. *Polygonum hydropiper* L. *Scirpus lacustris* L. *Scirpus maritimus* L. subsp.

								maritimus
								Typha angustifolia L.
								Typha latifolia L.
Shabla lake	L7	43.56788	28.56175	-3	-44	moderate	*Ceratophyllum demersum* L.	*Calystegia sepium* (L.) R.Br.
							Lemna minor L.	*Hydrocharis morsus-ranae* L.
							Myriophyllum spicatum L.	*Lycopus europaeus* L.
							Nuphar lutea Sm.	*Phragmites australis* (Cav.) Trin. ex Steud.
								Solanum dulcamara L.
								Sparganium erectum L.
Mandra reservoir	L7	42.2622	27.2554	5	-34	moderate	*Myriophyllum spicatum* L.	*Alisma plantago-aquatica* L.
							Najas marina L.	*Ruppia maritima* L.
							Potamogeton perfoliatus L.	
Alepu lake	L8	42.2118	27.4229	-0.5	-42	moderate	*Ceratophyllum demersum* L.	*Hydrocharis morsus-ranae* L.
							Potamogeton crispus L.	*Trapa natans* L.
Pomoriisko lake	L10	42.3416	27.3802	0				
Atanasovsko lake	L10	42.30297	27.203296	0				

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Toxicity of Essential Oils Isolated from Achillea millefolium L., Artemisia dracunculus L. and Heracleum persicum Desf. Against Adults of Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) in Islamic Republic of Iran

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Abstract. The environmental problems caused by overuse of synthetic insecticide have been the matter of concern in recent years. Essential oils from aromatic plants are recognized as proper alternatives to conventional insecticides. Therefore, this study was conducted to determine the Fumigant toxicity of essential oils from *Achillea millefolium*, *Artemisia dracunculus* and *Heracleum persicum* against adults of *Plodia interpunctella* under laboratory conditions and mortality was determined after 12, 24, 36 and 48 h from beginning of exposure. The essential oils were extracted from seeds of *H. persicum* and aerial parts from 1.5 cm of top of *A. millefolium*, *A. dracunculus* by hydrodistillation method using a Clevenger apparatus. All essential oil were highly effective against *P. interpunctella* and the mortality values reached 100% when the adults were exposed to 50, 65 and 80 µl/ l concentrations of *A. dracunculus*, *A. millefolium* and *H. persicum* essential oil, respectively. The LC_{50} (lethal concentration to kill 50% of the population) values of essential oils from *A. dracunculus*, *A. millefolium* and *H. persicum* were 22.24, 34.80 and 36.96 µl/ l after 24 h fumigation, respectively. On the other hand, *A. dracunculus* oil was more effective than the other essential oils against *P. interpunctella* adults. The LC_{50} values decreased with increasing of exposure times. In all cases, considerable differences in the mortality of insect to essential oils were observed with different concentrations and exposure times. These results suggest that the essential oils of *A. millefolium*, *A. dracunculus* and *H. persicum* have merit further studies as potential fumigants for the management of *P. interpunctella* or probably other stored-product insects.

Key words: *Achillea millefolium*, *Artemisia dracunculus*, *Heracleum persicum*, essential oil, Fumigant toxicity, *Plodia interpunctella.*

Introduction

Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), is distributed world-wide and is a serious stored-product pest of grain and seeds as well as flour and other milled products (NANSEN & PHILLIPS, 2004). It prefers to feed on broken grains and more especially on milled cereal, dried fruits and almonds, pistachios and walnuts and groundnuts. It is found in warehouses (PEREZ-MENDOZA & AGUILERA-PENA, 2004). In recent years, it was considered as the most important pest of stored pistachios in Iran which cause severe qualitative and quantitative losses in this fruit crop (SHOJAADDINI *et al.,* 2005). Because of its high incidence, synthetic insecticides have been used to control it. Synthetic pesticides have been considered the most effective and accessible means to control insect pests of stored products (HUANG & SUBRAMANYAM, 2005). The indiscriminate use of synthetic pesticides has given rise to many serious problems, including genetic resistance by pest species, toxic residues, increasing cost of application, pollution of storage environment, and hazard from handling (BARNARD *et al*., 1997; ISMAN, 2000; HANSEN & JENSEN, 2002; KOUL *et al*., 2008).In view of these facts, researchers for the last two decades or so have diverted their attention towards age-old practices of using alternative eco-friendly insect pest control methods which are readily biodegraded, less toxic to mammal, easy to use and specific in their action. Therefore, the use of essential oils extracted from aromatic plants to control insect pests has been investigated and is well documented (ISMAN, 2006; KOUL *et al*., 2008; RAJENDRAN & SRIRANJINI, 2008).

Heracleum persicum Desf. Belongs to Apiaceae family, known as "golpar", is native to Iran. Fruits of the plant are used as a constituent of the daily diet of general population in Iran. *Artemisia dracunculus* L. (Asteraceae), known as "tarkhun" in Iran, is a small shrubby perennial herb. It is cultivated for the use of its aromatic leaves in seasoning, salad, and soup. *Achillea millefolium* L. (Asteraceae), known as "yarrow", is a perennial herb that has been widely used in folk medicine in many countries (BENEDEK *et al*., 2008). Consequently, the objective of this research was to evaluate the insecticidal activities of essential oils from *A. dracunculus*, *A. millefolium* and *H. persicum* against *P. interpunctella* under laboratory conditions. On the other hands, the goal of this study was to seek much safer and cheaper agents for controlling insect pests.

Material and methods

Insect cultures and experimental conditions

The colony of *P. interpunctella* was reared on a diet of 80% ground rice, 10% glycerin, 5% yeast and 5% honey in plastic containers (30 cm length × 20 cm width × 8 cm height). Mouth of the containers was covered with fine mesh cloth for ventilation as well as to prevent escape of the insects. The cultures were maintained in the laboratory at 27 ± 1 °C, 60 ± 5% Relative Humidity (RH) and 16:8 h light: dark. Adult insects, 1-2 days old, were used for fumigant toxicity tests. Parent adults were obtained from laboratory stock cultures maintained at the Entomology Department, University of Urmia, Iran. All experimental procedures were carried out under the same environmental conditions as the cultures.

Plant materials and extraction of essential oils

The ripe seeds of *H. persicum* and aerial parts from 1.5 cm of top of *A. dracunculus* and *A. millefolium* were collected at the flowering stage from Ardabil city, Ardabil province, Iran. The specimen plants were air dried in the shade at room temperature (26-28 ºC) for 14 days. The essential oil was isolated from dried plant samples by hydrodistillation method using a Clevenger apparatus. Conditions of extraction were: 50 g of air-dried sample, 1:10 plant material/water volume ratio, 4 hrs distillation. Anhydrous sodium sulfate was used to remove water after extraction. Extracted oils were stored in a refrigerator at 4 ºC.

Insecticidal activity

The insecticidal effects of the essential oils were evaluated by fumigation method. The fumigant bioassays were conducted as described by NEGAHBAN *et al*. (2007), with slight modifications. In this study, tested insects were confined in the plastic tubes. Thus, there was no direct contact between the oil and the insects. Different concentrations were prepared to evaluate mortality of insects after an initial dose-setting experiment. *P. interpunctella* were exposed to *A. dracunculus* oil at 10, 14.8, 22.3, 33.4 and 50 µl/ l, to *A. millefolium* oil at 20, 27, 36.2, 48.5 and 65 µl/ l and to *H. persicum* oil at 25, 33.5, 44.7, 59.8 and 80 µl/ l. Each concentration was applied to filter paper stripe (4 × 5 cm, Whatman N° 1) and the impregnated filter papers were then attached to the screw caps of glass jars with volumes of 1 L. Ten insect adults were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with clothes mesh and each tube were placed at the bottom of glass jar. Caps of glass jars were screwed tightly. The jars were kept in the incubator and mortality was determined after 12, 24, 36 and 48 hrs from beginning of exposure. Each experiment was replicated for four times at each concentration. The control insects were maintained under the same conditions without any essential oil. When no leg or antennal movements were observed, insects were considered dead. The experiments were arranged in a completely randomize design and the data were analyzed with ANOVA. The means were separated using the Duncan's test at the 5% level. The LC_{50} values with their fiducial limits were calculated by probit analysis using the SPSS version 16.0 software package.

Results and Discussion

The essential oils from *A. dracunculus*, *A. millefolium* and *H. persicum* showed strong fumigant activity against *P. interpunctella* adults at different concentrations and exposure times. Figure 1 displays the mortality percentages of five different concentrations from essential oils in four times on *P. interpunctella*. This figure also shows that there are significant differences between insect mortality induced by essential oils with different concentrations and exposure times. These differences obtained by Duncan's test at $p \leq 0.05$. The mortality values reached 100% when the adults were exposed to 50, 65 and 80 µl/ l concentrations of *A. dracunculus*, *A. millefolium* and *H. persicum*, respectively (Fig. 1).

Essential oils showed variable toxicity to adults of *P. interpunctella*. The concentration for the essential oil to cause 50% mortality (LC_{50}) for *A. dracunculus* essential oil was 31.50 µl/ l after 12 h from commencement of fumigation (Table 1), whereas with *A. millefolium* and *H. persicum* essential oils 12 h LC_{50} values were 43.07 and 46.25 µl/ l respectively. The susceptibility of insect increased with exposure time and essential oil concentrations and LC_{50} values decreased within 48 h. On the other hand, increase susceptibility of insect associated with increase of the different concentrations of all oils and time of exposure. For example, LC_{50} value for *A. millefolium* essential oil decreased from 34.80 µl/ l at 24 h exposure time to 22.07 µl/ l after 48 h (Table 1). Based on LC_{50} values, essential oil of *A. dracunculus* was more potent than *A. millefolium* in the all times and it is obvious that *A. millefolium* oil had stronger toxicity than *H. persicum* oil on *P. interpunctella* (Table 1).

The failure to discover a significant new class of insecticides has led many researchers back to biodiscovery studies in the search for new and economically viable alternatives. It has been recognized that some plant-derived insect-control agents could be developed into products suitable for integrated pest management because they are selective to pests, have no or little harmful effect against non-target organisms or the environment (ISMAN, 2000). The most promising botanical groups are Meliaceae, Rutaceae, Asteraceae, Annonaceae, Lamiaceae, Aristolochiaceae and Malvaceae (REGNAULT-ROGER, 1997). *A. dracunculus* and *A. millefolium* are belonging to Asteraceae family and they are candidate for toxic agents on insect pests. However, the insecticidal activity of some essential oils from Apiaceae has been evaluated against a number of stored product insects (SAHAF *et al*., 2007; CHAUBEY, 2008; LOPEZ *et al*., 2008). In the present study the insecticidal activity of *H. persicum*, as one of the Apiaceae family, has been evaluated.

