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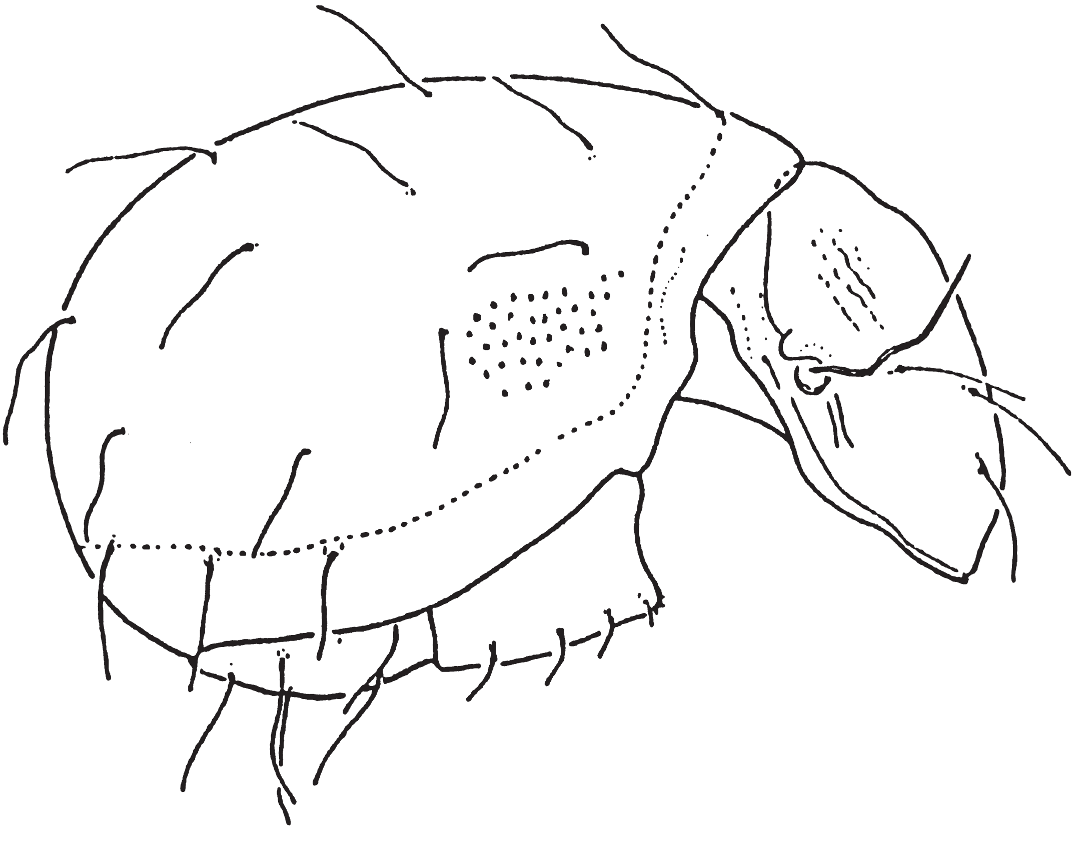

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ASSESSMENT OF YIELD LOSSES IN MUSTARD (*Brassica juncea* L.) DUE TO MUSTARD APHID (*Lipaphis erysimi* Kalt.) UNDER DIFFERENT THERMAL ENVIRONMENTS IN EASTERN CENTRAL INDIA

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Abstract. Mustard aphid (*Lipaphis erysimi.* Kalt.) has been reported as one of the devastating pests in realizing the potential productivity of Indian mustard (*Brassica juncea* L.). The experiment was carried out to assess the yield losses in mustard due to mustard aphids grown in different thermal environments under protected and unprotected conditions. To provide different thermal environments the crop was sown on 5 dates i.e. 08, 18 and 28 November and 08 and 18 December during winter seasons of 1995-96, 1996-97 and 1997-98. It was observed that yield attributes and yield of mustard was significantly decreased in delayed sowing even under protected conditions. On an average maximum seed yield of 1409 kg/ha was harvested when the crop was sown on 08 November under protected condition, while only 279 kg/ha seed yield was recorded under unprotected condition. Similarly the yield attributes and yields drastically reduced under unprotected condition as compared to protected one in all the thermal environments. On the mean basis 80.6, 81.4, 95.2 and 97.6 per cent yield loss was observed under unprotected condition as compared to protected condition in 08, 18, 28 November and 08 and 18 December sowing, respectively. It was also observed that the critical period of mustard exposure to aphids was found to be the 3rd week after aphid appearance when the crop was in flowering stage and hence the control measures have to be initiated before flowering. There was not much difference in natural aphid population/plant in the crop sown up to end of November while the aphid population increased suddenly in December sown crop.