The effect of many essential oils as insecticides in protecting *P. interpunctella* infestation has been studied, and this insect has shown susceptibility to the some plant derived chemicals. AYVAZ *et al*. (2010) stated that the essential oils from oregano, *Origanum onites* L., savory, *Satureja thymbra* L., and myrtle, *Myrtus communis* L. were highly effective against *P. interpunctella*. In accordance with this study, our earlier study indicated that the essential oil of *Agastache foeniculum* Kuntze had fumigant toxicity against *P. interpunctella* (EBADOLLAHI *et al.*, 2010) and LC_{50} value decreased from 16.535 µl/ l at 24 h to 6.690 µl/ l at 96 h exposure time.

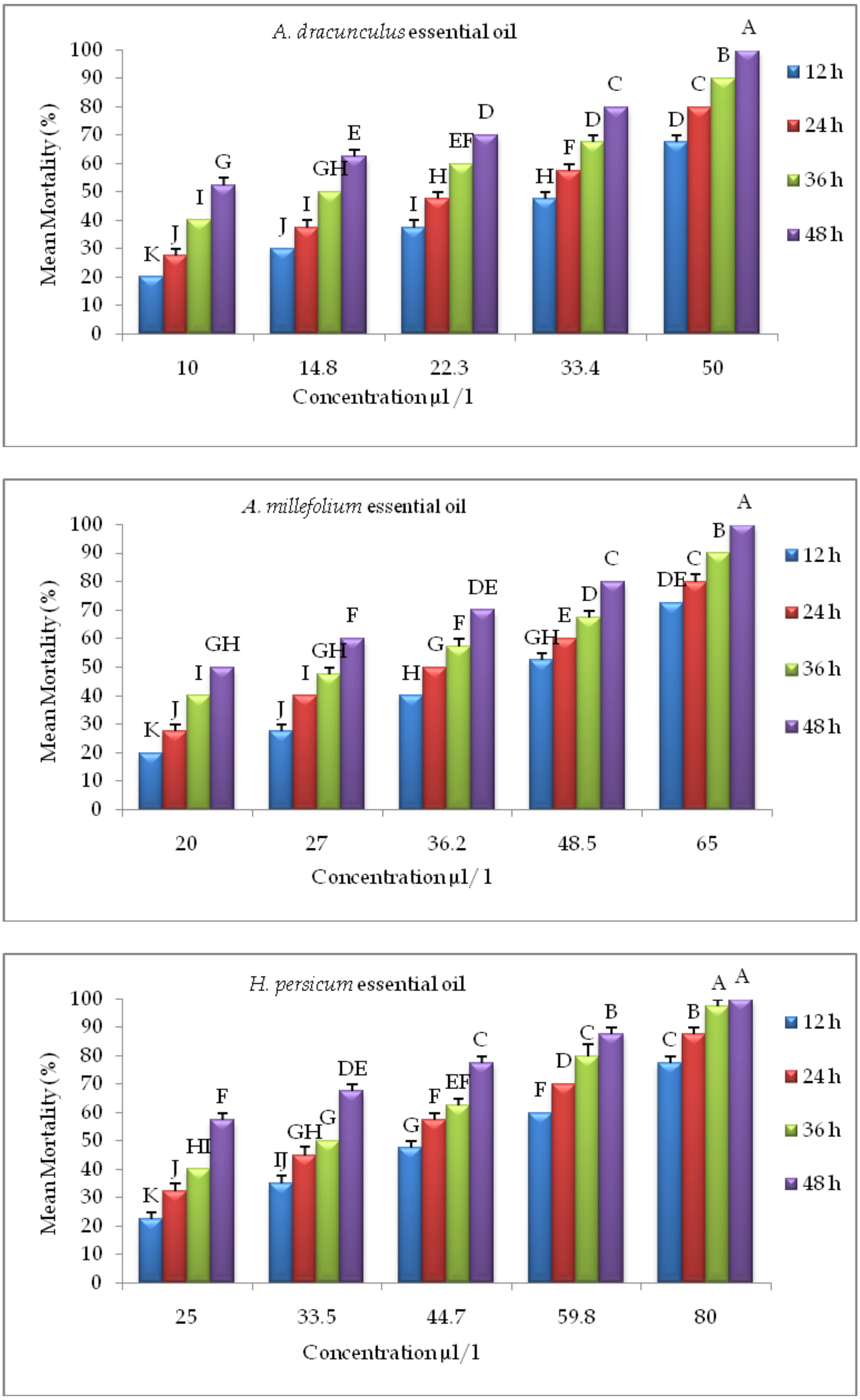

Fig. 1. Mean mortality (%) of *Plodia interpunctella* exposed to different concentrations of the essential oils from *Achillea millefolium*, *Artemisia dracunculus* and *Heracleum persicum*. Different letters on top of columns are significant differences according to Duncan's test at $p \leq 0.05$. Columns with the same letter are not significantly different. Vertical bars indicate standard error of the mean (±); very small values are not represented.

Table 1. Results of probit analysis from toxicity of the essential oils from *Achillea millefolium*, *Artemisia dracunculus* and *Heracleum persicum* against *Plodia interpunctella* at four different times.

Essential oil	Time (h)	LC_{50} (µl/l air)[a]	Slope	Intercept	Chi-square (χ^2)[b]
A. dracunculus	12	31.50 (24.75 – 46.04)	1.75	2.39	0.69
	24	22.24 (17.54 – 28.22)	1.91	2.43	1.32
	36	14.97 (10.44 – 18.80)	1.90	2.78	2.43
	48	10.68 (0.19 – 16.97)	2.22	2.71	5.57
A. millefolium	12	43.07 (37.0 – 52.62)	2.78	0.46	0.61
	24	34.80 (29.07 – 41.17)	2.62	0.97	0.82
	36	27.79 (21.74 – 32.69)	2.63	1.21	2.79
	48	22.07 (3.92 – 30.13)	3.20	0.69	5.33
H. persicum	12	46.25 (39.72 – 54.32)	2.89	0.19	0.24
	24	36.96 (30.64 – 42.60)	2.99	0.32	0.72
	36	32.42 (27.05 – 36.81)	3.62	-0.43	3.40
	48	23.72 (16.43 – 28.58)	3.30	0.47	3.05

[a]95% lower and upper fiducial limits are shown in parenthesis [b]df = 3

Similar to our results, MAHMOUDVAND *et al.* (2011) studied the fumigant toxicity of essential oils extracted from *Rosmarinus officinalis* L., *Mentha pulegium* L., *Zataria multiflora*, and *Citrus sinensis* (L.) Osbeck var. *hamlin* on adults of *P. interpunctella* and discovered that *Z. multiflora* and *R. officinalis* had fumigant toxicity on *P. interpunctella* adults. RAFIEI-KARAHROODI *et al.* (2011) investigated insecticidal effect of six native medicinal plants essential oil on *P. interpunctella*. They results demonstrated strong toxicity of essential oils extracted from *Melissa officinalis* L., *Mentha piperata* L., *Petroselinum sativum* Hoffmann, *Lavandula angustifolia* Mill., *Ziziphora clinopodioides* Lam., and *A. dracunculus*, on first instar larvae and eggs of *P. interpunctella*. These findings are parallel with the results of present study for susceptibility of *P. interpunctella* to plant essential oils.

Previous studies have showed that, in general, the toxicity of plant essential oils against stored product pests is related to their major components (TAPONDJOU *et al.*, 2002; SINGH *et al.*, 2003). 1,8-cineol, camphor and borneol in the *A. millefolium* essential oil (HAZIRI *et al.*, 2010), (Z)-anethole, methyl-eugenol, (Z)-β-ocimene and limonene in the *A. dracunculus* essential oil (AYOUGHI *et al.*, 2011) and (E)-anethole, γ-terpinene and limonene in the *H. persicum* essential oil (FIRUZIA *et al.*, 2010), were major components. LEE *et al.* (2001) showed 1,8-cineole (LC_{50} = 23.5 µl/l air) was the most toxic fumigant on *S. oryzae*, followed by limonene (LC_{50} = 61.5 µl/l air) and α-terpinene (LC_{50} = 71.2 µl/l air). CHANG & AHN (2002) studied fumigant activity of (E)-anethole *Blattella germanica*. (E)-Anethole caused 100% mortality at 0.398 mg cm-2 4 h after treatment. HUANG *et al.* (2002) indicated contact toxicity of eugenol, isoeugenol and methyleugenol on *Sitophilus zeamais* Motsch. and *Tribolium castaneum* (Herbst). PAPACHRISTOS *et al.* (2004) investigated relationship between the chemical composition of the essential oils from *Lavandula hybrida* Rev, *Rosmarinus officinalis* L and *Eucalyptus globulus* Labill and their insecticidal activity against *Acanthoscelides obtectus* Say. They found among the main constituents, only linalyl and terpinyl acetate were not active against

A. obtectus, while all the others (including teprinen-4-ol, camphor, 1,8-cineol, verbenone, p-cymene, S(-)limonene, R(+)limonene, γ-terpinene, α-terpineol) exhibited insecticidal activity against both male and female adults. OGENDO *et al.* (2008) introduced (Z)-β-ocimene as a toxic agent against adults of *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), *Oryzaephilus surinamensis* (L.), *Rhyzopertha dominica* (F.) and *Callosobruchus chinensis* (L.). Moreover, Repellency of Bornyl acetate, Borneol, Linalool, p-Cymene and Camphene against *Myzus persicae* (Sulzer) (Aphididae) was proved (MASATOSHI, 1998). Therefore, the insecticidal activity of *A. dracunculus*, *A. millefolium* and *H. persicum* essential oils could be related to these constituents.

The essential oils investigated in this study are used as pharmaceuticals and in flavoring and are therefore considered less harmful to humans than most conventional insecticides and they can use as safe fumigants for controlling *P. interpunctella*. However, the possibility of employing this natural insecticides in the management of Indian meal moth is plausible, but is worthy of further investigation. Future research should focus on residues on target commodity and the influence of any residues on product acceptability.

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Data on Population Dynamics of Three Syntopic Newt Species from Western Romania

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Abstract. We studied the population dynamics of three syntopic newt species [*Mesotriton alpestris* (Laurenti, 1768), *Lissotriton vulgaris* (Linnaeus, 1758) and *Triturus cristatus* (Laurenti, 1768)] in Zarand Mountains (Arad County, Romania). *M. alpestris* had the shortest aquatic phase, approximately two months, out of which the nuptial display was 2-3 weeks long. *L. vulgaris* and *T. cristatus* spent three months in the habitat, having a nuptial display of 2-3 weeks for *L. vulgaris,* and of 4-5 weeks for *T. cristatus*. *M. alpestris* had the highest degree of reproductive synchronization, while this was the lowest at *T. cristatus.* Males from all three species had a higher affinity for the aquatic habitat than females. The population size was estimated at 769 for *L. vulgaris*, 588 for *T. cristatus*, and 294 for *M. alpestris.* Balanced sex ratio was observed in the peak of breeding activity for all species.

Keywords: Salamandridae, aquatic phase, population dynamics, population size, sex ratio.

Introduction

There are five newt species in Romania: *Lissotriton vulgaris, Lissotriton montandoni, Mesotriton alpestris, Triturus cristatus, Triturus dobrogicus* (COGĂLNICEAU *et al.*, 2000). The conservation status of these five species is governed by the Government Emergency Ordinance 57/2007, which transposed into Romanian legislation the Birds Directive and Habitats Directive (O.U.G. 57/2007). The size of a population is an important parameter in assessing the conservation status of plant and animal species, as well as in the management of Natura 2000 network sites. There are few studies on newt population dynamics in Romania (CICORT-LUCACIU *et al.*, 2008, 2009, 2010; DOBRE *et al.*, 2009). The present study focuses on the dynamics of the number of individuals during repopulation (entering in the aquatic habitat) and depopulation (leaving the aquatic habitat) of an aquatic habitat from western Romania, used for reproduction by three newt species (*L. vulgaris, T. cristatus, M. alpestris*). Based on this data, we could then determine the size of the populations and sex-ratio. According to IUCN red list, all these three species are considered least concern (IUCN, 2011).

Material and methods

The study was carried out in the spring of 2006, when we went on field at four times: on the 9th of April, the 6th of May, the 20th of May, and on the 17th of June. On each occasion we tried to capture all newts present in the habitat. Newts were captured using nets with a round metal frame, fixed to the end of long metal rods. We noted the species, gender and age class (juvenile or adult) for each captured individual, followed by their release into the natural environment. Adults and juveniles were determined based on the presence (adults) or the absence (juveniles) of the secondary sexual characteristics (see in: KARLSSON *et al*., 2007). Elimination of individuals was the method used in order to assess population size (see in: COGĂLNICEANU, 1997), counting the individuals present in the habitat. The highest value within the four counts was considered to be the size of a population (juveniles were not included). The number of males and females recorded at a certain time allowed to calculate sex ratio. We observed the dynamics of the number of newts in the habitat during their aquatic phase. A population's presence in the habitat at a certain moment was expressed as the percentage of the number of individuals captured at that time, considered to the size of the population.

The habitat is located at an altitude of 450 m in Zarand Mountains, upstream from Madrigesti locality (46°10′01′′N/ 22°14′52′′E). A county road is interposed between the habitat and a valley. The habitat is a quasi-permanent pond, formed between a road and a slope covered by beech forest. It is fed by a small spring and by rain fall, with a maximum length of 12 m, and a maximum width of 3-4 m. One of its sides is touching the edge of the forest, here the substrate being covered by leaves. Above this deposit of leaves, the water level is 30-40 cm deep and there is no aquatic vegetation. *Typha* sp. clusters are present in the middle of the pond. The deepest regions (approximately 1 m) do not present vegetation. The bank near the forest is steeper, with large clumps of *Juncus* sp. The bank close to the road is completely devoid of aquatic vegetation, being flooded in spring. The most relevant anthropogenic factor affecting this habitat is bathing of cattle from nearby households. On the other side of the road, close to this habitat, there is a group of four small ponds, with sizes of about 2-4 m^2. Unlike the main habitat, these are temporary ones, without aquatic vegetation.

Results

Out of the three newt populations, only *M. alpestris* was not present in all four field work periods. The aquatic phase of this species was approximately one month shorter than that of the other two species. In the first two periods, *L. vulgaris* populated only the banks. It was only in the third period (20th May) when we observed individuals from this species to be distributed throughout the habitat, and to form reproduction pairs. Moreover, in the first two periods of field work (9th April, 6th May), some individuals inhabited the small ponds, where no individuals from the other two species were present. In the second period (6th May), numerous newts were migrating from the small ponds into the large pond. In this period we found newts as victims of road traffic. Unlike *L. vulgaris*, *T. cristatus* inhabited the pond in a relatively uniform manner from the beginning of its aquatic phase. Breeding pairs of this species were observed starting from the second period (6th May) of the field work. The situation is different in case of *M. alpestris*. They also formed breeding pairs in the second period of work, but the pairs did not last until the third period. *M. alpestris* was found in the deepest regions of the habitat.

On the 20th of May we recorded the peak for the number of individuals of *L. vulgaris* and *T. cristatus*, while for *M. alpestris* the peak number was on the 6th of May (Table 1). The peak periods are the same considering gender, and also, age of individuals. Therefore, the size of the surveyed populations was 769 for *L. vulgaris*, 588 for *T. cristatus*, and 294 for *M. alpestris*.

The relative abundance of the individuals differs between the periods. For *T. cristatus* the relative abundance was low in the first period (Table 2). For *M. alpestris* this value is high, including the last period (Table 2), this species leaving the water in a short interval. Sex ratio was 0.96 for *L. vulgaris*, 0.73 for *T. cristatus*, and 0.71 for *M. alpestris* (Table 3).

Table 1. *Number of individuals captured in the study periods. The maximum numbers are given as the population sizes (juveniles were not considered – see the text).*

Species	Gender / Age class	9 April	6 May	20 May	17 June
Lissotriton vulgaris	males	81	108	377	121
	females	75	88	392	131
	adults	156	196	769*	252
	juveniles	-	-	5	-
Triturus cristatus	males	29	73	249	73
	females	32	61	339	111
	adults	61	134	588*	184
	juveniles	1	-	9	4
Mesotriton alpestris	males	28	122	102	-
	females	36	172	124	-
	adults	64	294*	226	-
	juveniles	-	-	-	-

* population size

Table 2. *Relative abundance of individuals in the four study periods. (100% was considered the recorded peaks for the number of individuals – see Table 1)*

Species	Gender	9 April	6 May	20 May	17 June
Lissotriton vulgaris	males	21.48%	28.64%	100%	32.09%
	females	19.13%	22.44%	100%	33.41%
	total	20.30%	25.54%	100%	32.75%
Triturus cristatus	males	11.64%	29.31%	100%	29.31%
	females	9.43%	17.99%	100%	32.74%
	total	10.53%	23.65%	100%	31.02%
Mesotriton alpestris	males	22.95%	100%	83.60%	-
	females	20.93%	100%	72.09%	-
	total	21.94%	100%	77.84%	-

Table 3. *Sex ratios (males/females) in different study periods and marked with asterisk for the whole population*

Species	9 April	6 May	20 May	17 June
Lissotriton vulgaris	1.08	1.23	0.96*	0.92
Triturus cristatus	0.91	1.20	0.73*	0.66
Mesotriton alpestris	0.78	0.71*	0.82	

Discussion

M. alpestris had a more synchronous reproduction than *L. vulgaris* and *T. cristatus*. Out of the latter two, the reproduction of *T. cristatus* was the most asynchronous, this being the only species which formed pairs for reproduction during two field work periods. It seems that *L. vulgaris* was affected by the low temperatures in the deeper regions of the habitat, recorded at the beginning of the aquatic phase and so populated only the banks. The larger size of *T. cristatus* did not allow them to occupy the marginal areas of the habitat, unlike *L. vulgaris*. The preference of *L. vulgaris* for areas immediately next to the bank was previously reported, as well (GHIRA, 2007). In these regions, the water is not so deep and heats up easier. Later this period, *L. vulgaris* had a uniform distribution, with individuals forming pairs only during a single period of work, similarly to *M. alpestris*. However, there is a difference between the populations of the two species in this regard, given the fact that similar to *T. cristatus, L. vulgaris* had a longer aquatic phase.

M. alpestris had the shortest aquatic phase. This is probably a consequence of the temporary character of the small habitats characteristic to the mountain regions in Romania in which this species is frequently found (STRUGARIU *et al.*, 2006; GHERGHEL *et al.*, 2008; SOS *et al.*, 2008; COVACIU-MARCOV *et al.*, 2009 a, b). Nevertheless, a study undertaken in the Apennines Mountains (FASOLA & CANOVA, 1992) reports that out of the syntopic populations from these three species, *M. alpestris* was the only one present in water throughout the year. Such cases were reported in Romania as well (COGĂLNICEANU *et al.*, 2000). Those populations inhabit high altitude lakes, where the surface is covered with ice for a longer time of the year.

L. vulgaris population had the highest number of individuals. This was reported in other cases as well, comparing with *T. cristatus* (CICORT-LUCACIU *et al.*, 2009; DOBRE *et al.*, 2009) or with *T. dobrogicus* (CICORT-LUCACIU *et al.*, 2008). A decreasing body size during speciation is a process of specialization to use food and habitat (JOLY & GIACOMA, 1992). The smaller size is an advantage for *L. vulgaris*, allowing a higher number of individuals within a limited space. Considering the reports about the habitats populated by *L. montandoni* and *T. cristatus*, we observe that this rule is generally valid within syntopic *Lissotriton* and *Triturus* populations (CICORT-LUCACIU *et al.*, 2010). Generally the number of *Triturus karelinii* populations is nearly always lower than that of the coexisting newt species (MERMER *et al.,* 2008), underlining the influence of the larger size of the crested newts. Furthermore, southern crested newts prefer relatively deeper waters for breeding, compared to other newt species (TARKHNISHVILI & GOKHELASHVILI, 1999; MERMER *et al.*, 2008), this preference being valid also in the case of *T. cristatus* population from Madrigesti. However, there are cases in which crested newts are more numerous that other newt species to which they co-habitate with (ŠIZLING & ZAVADIL, 2001).