Keywords: *mustard, Lipaphis erysimi, avoidable losses, yield.*

Introduction

In the eastern part of Central India insect-pests is one of the major limiting factors influencing oilseed production especially mustard yield. About three-dozen insect-pests have been found infesting mustard crop in India [12]. On the basis of economic importance mustard aphids are considered to be the key pests, out of three species of aphids namely *Lipaphis erysimi* Kalt., Myzus persicae Sulzer and *Brevicoryne brassicae* Linn., the first one is the most serious, cosmopolitan and has attained the importance of national pest and causes yield loss from 35.4 to 91.3% [4, 21]. The peak activity of pest is observed between January and March in different locations. This variation in the incidence is largely governed by weather parameters. Under the hypothesis of integrated pest management, alteration in sowing time provides wider opportunity to minimize the

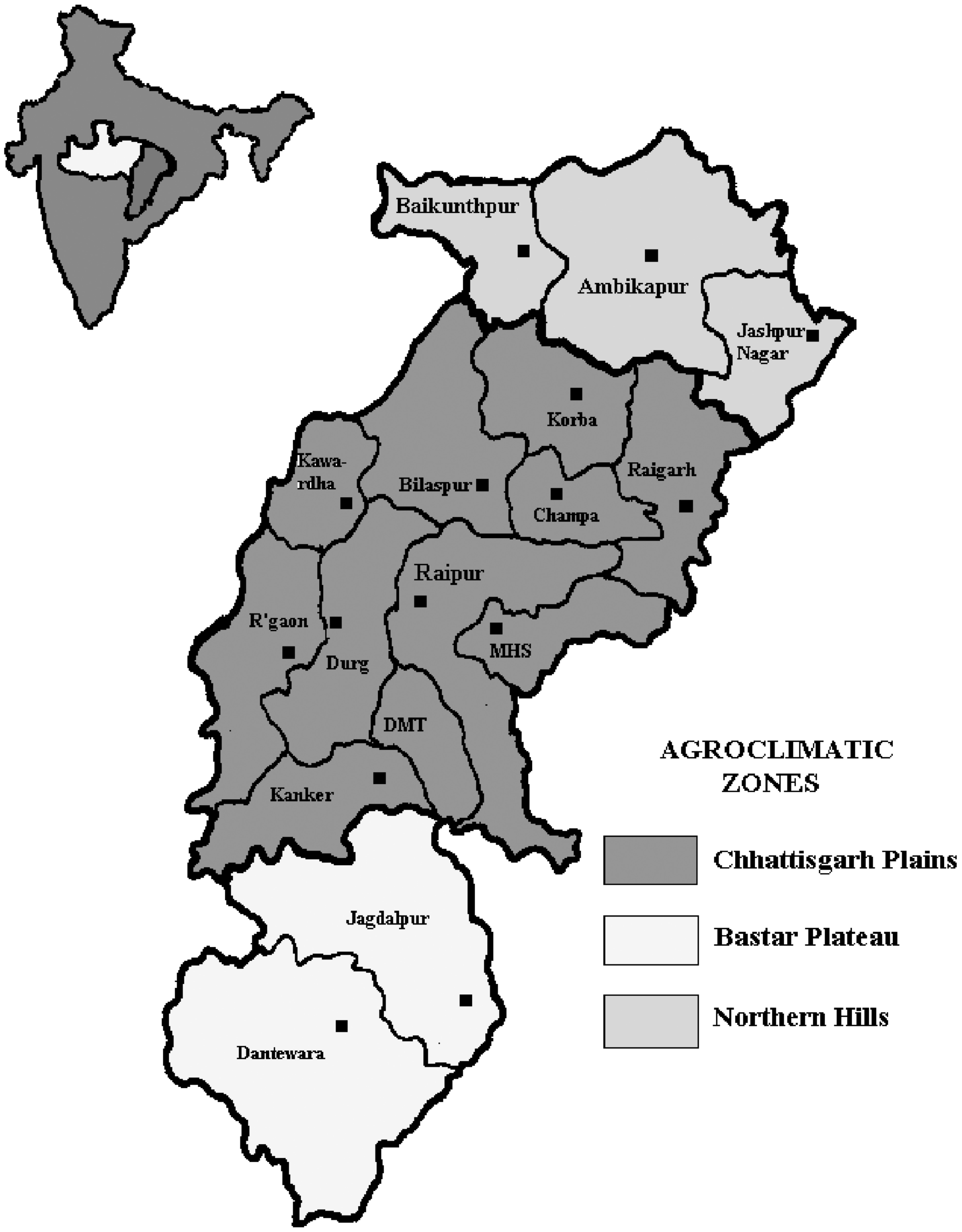

Figure 1. Location and agroclimatic zones of Chhattisgarh in India

damage due to insect pest because the susceptible crop stage is not coinciding and escapes the peak pest population. In agro-ecological conditions of northern India the crop sown before 20th October suffered less damage from aphid than sown later [2, 20, 22]. In eastern part of Central India i.e. Chhattisgarh State (*Figure 1*) mustard is widely grown under irrigated conditions in rice based cropping system. The productivity of mustard in this state is far below as compared to National average, as the sowing is often delayed due to late harvesting of rice crop and thereby field preparation for sowing. Apart from the delayed sowing the winter span in Chhattisgarh State is also shorter as compared to traditional mustard growing Northern India. The information pertaining to the extent of losses due to key pest of mustard is very meagre in this region. In view of this the present investigation was undertaken to assess the yield losses due to mustard aphid under different thermal environments.

Materials and Methods

A field experiment was conducted for 3 years during the winter seasons of 1995-96, 1996-97 and 1997-98 at Regional Agriculture Research Station, Indira Gandhi Agricultural University, Raigarh. The investigation was aimed to assess the losses in yield due to aphids under different thermal environments. Two separate experiments were laid out in randomised block design with four replications. The crop was sown at five dates representing five thermal environments i.e. November 08, 18, 28 and December 08 and 18 in each year under protected and unprotected conditions. The soil of the experimental site was sandy loam in