There is an evident difference in the behaviour of males and females in the case of populations where the process of populating the habitat takes an evolution in time before reaching the peak number (*L. vulgaris* and *T. cristatus*). Males dominate females numerically at the beginning of the aquatic period, although females dominate males considering the structure of the population. Reproducing adults are, in general, faithful to the same aquatic habitat every year (JOLY & MIAUD, 1989). However, juveniles inhabit other aquatic habitats besides the one they originate from (BELL, 1977; GILL, 1978a; JOLY & MIAUD, 1989; LANGTON *et al.*, 2001). Therefore, when determining the size of population we did not take into account the number of juveniles. The difficulty of counting the juveniles was previously signalled (ARNTZEN & TEUNIS, 1993). Still, captured juveniles provide some information about the species' behaviour. We only found juveniles from species with a longer aquatic phase (*L. vulgaris* and *T. cristatus*). Probably this is a consequence of shorter aquatic phase for *M. alpestris*. The highest number of both adults and juveniles was recorded on the 20th of May. This was the case with both populations from this study, as well as with some *L. vulgaris* and *T. dobrogicus* populations from Western Plain (CICORT-LUCACIU *et al.*, 2008).

There are no big differences between *L. vulgaris* and *T. cristatus* with regard to leaving the habitat. Studies from Oas Mountains, Romania also report the size of *T. cristatus* as a limiting factor when repopulating the habitat (CICORT-LUCACIU *et al.*, 2010). In the beginning of the aquatic period the males` relative abundance was higher than the females`. A previous study mentions that males from syntopic *L.*

vulgaris and *L. helveticus* populations entered the water before females (HARRISON *et al*., 1983). The same study reports *L. helveticus* females leaving the water at a significantly later point. This behaviour was also observed while comparing values of sex ratio at the beginning and at the end of the aquatic phase (ARNTZEN, 2002). It was found that sex ratio is in favour of males at the beginning of the aquatic phase, and of females at the end of it. However, another study reports *T. cristatus* males to be in the water before females, and leaving the water after females (VERRELL & HALLIDAY, 1985). Other studies from north-western Romania also describe that newt populations follow this rule in the beginning of their aquatic phase (CICORT-LUCACIU *et al*., 2008, 2009, 2010). Considering *M. alpestris,* the percentage of individuals present in the habitat at the moment of our last count was too high to reflect the behaviour of depopulating the habitat.

The time spent in water without having a reproductive activity may be extended under favorable feeding conditions. Feeding studies indicate that, from the three species in question, *T. cristatus* had the highest feeding activity (e.g. COVACIU-MARCOV *et al*., 2010). So, we can consider that this species has the highest affinity to the aquatic habitat, in other cases it spending more time in water than *L. vulgaris* (GRIFFITHS & MYLOTTE, 1987). This was the only species present in the habitat in August as well, with a few individuals. A study in the Apennines reports that *T. cristatus* remained in water 3 to 4 months after the end of the reproductive period, while *L. vulgaris* used the aquatic habitat exclusively for reproduction (FASOLA & CANOVA, 1992). The relation between the relative abundance of individuals and the population size shows that males had higher presence in the habitat than females. Their affinity to the aquatic environment is probably a consequence of the presence of dorsal crest, which helps mobility in the water.

Sex ratio is approximately equal in the case of all three newt species. However, females were more numerous than males considering the structure of all three newt populations. There are previous studies which report an almost balanced sex ratio for newts (e.g. HAGSTROM, 1979; ARNTZEN, 2002; JOHNSON, 2002; MERMER *et al*., 2008). Whenever there is a slight difference, females are more numerous (e.g. HARRISON *et al*., 1983; KARLSSON *et al*., 2007, MERMER *et al*., 2008, ÇIÇEK & AYAZ, 2011). In very rare cases males outnumber females (GILL, 1978b). Several factors are known to favour females in sex ratio. The number of females increases when the temperature drops below optimum (WALLACE & WALLACE, 2000). Another study mentions that mortality of *L. vulgaris* males during winter is higher than that of females (BELL, 1977). Out of 15 newt populations previously studied in Romania (CICORT-LUCACIU *et al*., 2008, 2009, 2010; DOBRE *et al*., 2009), 10 had of approximately balanced sex ratios. In three cases, the sex ratio was female biased (CICORT-LUCACIU *et al*., 2008, 2010), while in two populations sex ratio was male biased (DOBRE *et al*., 2009; CICORT-LUCACIU *et al*., 2010).

In conclusion, the newts form Madrigesti behave in the aquatic period, following the rules established previously in the case of other populations from Romania (CICORT-LUCACIU *et al.,* 2008, 2009, 2010). *L. vulgaris* has the largest population and *M. alpestris* has the shortest aquatic period. These results show the uniformity of the factors that act upon the newts in the aquatic period indifferent of the geographical region.

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Preliminary Information on the Vertebrate Fauna (Animalia: Vertebrata) of the NATURA2000 Site "Rice Fields Tsalapitsa" (Bulgaria)

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Abstract. The study was carried out by tree visits in different areas of the Natura 2000 site "Rice Fields Tsalapitsa" BG 0002086 (West of Plovdiv city, near village of Tsalapitsa). Our preliminary research showed that in the area vertebrates with high conservation status occurred included in the Bulgarian Bidiversity Act, Appendix II and III, Appendix II of the Bern convention, Natura 2000 species list, the Bonn convention, Appendix II, the Convention of the International Trade of Endangered Species, and the list of the International Union for Conservation of the Nature. This information will help in future preparation of the management plan of the Natura 2000 site and showed the need of studies focused on such areas which will help undertaking adequate measures for their proper way of conservation.

Key words: Natura 2000, rice fields, Amphibia, Reptilia, Aves, Mammalia

Introduction

In the more and more rising problem with the water resources on Earth more attention is paid on the anthropogenic wetland habitats. Some of them are considered as a high priority for conservation holding a specific and rich biological diversity. In spite of that in Bulgaria there were no studies focused exactly on the rice fields and their fauna and flora, but in the mean time such habitats were determined by some foreign authors as very important for a variety of vertebrate animals (TRÉCA, 1992, FUJIOCA *et al*., 2004).

In this paper we represent first data on the species diversity of vertebrate animals and their conservation status in the rice fields "Tsalapitsa" in Bulgaria.

Materials and methods

The study was carried out by tree visits on 17 and 18.06.2010 in different areas of the NATURA 2000 site "Rice Fields Tsalapitsa" BG 0002086 (West of Plovdiv city, near village of Tsalapitsa).

Most of the species were registered by direct observations (using binoculars), and some by their tracks and signs (as excrements and pellets). The small mammals (insectivores and rodents) were registered by analyzing pellet contents of Barn Owl (*Tyto alba* Mill.) collected in the region. These species we consider as "possibly inhabiting the area", because of the specifications of the behaviour and the size of the hunting territory of these owls.

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The habitats where the species were registered were also noted down: 1. water basins with rice (*Oryza sativa*), 2. canals with rush (*Typha* sp.), 3. maize (*Zea mays*) crops, 4. dykes with grass vegetation and single trees, and 5. undetermined habitat (for the small mammals found in owl pellets).

Results and Discussion

During our visits of NATURA 2000 site "Rice Fields Tsalapitsa" in June 2010 we have registered 52 species of vertebrate animals: 3 amphibian species, 2 reptiles, 37 birds, and 10 mammals (Table 1 and 2).

According to their conservation status the species included in the Bulgarian Biodiversity Act, Appendix III, strictly protected species predominated (34), followed by those in Appendix II of the Bern convention (29). The NATURA 2000 species of priority were 19, and those with habitats with a priority for conservation (Bulgarian Biodiversity Act, Appendix II) were 14. The species included in the Bonn convention were from Appendix II – 13 species. Relatively small number of species figured in the Convention of the International Trade of Endangered Species (CITES) – 6 species. In the list of the International Union for Conservation of the Nature (IUCN) 2 species were registered and 6 species in the area were without any conservation status.

In the study area we registered breeding of 19 bird species (Table 1), some of them with high conservation status as *Glareola pratincola* and *Himantopus himantopus*. For some other birds the rice fields was an important feeding site like *Ciconia nigra*, *Ciconia ciconia*, *Nycticorax nycticorax*, *Ardea cinerea*, and *Egretta garzetta*.

Conclusions

Our preliminary research showed that in the area of the NATURA 2000 site "Rice Fields Tsalapitsa" BG 0002086 vertebrates having high conservation status occurred included in the Bulgarian Biodiversity Act, Appendix II and III, Appendix II of the Bern convention, NATURA 2000 species, the Bonn convention, Appendix II, the Convention of the International Trade of Endangered Species, and the list of the International Union for Conservation of the Nature.

This information will help in future preparation of the management plan of the NATURA 2000 site and showed the need of studies focused on such areas which will help undertaking adequate measures for their proper way of conservation.

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Table 1. List of the bird species registered in the Natura 2000 site "Rice Fields Tsalapitsa" BG 0002086. Abbreviations and habitat numbers were explained in the text.

Species	Biodiversity Act	IUCN red list	BERN	BONN	CITES	Natura 2000	Habitat	Breeding in the region
Aves								
Ixobrychus minutus Linnaeus,1766	II, III		II	II		*	2	yes
Nycticorax nycticorax Linnaeus,1758	II, III		II			*	1	no
Egretta garzetta Linnaeus,1766	II, III		II			*	1	no
Ardea cinerea Linnaeus,1758	III		III			*	1	no
Ciconia ciconia Linnaeus,1758	II, III		II	II		*	1, 2, 3	no
Ciconia nigra Linnaeus,1758	II, III		II	II	II	*	1, 3	no
Anas platyrhynchos Linnaeus,1758			III				1	?
Circus aeroginosus Linnaeus,1758	III		II	II		*	2	?
Buteo buteo Linnaeus,1758	II, III		II	II	II	*	5	no
Falco tinnunculus Linnaeus,1758	III		II	II	II	*	5	?
Perdix perdix Linnaeus,1758			III				4	?
Gallinula chloropus Linnaeus,1758	III		III			*	1, 2	yes
Himantopus himantopus Linnaeus,1758	III		II	II		*	1, 3	yes
Glareola pratincola Linnaeus,1766	II, III		II	II		*	1, 3	yes
Vanellus vanellus Linnaeus,1758	III		III	II		*	1, 3	yes
Tringa ochropus Linnaeus,1758	III		II	II		*	1, 3	?
Larus cachinans Pallas,1811			III			*	1, 5	no
Cuculus canorus Linnaeus,1758	III		III				2, 4	yes
Tyto alba Scopoli,1769	III		II		II		4	?
Athene noctua Scopoli,1769	III		II		II		4	yes
Alcedo atthis Linnaeus,1758	II, III		II			*	1	?
Upupa epops Linnaeus,1758	III		II				4	?
Alauda arvensis Linnaeus,1758	III		III				3, 5	yes
Galerida cristata Linnaeus,1758	III		III				3, 5	yes
Hirundo rustica Linnaeus,1758	III		II				1, 2, 3	yes
Motacilla flava Linnaeus,1758	III		II				1, 2, 3	yes
Luscinia megarhynchos Brehm,1831	III		II	II			4	yes
Acrocephalus arundinaceus Linnaeus,1758	III		II	II			2	yes
Hippolais pallida Hemprich & Ehrenberg,1833	III		II	II			4	yes
Lanius minor Gmelin,1788	II, III		II			*	4	yes
Pica pica Linneus,1758			III				2, 3, 4	yes
Corvus corone Linnaeus,1758							3, 5	?
Sturnus vulgaris Linnaeus,1758			III				4, 5	?
Passer hispaniolensis Temminck,1820	III		III				4	yes
Carduelis carduelis Linnaeus,1758	III		II				4, 5	?
Emberiza calandra Linnaeus,1758	II, III		II				3, 4	yes
Emberiza melanocephala Scopoli,1769	III		II				4	yes

Table 2. List of the bird species registered in the Natura 2000 site "Rice Fields Tsalapitsa" BG 0002086. Abbreviations and habitat numbers were explained in the text. With grey color were marked the species found only in pellets of Barn Owl (*Tyto alba*) and considered as "possibly inhabiting the area".