texture representing the 40 per cent area in the state. The average initial nutrient status of the soil was low in available N (230.1 kg/ha) and P (10.7 kg/ha) and medium in K (342.0 kg/ha) with pH 6.9. A fertilizer dose of 80 kg N, 21.8 kg P and 24.9 kg K/ha was given to all the treatments uniformly. Half of N and entire quantity of P and K were applied at the time of sowing, remaining N was given after 35 days of sowing at flower bud initiation stage. Three irrigations were given to the crop at rosette, 50% flowering and pod filling stage. Under protected condition plant protection measures were adopted to protect the crop from aphid damage by spraying endosulfan at 1.5 ml/litre. Ten plants were randomly selected to record observations on yield attributes, whereas the yield was recorded from net plot and finally computed in kg per hectare. A total of 8.6, 5.0 and 265.5 mm rainfall were received during the cropping season of 1995-96, 1996-97 and 1997-98, respectively. The relationship between seed yield and aphid population with delay in sowing in days was examined through regression analysis in linear model. All the data were tabulated and analysed statistically as per the procedure suggested by Chandel [6] and Panse and Sukhatme [11]. The ‘F’ (Fisher's) test was used for judging the significance of the treatment mean at 5 per cent probability level. Whenever ‘F’ test showed significant difference, the differences between treatment means were further tested using critical difference (CD) values.

To compare different mean value of treatments, critical difference (CD) values were calculated as follow:

(i) $\text{SEm} \pm \; = \; \sqrt{\text{Ems}/n}$

Where
SEm ± = Standard Error of mean
Ems = Error mean square
n = number of observations on which the mean value is based
(ii) CD (P=0.05) = SEm x ‘t’ (at 5%) for Error degree of freedom

Aphid count

Observations on insect pests along with natural enemies were recorded on randomly selected 5 plants/plot at weekly interval. At the vegetative stage of the crop, aphid (*Lipaphis erysimi* Kalt.) population was recorded on three leaves (upper, middle and lower) and at flowering, pod filling and maturity stages of the crop, it was observed on 4 twigs of 10 cm/plant including central shoot on 5 plants/plot. All 20 twigs/plot were observed to record the aphid population at these crop stages. The extent of leaf damage by flea beetle (*Phyllotreta cruciferae* Goeze) and the sawfly (*Athalia lugensproxima* Klug) larvae were assessed in terms of percentage leaf area damaged by foliage feeders. Coccinellid predators (*Menochilus sexmaculatus* Fabr. and *Coccinella septempunctata* Linn.) were also observed on whole plant basis.