Species	Biodiversity Act	IUCN red list	BERN	BONN	CITES	Natura 2000	Habitat
Amphibia							
Bombina bombina Linnaeus, 1761	II		II			*	1
Rana ridibunda Pallas, 1771							1, 2, 4
Hyla arborea Linnaeus, 1758	II, III	*	II				2
Reptilia							
Podarcis taurica			II				4
Lacerta trilineata			II				4
Mammalia							
Crocidura leucodon (Hermann, 1780)			III				5
Neomys anomalus Cabrera, 1907			III				5
Mustela nivalis Linnaeus, 1766	III		III				4
Lutra lutra Linnaeus, 1758	II, III	*	II		I	*	1, 2
Vulpes vulpes Linnaeus, 1758							3
Lepus europeus Pallas, 1778			III				3
Myocastor coypus (Molina, 1782)							1, 2
Mus musculus Linnaeus, 1758							5
Arvicola terrestris (Linnaeus, 1758)							2
Microtus arvalis (Pallas, 1778) - *complex*							5

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Shell Size of the Freshwater Snail Physella acuta (Draparnaud, 1805) Collected from Water Vegetation: A Case Study from South-East Bulgaria

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Abstract. The specimens of the freshwater snail *Physella acuta* collected from Southeastern part of Bulgaria during the cold period as a whole were with mean shell height of 5.7 mm. During spring and summer it was more than twice lower, 2.3 mm. The ratio of the size groups was more equally spread during cold seasons rather than in warm ones. The variation index during cold seasons is about eight times higher than in the warm ones (Var = 3.96 and 0.58, respectively). On both plant species the gastropods had similar mean shell heights. For *C. demersum* it was 4.8 mm (min-max = 2.5-8.9 mm), and for *E. canadensis* 7.2 mm (min-max = 3.6-10.0 mm).

Key words: freshwater, quantity, gastropods, size, vegetation.

Introduction

The studies on the Bulgarian freshwater snails have started from the work of MOUSSON (1859), and continued with many others mainly focused on the taxonomy and diversity of species in various regions of the country (for example: BOURGUIGNAT, 1870, 1880; WAGNER, 1927; URBAŃSKI, 1960; ANGELOV, 1959, 1965, 1967, 1972, 1976).

Some ecological notes on the freshwater snails were given as a result of hydrobiological works (as RUSSEV *et al.*, 1998; KIRIN *et al.*, 2003, and many others) or synopses (ANGELOV, 2000; HUBENOV, 2005, 2006). Recently the first data on the habitats (GEORGIEV, 2005a, 2005b, 2006, 2008, GEORGIEV & STOYCHEVA, 2009), and species diversity, especially this one of the family *Hydrobiidae* Troschel, 1857 was intensively studied (GLÖER & PEŠIĆ, 2006; ZETTLER, 2008; IRIKOV & GEORGIEV, 2008; GEORGIEV & STOYCHEVA, 2008, 2011; GLÖER & GEORGIEV, 2009; 2011; GEORGIEV, 2009, 2011a, 2011b, 2011c, 2011d; GEORGIEV & GLÖER, 2011).

In Bulgaria there is a lack of detailed investigations regarding the ecology of the freshwater molluscs, while in the same time the foreign literature is quite rich on such kind of researches. Some of the most signify-cant aspects of the ecology of freshwater gastropods are their relations with the aquatic plants. Both are quite sensitive to water pollution, and are often used as bio-indicators (GECHEVA & YURUKOVA, 2008). Focus on this question was made by the works of VASILEVA *et al.* (2009, 2011) but not considering the size of the gastropods and their age groups.

The aim of this study is to investigate the size characteristic of the populations of *Physella acuta* (Draparnaud, 1805) dwelling on different water macrophytes during the cold and warm seasons in South-East Bulgaria.

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Material and methods

The research was conducted through the period 2008 - 2009 in the Upper Thracian Lowland: Maritsa River in the city of Plovdiv, flood area near the bridge at UFT, N42°09` E24°43`; Eastern Rhodopes: Varbitsa River at around 3 km south of the town of Kardzhali, N41°34` E25°23`. The field trips were made from 19.02.2009 until 12.11.2009.

The mollusks were collected by hand or with a sack, along with the aquatic vegetation and were transported to the laboratory. The material was collected from total of 1207 g herbage biomass from the plant species: *Ceratophyllum demersum* L. – Rigid Hornwort (Varbitsa River: 150 g, and Maritsa River: 458g), and *Elodea canadensis* Michx. – Pondweed (Maritsa River: 599 g). The analysis of the results was made according to the plant species and season (cold – autumn and winter, and warm – spring and summer).

The material (total of 204 specimens) was separated from the plants by hand and by running water. The shells of the molluscs were measured (only the shell height was considered) and determined by GLÖER & MEIER-BROOK (2003) and a reference collection. The size groups were considered according to 1 mm. The index of variation was calculated using the program MS Excel.

Results and Discussion

The specimens collected during the cold period as a whole (number of specimens N = 39) were with mean shell height of 5.7 mm (min-max = 2.5-10.0 mm). During spring and summer (number of specimens N = 165) it was more than twice lower, 2.3 mm (min-max = 1.0-4.3 mm).

During the warm season specimens (in the following, in parentheses % of the total number of collected specimens) with shell height of 1-3 mm dominated (80.00%), and the most numerous was the group size 1-2 mm (49.70%l). Lowest percentage had the snails with shell height of 4-5 mm (1.82%). Specimens with shells higher than 5 mm were not registered. Such we found during the cold seasons, those with shell height between 3 and 8 mm (79.49%). Lower percent had the group 2-3 mm (7.69%), and specimens shorter than 2 mm were not collected. Accepting the maximal sizes of the species pointed by GLÖER & MEIER-BROOK (2003), of 8-12 mm shell height we consider that during warm seasons on the water vegetation studied the juvenile specimens dominate, and during the cold period subadults are the most frequent, and some adults could also be found. The ratio of the size groups was more equally spread during cold seasons rather than in warm ones (Table 1, 2).

The variation index during cold seasons is about eight times higher than in the warm ones (Var = 3.96 and 0.58, respectively).

On both plant species the gastropods had similar mean shell heights. For *Ceratophyllum demersum* it was 4.8 mm (min-max = 2.5-8.9 mm), and for *Elodea canadensis* 7.2 mm (min-max = 3.6-10.0 mm).

Table 1. Number and percent of the size groups of *Physella acuta* on the freshwater macrophytes during spring and summer.

Size group	Number of specimens	%
1-2 mm	82	49.70
2.1-3 mm	50	30.30
3.1-4 mm	30	18.18
4.1-5 mm	3	1.82
Total	**165**	**100.00**

Table 2. Number and percent of the size groups of *Physella acuta* on the freshwater macrophytes during autumn and winter.

Size group	Number of specimens	%
2.1-3 mm	3	7.69
3.1-4 mm	7	17.95
4.1-5 mm	5	12.82
5.1-6 mm	8	20.51
6.1-7 mm	6	15.38
7.1-8 mm	5	12.82
8.1-9 mm	2	5.13
9.1-10 mm	3	7.69
Total	**39**	**100.00**

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Review on Periphyton as Mediator of Nutrient Transfer in Aquatic Ecosystems

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Abstract. In the studies of aquatic ecology, periphyton has been uncared for despite its vital role in nutrient uptake and transfer to the upper trophic organisms. Being the component of food chain as attached organism it takes part in nutrient cycling in the ecosystem like that of suspended planktonic counterparts. The present review, with an aim to understand the role of periphyton in nutrient transfer from benthic environment to upper trophic level, focuses many aspects of periphyton-nutrient relationship based on available literatures. It also attempts to redefine periphyton, as a part of biofilm, harboring nutrient components like protein, fat and carbohydrate preferably in its extracellular polymeric substance (EPS), cyanobacteria, diatom and other algal communities. In addition to physical processes, nutrient uptake by periphyton is catalyzed by enzymes like Nitrogen Reductase and Alkaline Phosphatase from the environment. This uptake and transfer is further regulated by periphytic C: nutrient (N or P) stoichiometry, colonization time, distribution of periphyton cover on sediments and macrophytes, macronutrient concentration, grazing, sloughing, temperature, and advective transport. The Carbon (C) sources of periphyton are mainly dissolve organic matter and photosynthetic C that enters into higher trophic levels through predation and transfers as C-rich nutrient components. Despite of emerging interests on utilizing periphyton as nutrient transfer tool in aquatic ecosystem, the major challenges ahead for modern aquatic biologists lies on determining nutrient uptake and transfer rate of periphyton, periphytic growth and simulating nutrient models of periphyton to figure a complete energy cycle in aquatic ecosystem.

Key words: Biofilm, bacteria, algae, nutrient stoichiometry, diffusive boundary layer, periphytic succession.

Introduction

In an aquatic food chain, the significance of food quality of primary producers for well-being of zooplankton and fishes has always been the focus of applied research. In determining food quality, attention was paid on two parameters, firstly, nitrogen (N): phosphorus (P) stoichiometry (URABE *et al.*, 1997) and secondly, Carbon (C) as DOC, carbohydrate and long-chain polyunsaturated fatty acids (PUFAs) (BRETT & MÜLLER-NAVARRA, 1997; WEERS & GULATI, 1997). The sestonic algal community has already been characterized as rich source for ω-3 PUFAs for higher trophic levels through extensive studies (See review of SAIKIA & NANDI, 2010). However, the epiphytic or attached life forms on aquatic substrates/plants (i.e. periphyton), being similar in genetic origin but occupying different ecological grade, requires wider attention in the context of food quality. The recently developed aquaculture technologies (KESHAVANATH *et al.*, 2001; SAIKIA & DAS, 2009) have experimentally proved the potentiality of periphyton as good source of quality food for stocked fish. On this

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background, certain basic questions need critical review in comprehending the nature of nutrient transfer ability of the periphytic life forms in aquatic food chain viz., (i) How does epiphytic or periphytic community act as food source for the grazers/detritus feeders? (ii) How the uptaken C, N and P in periphyton are being transferred into aquatic food chain and (iii) What are the stoichiometric consequences for uptake and transfer of nutrients in a periphyton based food chain? The present review is an effort to realize all those questions in detail as well as to present a comprehensive synthesis of fact on the background of available literature.

Biofilm or periphyton?

The terms 'biofilm' and 'periphyton' are often used interchangeably for all epiphytic microorganisms. While reviewing the present topic, it becomes a dilemma in referring the terminology is to be used throughout the discussion and therefore, both the terms need justified clarification to start with. Even though both the terms are used mostly as synonymous, however there exists narrow but significant difference from compositional as well as ecological point of view. The term 'biofilm' was coined and described in 1978 (COSTERTON *et al.*, 1978) that denotes to an aggregation of bacteria, algae, fungi and protozoa enclosed in a matrix consisting of a mixture of polymeric compounds, primarily polysaccharides, generally referred to as extracellular polymeric substance (EPS). The formation of biofilm is a prerequisite for the existence of all microbial aggregates (FLEMMING & WINGENDER, 2001a; SUTHERLAND, 2001) and it is an essential step in the survival of bacterial populations (VAN HULLEBUSCH, 2003). The proportion of EPS in biofilms can comprise between approximately 50-90% of the total organic matter (DONLAN, 2002; FLEMMING & WINGENDER, 2001b). In addition to polysaccharide, biofilms also consist of proteins, nucleic acids, lipids and humic substances. The composition and quantity of the EPS may also vary depending on the type of microorganisms, age of the biofilms and the different environmental conditions under which the biofilms exist (MAYER *et al.*, 1999). To its true meaning, microbial biofilms, which 'may' exclude eukaryotic primary producers, and thereby mostly includes decomposers and pioneer colonizing groups of early successional stages, can develop on a number of different surfaces, such as natural aquatic and soil environments, living tissues (e.g. gut lumen), medical devices or industrial or potable water piping systems (DONLAN, 2002; FLEMMING & WINGENDER, 2001a) etc.

However, the term "periphyton" though often used to describe microorganisms such as algae and bacteria growing in association with substrata (STEVENSON, 1996), has specificity, mostly in terms of nutrient dynamics in ecosystem. Close to such objectivity, WETZEL (1983a) defined it as the micro 'floral' community living attached to the substrate inside water. These micro flora plays an important role in water bodies, not only by being important primary producers (VADEBONCOEUR *et al.*, 2001; LIBORIUSSEN & JEPPESEN, 2003) and serving as an energy source for higher trophic levels (HECKY & HESSLEIN, 1995), but also by affecting the nutrient turnover (WETZEL, 1993) and the transfer of nutrients between the benthic and the pelagic zone (VANDER ZANDEN & VADEBONCOEUR, 2002). The substrate selectivity of periphyton commonly includes submersed plants or plant parts, rocks and sediments. Such substrate selection denotes periphyton's role in transferring and 'trophic upgrading' of nutrients available in the benthic environment either directly or indirectly. This property embodies periphyton under extensive research to design as a tool for biofiltering excess nutrient from polluted waters and for efficient nutrient transfer from primary to higher trophic levels. Therefore, periphyton, though a form of biofilm appears at a later stage of succession should be discussed under the preview of nutrient exchange between benthic and pelagic ecosystems. However, bacterial colonization and EPS formation are preconditions for periphyton colonization on any substrate. The commonly referred periphytic groups in relation to nutrient transfer through trophic levels are algae.

Nutrient composition of Periphyton

The basic foundation of ecosystem lies on the availability of food as C and macronutrients (viz. N and P). According to WHAL (1989), periphyton is formed following a settling pattern, which can be divided into four phases: (i) adsorption of dissolved organic compounds, i.e. macromolecules that attach to submersed surfaces, being a spontaneous physical–chemical process; (ii) bacterial settling – after colonization, bacteria start to produce extracellular polymeric substances (EPS), that protect them against predators, and increase their resistance to the radiation and dehydration; (iii) colonization by eukaryotic unicellular microorganisms, mainly protozoan, microalgae and cyanobacteria and (iv) settling of eukaryotic multicellular organisms. Therefore, the succession procedure of periphyton initiates accumulation of variable sources of nutrients as C, microbial protein, lipid and P in its complex.

In aquatic environments, bacterial EPS which is a precondition of periphyton colonization on natural substrate exist as a part of dissolved organic matter (LIGNELL, 1990) and in particulate matter (DECHO, 1990, 2000; PASSOW *et al*., 1994). FREEMAN & LOCK (1995) proposed that EPS of bacterial component acts as rich organic C storage. It has been shown to be polyanionic by nature (COSTERTON *et al*., 1978) and is believed to permit nutrient entrapment through ion exchange processes (FREEMAN *et al*., 1995). Such entrapment mechanism permits the storage of organic C in the biofilm. That is why EPS acts as an important supplier of C demand for many organisms that feed on periphytic aggregates (DECHO & MORIARTY, 1990; BAIRD & THISTLE, 1986; HOSKINS *et al*., 2003). Among the bacterial fractions, Cyanobacteria are important primary producers, many species of which are able to fix atmospheric N_2 (STEWART, 1973; WHITTON & POTTS, 1982). Chemical screening of many laboratory grown, commercially viable, marine cyanobacteria has revealed that they have a high nutritional value, in terms of protein (VENKATARAMAN, 1993). CHOI & MARKAKIS (1981) found 63% of crude protein content from *Anabaena flos aqua,* a very common periphytic candidate.

Other algal communities also play a key role in periphyton formation and nutrient addition to periphytic complex through their surfaces that provide potential habitats for several bacteria from early successional stages. A study on algal bacterial interactions revealed that in the case of submerged plant surfaces, bacterial abundance is significantly higher in areas of diatom colonization (DONNELLY & HERBERT, 1999). These bacteria involved in the community metabolism of periphyton can trap not only dissolved organic materials and debris drifting from the water body but also the metabolic products released by bacteria in algal species (MAKK *et al*., 2003). Such algal bacteria interaction turns periphytic organic matrix as a source of polysaccharides, proteins, nucleic acid and other polymers (DAVEY & O'TOOLE, 2000).

In algal-based food webs the abundances of essential elements (e.g. N, P) and organic compounds (e.g. fatty acids and amino acids) are thought to play a large role in determining algal food quality (STERNER & HESSEN, 1994; BRETT & MÜLLER-NAVARRA, 1997). Being dominated by algal members as secondary colonizer in periphyton formation, food webs regulated by these eukaryotic components are not a deviation of such possibility. Therefore, all microorganisms present in the periphyton regime represent a complementary food source, providing essential nutrients like polyunsaturated fatty acid (PUFA), sterols, amino acids, vitamins and pigment that help a better development of post successional organisms (THOMPSON *et al*., 2002).

The nutrient quality and availability on periphyton varies with several factors like grazing pressure, algal and bacterial taxonomic composition, nutrient level of environment, environmental purity, and most significantly to substrate type (MAKAREVICH *et al*., 1993; AZIM *et al*., 2002). MONTGOMERY & GERKING (1980) reported proximate composition of 16 periphytic algae grown on granite boulders suspended in the Gulf of California. Protein, lipid, carbohydrate and ash contents of these epilithic algae were 8-10, 2-5, 52-60, and 25-38% respectively. An average protein content of 15% was estimated in periphyton collected from coral reef (POLUNIN, 1988). DEMPSTER *et al.* (1995) reported 28-55% protein and 5-18% lipid in some algal species of periphytic nature. AZIM *et al.* (2001a) estimated 27.19% crude protein from periphyton grown on bamboo substrate. He also recorded 14.63% protein in Hizol (*Barringtonia* sp.) branches, 18.74% on Kanchi (bamboo side shoot), and 12.69% protein on jute stick. KESHAVANATH *et al.* (2004) also recorded protein level of 19.27-35.56% in periphyton. LEDGER & HILDREW (1998) recorded as low as 2-3% protein, 0.04-0.29% lipid, and 29–33% carbohydrate in periphyton grown on stones. BECKER (2007), in a recent study, reported protein, lipid and fat content of some algae as 35-63%, 10-57% and 2-22% respectively.

AZIM *et al.* (2001a) observed periphytic fat content as 5.43%, 0.35%, and 2.75%, respectively on substrates Hizol, Kanchi, and Jute stick. The ash content also shows variation with a range from 17.45–41%. AZIM *et al.* (2001b) observed ash content from periphyton on bamboo (29%), Hizol (41%), Kanchi (29%), and jute stick (31.12%). Ash content of periphyton is known to increase as the community grows older (HUCHETTE *et al.*, 2000). NIELSEN *et al.* (1997) found the EPS of biofilm accounted for 50-80% of the total organic matter, therefore, high amount of carbohydrate.

Thus, all sorts of nutrient components make their representation on the periphytic microhabitat. As periphytic microhabitat is constituted of heterogeneous prokaryotic as well as eukaryotic epiphytic microbial communities, the interactions of periphytic microhabitat might be more intraspecific than interspecific. Such interactions could enable addition of variable food qualities to the periphytic communities as a whole. Probably, these anthropogenic natures of periphyton stimulates survival and growth rates of several cultivated organisms on consumption (AZIM *et al.*, 2002; BRATVOLD & BROWDY, 2001; MRIDULA *et al.*, 2003; KESHAVANATH *et al.*, 2004). AZIM *et al.* (2001a) reported that periphyton alone can support fish production of 5000 kg/ha^{-1}year^{-1}. BALLESTER *et al.* (2007) demonstrated that the consumption of periphyton by the pink-shrimp *Farfantepenaeus paulensis* can enhance its survival and growth rates. ABREU *et al.* (2007) by using stable isotope analysis (d13 C and d15 N) demonstrated that periphyton contribution to the pink-shrimp *F. paulensis* growth represents 49% of carbon and 70% of nitrogen of shrimp demand.

How does periphyton uptake nutrients?