Weather

The daily weather parameters viz maximum and minimum temperature, morning and evening relative humidity and rainfall that have distinct influence in the multiplication of aphid population were collected and the weekly pattern of these parameters were illustrated through *Figure 2.* The steps down regression equations between aphid populations with different weather parameters were also worked out to predict the influence of rainfall, temperature and humidity on aphid multiplication using Statistical Software SPAR 1.

Results and discussion

Crop phenology

The days taken for different physiological events as influenced by different thermal environments are presented in *Table 1.* It was obvious from the data that the duration for emergence and 50% flowering was delayed due to delay in sowing from 08 November to 18 December. The days taken for emergence delayed by 2 days while the days taken for 50% flowering was extended by 5 days. The prolonged duration for emergence and 50% flowering under delayed sowing was mainly attributed due to lower temperature during the germination and vegetative phase of the crop. After the 50% flowering it was observed that the duration for start and end of seed filling was shortened by 3 and 13 days respectively in 18 December sowing as compared to 08 November sowing. Finally the duration for physiological maturity was shortened by 17 days and causes forced maturity due to higher temperature at the time of grain filling. The increase in duration of vegetative phase and decrease in reproductive and maturity phase as observed in the present study were also reported by Singh et al. [23] under the agro-climatic conditions of Hisar, situated in Northern India.

Phenological stages (Days after sowing)	Dates of sowing				
	08 Nov.	**18 Nov.**	**28 Nov.**	**08 Dec.**	**18 Dec.**
P_1 – Emergence	4	4	4	5	6
P_2 - 50% flowering	45	45	46	48	50
P_3 - Start of seed filling	55	54	55	56	57
P_4 - End of seed filling	95	94	92	89	84
P_5 - Physiological maturity	108	106	102	98	91

Table 1. *Phenology of mustard as influenced by different thermal environments under protected conditions.*

Yield attributes and yield

The data presented in *Table 2* revealed that the yield attributes were significantly influenced due to different thermal environments. Significantly higher values of yield attributes i.e. plant height, branches/plant, siliquae/plant, seeds/siliqua and test weight were recorded with earlier sowing on 08 November as compared to sowing on 18 and 28 November and 08 and 18 December. All these parameters were drastically reduced as the sowing was delayed from 08 November to 18 November onwards. Sharma et al. [17] also reported decline in yield attributing characters when sowing was done after 25 October under the agroclimatic conditions of Gwalior in Central India. Whereas, Chandrakar and Urkurkar [7] reported significant decrease in yield attributes when the crop was sown after 23 November under Raipur conditions. Reddy and Kumar [13], Singh et al. [23] and Singh and Singh [24] also reported the similar results.

Table 2. *Yield attributes and yield of mustard as influenced by different thermal environments under protected condition (mean of 3 years)*

Sowing dates	Plant height (cm)	Branches/ plant	Siliquae/ plant	Seeds/ siliqua	1000 seed weight (g)	Oil content (%)	Seed yield (kg/ha)	
							Pro	**Unpro**
D_1-08 Nov.	151.8	10.8	211.8	17.5	5.93	38.9	1409	279
D_2-18 Nov.	136.3	8.0	169.8	15.6	4.93	37.9	842	152
D_3-28 Nov.	116.9	5.6	80.8	13.3	4.43	37.5	515	77
D_4-08 Dec.	99.3	4.5	43.4	11.4	3.83	37.2	330	15
D_5-18 Dec.	89.7	3.4	28.2	8.0	3.10	36.9	198	05
S Em$\pm$	4.3	0.4	6.5	0.5	0.20	0.5	43	30
CD ($p= 0.05$)	13.2	1.3	19.7	1.7	0.60	1.1	128	89