Periphyton assemblages can play significant roles in uptake of ambient macronutrients since they can trap particulate material from the water column (ADEY *et al.*, 1993). Such macronutrient uptake values are potentially influenced by the degree and distribution of periphyton cover on substrate in addition to ambient macronutrient concentration, grazing, sloughing, temperature, and advective transport. Such macronutrient uptake by periphyton and other benthic autotrophs is controlled by three principal processes that occur in series: (1) diffusion from the ambience into the viscous sub layer of the periphytic boundary layer; (2) slower transport, dominated by molecular diffusion, through the inner portion of the viscous sub layer (the diffusive boundary layer, or DBL) to periphyton cell surfaces; and (3) membrane transport from cell surfaces into cells. In relation to streams, LARNED *et al.* (2004) proposed three criteria of periphytic nutrient uptake. These are: (1) when the canopy is submerged within the DBL covering the substratum, uptake is controlled by the thickness of this DBL; (2) when canopy height is greater than DBL, but comparable with the substratum DBL thickness, uptake is controlled jointly by the substratum DBL and by individual DBLs surrounding the periphyton elements that protrude above the substratum DBL; and (3) when the substratum DBL is very thin and most of the canopy protrudes above it, uptake is controlled by the DBLs surrounding periphyton elements.

The rationale of nutrient uptake is further influenced by the activity of enzymes prevalent in periphytic body. The enzymatic uptakes of N and P by periphyton in algae are regulated in response to available macronutrients and are thus act as a physiological index of nutrient status (BEARDALL *et al*., 2001).

Inorganic N assimilation by algae follows the following pathway:

$$NO^{3-} \xrightarrow{NR} NO^{2-} \rightarrow NH^{4+} \rightarrow N\ uptake \rightarrow amino\ acids\ and\ proteins$$

Nitrate Reductase (NR) catalyzes the initial reduction of NO^{3-} to NO^{2-}, mostly available from decomposers from understory and overstory bacterial components, which is believed to be the rate limiting step in uptake and assimilation of NO^{3-} into amino acids and proteins. The activity of NR is regulated in response to available NO^{3-}, NO^{2-} and NH^{4+}; NR expression dependent on NO^{3-} and light and is suppressed by high ambient concentrations of NH^{4+} in most algae (BERGES *et al*., 1995; YOUNG *et al*., 2005).

Growth of microalgae consumes P as an essential element needed for cellular constituents such as phospholipids, nucleotides, and nucleic acids (MIYACHI *et al*., 1964). Much of the P-fraction available in the aquatic environment is not available for uptake by algae because it is bound to organic chelators. A widely distributed enzyme which helps cleave orthophosphate from the organic chelator is alkaline phosphatase (AP). The expression of AP activity is greatly elevated under conditions of low P availability, means when it is P limiting (DYHRMAN & PALENIK, 1997). The overall process is

$$Organically\text{-}bound\ PO_4^{3-} \xrightarrow{AP} free\ PO_4^{3-} \rightarrow P\ uptake$$

These uptake qualities of periphyton help it to act as a bioeliminator to improve water quality (SLÁDEČKOVÁ & MATULOVÁ, 1998). This is why the periphytic communities serve important regulatory functions that can drastically alter rates and pathways of ecosystem biogeochemical cycling (WETZEL, 1983b). CRISPIM *et al*. (2009) reported that periphyton is highly effective in removing nutrients through nutrient uptake efficiency, and, to a less extent, macrophytes sheltering epiphytes also play an important role. In a comparative study, they observed that macrophytes efficiently capture dissolved N, but not P from the water, whereas periphyton is efficient in capturing both.

A second biological mechanism of nutrient uptake is luxury P-uptake. Luxury P-uptake is the storage of P within the biomass in the form of polyphosphate. Polyphosphate can be present as acid-soluble or acid-insoluble polyphosphate. Acid-soluble polyphosphate is actively involved in metabolism, while acid-insoluble polyphosphate is stored for when the external phosphate concentration becomes limiting (MIYACHI *et al*., 1964).

Periphytic nutrient transfer

C source and transfer

Aquatic macrophytes and periphyton remove soluble nutrients from the ambient water during their growth phase. Nutrients acquired by periphyton may be released to the environment back via several processes. First, macrophyte and epiphytes release soluble nutrients by respiration and lysis and particulate nutrients back into the water column by sloughing, scour and dislodgement. A fraction of the senescence and periphytic detritus as dead particulate organic matter is mineralized either within the periphyton mat or in the water column to release soluble nutrients. Nutrients released into the water column are subjected to downstream transport while those retained in the periphyton mat are not. In stream, this phenomenon is called "nutrient spiralling" (NEWBOLD *et al*., 1981). Nutrients retained in the periphyton, in this way, could have two possible fates, viz. total loss to the sediment or transfer to upper trophic levels. Further, the transfer of periphytic nutrient to upper trophic levels has two possible pathways. First, direct nutrient uptake from environment and natural substrate and transfer to immediate grazer (SAIKIA *et al.,* in press). Second, through de novo synthesis of metabolic products as raw material and trophic upgrading to immediate predator (SAIKIA & NANDI, 2010).

The colonization of bacterial biofilms is the first phase towards periphyton growth on all wetted surfaces in aquatic ecosystems. The source of C in periphyton establishes from the time of initiation of bacterial biofilm formation. The bacteria, as initial colonizer on substrate develop micro-colonies with EPS (COSTERION, 1984). Through this EPS, bacteria provide a significant source of C to biofilm complex (PEARL, 1978; HOBBI & LEE, 1980) (Fig. 1). It thus represents a trophic link between dissolved organic and inorganic substrates in the water column and the higher trophic levels of the ecosystem (HYNES, 1970). Two such substrates, the colloidal and dissolved organic C (DOC) are known to relocate as energy source for the microorganisms in those biofilms (LOCK & FORD, 1985). In general, the bacterial C reserve of biofilm generates through three mechanisms. The first mechanism supplies energy during substrate scarcity. During first-cryptic growth, the dying bacteria "leak" metabolizable substrates to immediate neighbours of periphyton strata. This property not only protects the neighbours from starvation but may also permit their multiplication (POSTGATE, 1976). In a growing biofilm, cynobacteria and other early colonized algae share this C source. In aged periphytic assembly, the old mostly filamentous periphytic layer receives such C from bacterial ecomposition. The second mechanism consists of endogenous energy reserves such as poly-β-hydroxy alkanoate (PHA). These reserves consist of C that is accumulated inside the microbial cell and which can be mobilized to ensure survival during starvation (DAWES & SENIOR, 1973). This mechanism could also play an important role in the starvation responses of biofilm bacteria. The third organic C storage, is the polysaccharide matrix (FREEMAN & LOCK, 1995). The matrix is polyanionic by nature (COSTERTON *et al.*, 1978) and is believed to permit nutrient entrapment through ion exchange processes (FREEMAN *et al.*, 1995). FREEMAN & LOCK (1995) proposed that the entrapment mechanism may also permit the storage of organic C in the biofilm.

The bacterial C may enter to next trophic group as complex C rich compound (Fig. 1). The C rich compound under extensive research now a day is the Fatty acid (FA) component of algae. Being dominated by algae, FA contributes to food quality in the mature periphytic assembly. Although the biosynthesis of FA is just beginning to be understood, it is well known that saturated fatty acids (SAFA) and monounsaturated fatty acids (MUFA) are the major components of neutral lipids. These lipids function mainly as energy storage reserves, which, in algae, generally increase as a result of exposures to stressful environmental conditions, such as high temperature, nutrient extremes and harsh light conditions. In contrast, PUFAs affect many physiological processes and are major constituents of polar lipids, which are present in cell and chloroplast membranes. Although recent studies have shown that some organisms, such as the nematode *Caerhabditis elegans*, can synthesize PUFA containing more than 20 carbon atoms directly from SAFA and MUFA (WALLIS *et al.*, 2002), most animals cannot synthesize essential fatty acids (EFA) de novo from linoleic acid (18:2ω6) and a-linolenic acid (18:3ω3) in sufficient amounts to achieve optimal physiological performance (CUNNANE, 1996; ARTS *et al.*, 2001). The dominance of algae in periphytic canopy provides a rich source of C in the form FA to periphyton grazing animals.

As food chain proceeds, C is transferred from periphyton to grazers through predation (Fig. 1). The trophic interactions between periphyton and consumers (predators) are mediated through direct and indirect predation mechanisms (ELSER & URABE, 1999; HILLEBRAND & KAHLERT, 2001). Several studies, on questioning the uniformity of C utilization by predators from periphyton, instead of being rich source of nutrients at this stage, observed consumers to show a disproportionate relia-

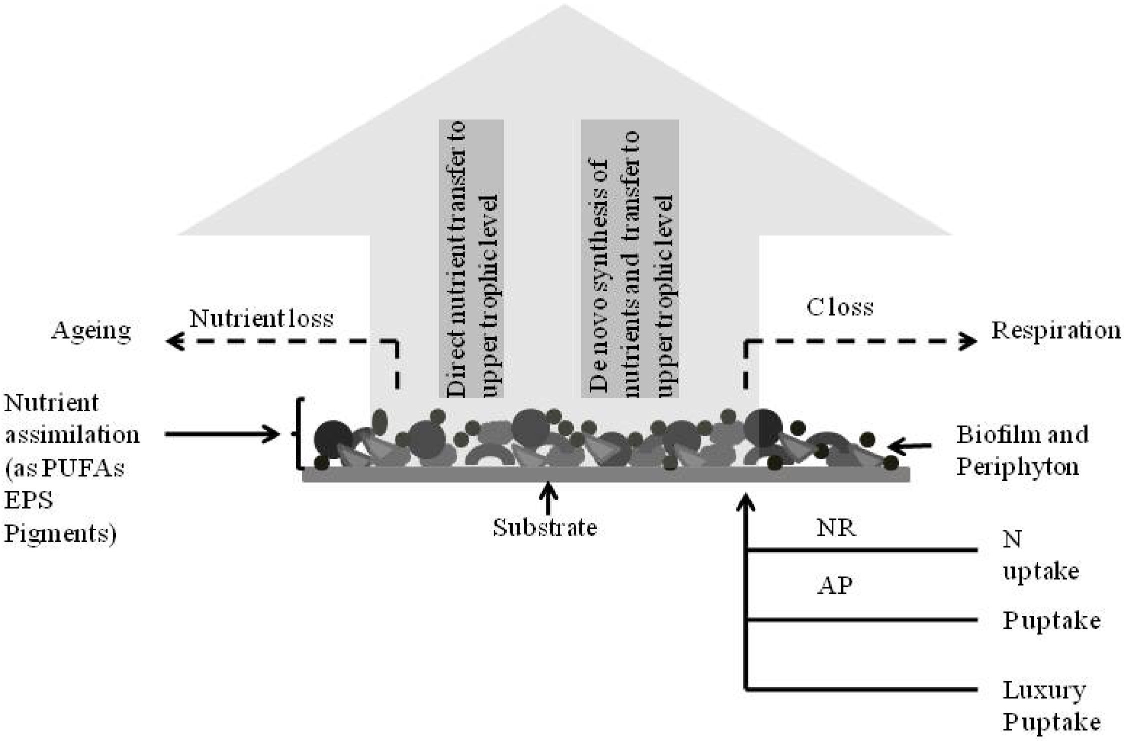

Fig 1. Periphyton as source and mediator of nutrient transfer between environment and higher trophic levels. Here NR, Nitrate reductase, AP, Alkaline phospatase, N, nitrogen, P, Phosphate and C, Carbon.

nce on periphyton C, even when standing consumer biomass is low (HECKY & HESSLEIN, 1995; JAMES *et al*., 2000).

Such reliance, especially of littoral zone food webs on algal periphytic sources of C contrasts with findings from the pelagic zones of several lakes, wherein allochthonous carbon sources have often been shown to fuel planktonic food webs (JONES *et al*., 1998; JANSSON *et al*., 2000). However, in shallow lakes with low planktonic productivity, periphyton is often the dominant C source for consumers (HECKY & HESSLEIN, 1995; JAMES *et al*., 2000). Some invertebrate taxa (such as aquatic insects, Hemiptera and Trichoptera, and the freshwater shrimp, Caridina) that are generally reported to use allochthonous C sources (MIHUC & TOETZ, 1994) attain up to 65% of their dietary C from periphyton.

The PUFAs, in particular 20:5ω3, seem to be ubiquitous among aquatic insects in temperate streams (HANSON *et al*., 1985; BELL *et al*., 1994; GHIONI *et al*., 1996; SUSHCHIK *et al*., 2003). Evidence from marine and lacustrine systems indicates that invertebrates in these systems obtain PUFAs primarily from algae (AHLGREN *et al*., 1992). At some times of the year, benthic algal communities, which are composed of a variety of taxa over an annual cycle (WEHR, 1981), can be a greater C source and, in particular, a higher-quality food source (i.e. PUFA rich) than terrestrial matter for the proper development and reproduction of macroinvertebrates (LAMBERTI, 1996). Algal food sources, especially the benthic algae, can remain qualitatively important throughout the year, even when their quantities are small, because of their higher protein and lipid content (LAMBERTI, 1996).

Nutrient (N:P) stoichiometry and transfer

Ecological nutrient stiochiometry considers how the relative proportions of nutrients affects their biological transformation in ecological interactions. In case of aquatic ecosystem, the elemental composition of N and P are mostly considered as nutrient stoichiometry measure with special reference to producers and consumers. In natural phytoplankton, the critical supply ratios of nitrogen to phosphorus (N: P) varies roughly from 7:1

to 45:1 atomic ratio i.e. 4.4:1 to 19.4:1 mass ratio (SUTTLE & HARRISON, 1988). The optimal ratio of N: P varies among species. The typical atomic ratio of 16:1 (Redfield ratio) is found in phytoplankton (REDFIELD, 1958). Generally the mass ratio of 7.2 1(N:P) is used as optimal ratio. Macro-algae tend to be more enriched in N, with an N: P ratio of 30:1 (ATKINSON & SMITH, 1983). Low ratios of N: P (usually <10:1) may indicate N-limitation, whereas higher values (>20-30:1) may indicate P limitation (RHEE, 1978; VYMAZAL, 1995).

As periphytic heterobiota (bacteria and other heterotrophs) are closely attached to organic and inorganic substrates, the organisms expose to all resources from bottom as well as upper aquatic column. The three major nutrient sources for periphytic heterobiota are water column, substrates and groundwater (BURKHOLDER, 1996; WHITE & HENDRICKS, 2000). A change in nutrient availability in any of these sources changes the N:P ratio of periphytic heterobiota. In addition, heterotrophic bacteria that are growing rapidly tend to have lower C:N ratio (CHRZANOWSKI *et al.*, 1996). During initial colonization phase, r-strategic bacterial species generally predominant on substrates (KRIŠTŮFEK *et al.*, 2005). In this case, bacterial C:P ratio shows less variation to C:N ratio (KIRCHMAN, 2000). The bacterial periphytic heterotrophs, therefore, can reduce the elemental stress not only among themselves but also between them and the substrates (CHRZANOWSKI *et al.*, 1996). The luxury P-uptake of bacteria adds additional dimension for P storage in bacterial cell influencing P uptake and transfer. FROST *et al.* (2002), therefore, justified periphytic bacteria as elastic elemental manager for nutrient stoichiometry.

Periphytic bacteria serve as important nutrient source at the base of periphytic food web even though allochthonus C input to benthic habitat is low and light penetration is high. According to light: nutrient hypothesis (STERNER *et al.*, 1997) light influence the autotrophic input of C (C fixation) and then effects C:N and C:P ratios of periphytic community itself and to consumers. However, to hypothesize that autotrophic input of C to effect C:N and C:P ratios, light: nutrient hypothesis received little experimental support (FROST & ELSER, 2002; HUGGINS *et al.*, 2004). However, the light: nutrient hypothesis may be, undoubtedly, effective in shallow wetlands.

Accumulation of organic matter along with periphyton colonization on substrates is another way to regulate nutrient stoichiometry through periphyton. The particulate organic matter (POM) tends to exhibit lower C:P ratio with decrease in particle size (SINSABAUGH & LINKINS, 1990). The fine particulate organic matter (FPOM) acquires higher nutrient content and lower C: nutrient ratio (BONIN *et al.*, 2000; CROSS *et al.*, 2003). The littoral organic matter with <1mm in diameter, therefore, a source of nutrient input to peripyton. During the late successional stages of periphyton in pond and rice fields, when decomposition rate is high, decrease in chlorophyll-a indicates possibility of accumulation of such organic particle on substrate (KESHAVANATH *et al.*, 2001; SAIKIA & DAS, 2009). Detritivory on periphyton at an optimum accumulation period of FPOM could ensure higher rate of nutrient transfer to consumers.

Conclusion

The overall transfer of nutrient through periphyton is highly dependent on consumer's feed selectivity and it's type, homeostasis, resistance or preference etc. Despite all these functional forces, periphyton ultimately forms an ecologically quantifiable as well as additional trophic level in all the aquatic ecosystems. Further, compared to suspended phytoplankton, periphyton complex plays more significant role in most of the functional aspects of aquatic ecosystem like regulation of eutrophic condition, maintenance of a two dimensional stable nutrient resource flow, performing as nutrient retention tool and excess nutrient removal agent, indicator of pollution and nutrient levels in the system as a whole. However, the major challenges, towards sustainable utilization of knowledge on periphyton and developing the models of application of the knowledge, lie primarily with progresses in quantifying parameters like its growth rate, nutrient uptake and transfer rate, C recycling rate etc. Therefore, current need of modern aquatic ecologists is to focus on exploration of less basic ecological processes linked with periphytic (attached) life forms for formulating utilizable ecological designs with simulative approach, rather confining only to the planktonic (Suspended) life forms. In the aquatic ecosystem, the knowledge of 'nutrient cycling' and 'trophic energy transfer' seems to remain incomplete if study on periphyton is ignored. Therefore, the contribution of periphyton must be accounted in addition to zooplankton and phytoplankton for evaluating over all material recycling and energy flow to the food web in any aquatic ecosystem in general .

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ECOLOGIA BALKANICA - INSTRUCTIONS TO AUTHORS - 2012

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A book:

AUTHOR A. 2000. *Title of the book.* Vol. I. Place of publication. Publishing house. 220 p.

Proceedings or book chapter:

AUTHOR A., B. AUTHOR 1990. Title of the contribution. - In: Author A. (Ed.): *Title of the book or proceedings.* Place of publication. Publishing house, pp. 235-265.

Software:

STATSOFT INC. 2004. STATISTICA (Data analysis software system), Vers. 7. Computer software. [http://www.statsoft.com].

GARMIN LTD. 2007. MapSource, Vers. 6.12. Computer software. [http://www.garmin.com]

Website:

FAUNA EUROPAEA. 2007. Invertebrates. Fauna Europaea. Vers. 1.1. Available at: [http://www.faunaeur.org]. Accessed: 12.10.2009.

In case of papers written in other than Latin letters, if there is an English (or German, or French) title in the summary, it may be used. If there is not such a summary, the author’s must be transcribed and the title of the paper must be translated into English and put in square brackets. If the name of the journal is also not in Latin letters it also should be transcribed. This should be noted in round brackets at the end of the paragraph, for instance: (In Bulgarian, English summary).

Example:

ANGELOV P. 1960. Communications entomologiques. I. Recherches sur la nourriture de certaines espèces de grenouilles. – *Godishnik na muzeite v grad Plovdiv*, 3: 333-337. (In Bulgarian, Russian and French summary).

KOROVIN V. 2004. [Golden Eagle (*Aquila heliaca*). Birds in agricultural landscapes of the Ural]. Ekaterinburg, Published by Ural University, 57 p. (In Russian).

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For special symbols (Greek letters, symbols for male and female etc.) use the Symbol list on the Insert menu in Microsoft Word with the following preferable fonts: Symbol, Webdings, Wingdings, Wingdings 2 and Wingdings 3. Degree symbols (°) must be used (from the Symbol list) and not superscript letter "o" or number "0". Multiplication symbols must be used (×) and not small "x" letters. Spaces must be inserted between numbers and units (e.g., 3 kg) and between numbers and mathematical symbols (+, –, ×, =, <, >), but not between numbers and percent symbols (e.g., 45%).

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Mean values should always be accompanied by some measure of variation. If the goal is to describe variation among individuals that contribute to the mean standard deviation (SD) must be used. When the aim is to illustrate the precision of the mean standard errors (SE) should be given. The last paragraph of Materials and Methods section should briefly present the significance test used. Quote when possible the used software. Real *p* values must be quoted both at significance or non-significance. The use of the sign is acceptable only at low values of *p* (e.g. $p<0.0001$).

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The authors of articles that are based on experiments that caused injuries or death of animals should explain and justify the grounds of the study and state that the scientific results of the study is at least in trade-off with the sufferings caused. In the Materials and Methods of the manuscript, authors should detail as precisely the conditions of maintenance, transport, anaesthesia, and marking of animals. When available, references should be added to justify that the techniques used were not invasive. When alternative non-harming techniques exist, but were not used, the manuscripts may not be considered for publication.

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