Pro = Protected, Unpro = Unprotected, S Em + = Standard Error of mean and CD = Critical difference

Significant decrease in seed yield was observed with successive delay in sowing from 08 November to 18 December at 10 days interval during all the years of study. On the mean basis sowing on 08 November produced seed yield of 1409 kg/ha that was 40.2, 63.4, 76.6 and 85.9 per cent higher then the seed yield of sowing on 18 and 28 November and 08 and

18 December, respectively (*Table 2*). Significant decrease in seed yield in delayed sowing may be due to shortening of growing period of the crop due to rise in temperature during grain filling and maturity. Sharma et al. [17], Butter and Aulakh [5], Sarmah [16], Sonani et al. [25], Tomar and Mishra [27] and Upadhyay [28] also reported similar results. The predicted pattern of decrease in grain yield was well fitted with the observed values. The rate of decrease in grain yield was higher in 1995-96 with coefficient of determination of 0.97 (*Figure 3*). The rate of decrease in grain yield was 37.1 kg/ha for every one-day delay in sowing in 1995-96. On the other hand the rate of decrease in grain yield was found to be 28.66 and 22.20 kg/ha/day in 1996-97 and 1997-98 with R^2 values of 0.83 and 0.77 respectively. Oil content in seed did not influence much during first year of study. But, significantly higher oil content was recorded with 08 November sowing as compared to 28 November and 08 or 18 December sowing. However, it was on par with sowing of crop on 18 November. Higher oil content in early sown crop may be due to favourable prolonged environmental conditions for better growth and development of the crop, which enhanced the oil content. These results are in agreement with the findings of Kurmi and Kalita [9], Sarmah (16), Sharma et al. [17] and Singh and Singh [24].

Avoidable losses in yield

The seed yield production was drastically reduced under unprotected condition as compared to protected condition in all the thermal environments (*Table 3*). Highest seed yield was obtained from earliest sowing on 8 November under protected condition. Seed yield of 1591, 1475 and 1161 kg/ha was recorded with 08 November sowing during 1995-96, 1996-97 and 1997-98, respectively (*Table 3*). The seed yield decreased considerably in 18 and 28 November and 08 and 18 December sown crop. Higher seed yield in early sowing was due to higher number of siliquae/plant, seed/siliqua and 1000 seed weight. It was significantly higher over all other treatments. During all the three years of study the seed yield was lowest in unprotected condition. The yield loss varied from 76.0 to 92.7 % in 1995-96, 81.5 to 100.0 % in 1996-97 and 84.4 to 100.0 % in 1997-98. Thus, it was observed that there was 100 per cent yield loss of mustard without proper and timely plant protection under delayed sowing on 18 December as the crop could not sustain the infestation and failed to survive. On an average there was 80.0 to 97.6 percent yield loss without plant protection. Singh and Sachan [21] also reported the avoidable losses due to mustard aphid up to 69.6 per cent. Similarly Bakhetia [1] observed 57.8 to 80.6 per cent yield loss due to mustard aphid and Suri et al. [26] observed 42.1 per cent yield loss under different agroclimatic conditions. Jadhav and Singh [8] also reported the similar results. The additional yield from protected plot as compared to unprotected plot ranges from 1209 to 192, 1202 to 215 and 980 to 175 kg/ha in 1995-96, 1996-97 and 1997-98 respectively under different thermal environments.

Population dynamics of aphids and predators

The data on population dynamics of mustard aphid (*Lipaphis erysimi*) as given in *Table 4* revealed that there was significant variation in aphid population under different thermal environments. The minimum number of aphids was recorded when the crop was sown on 08 November and gradually increased as the sowing was delayed. There was not much difference in aphid population with the crop sown in the month of November,

Table 3. Avoidable yield losses under different thermal environments due to mustard aphid.

Sowing dates	**Seed yield (kg/ha)**		**Additional yield over unprotected (kg/ha)**	**Avoidable losses in seed yield (%)**
	Protected	**Unprotected**		
1995-96				
D_1 - Nov. 08, 1995	1591	382	1209	76.0
D_2 - Nov. 18, 1995	1280	206	1074	83.9
D_3 - Nov. 28, 1995	795	167	628	79.0
D_4 - Dec. 08, 1995	338	37	301	89.1
D_5 - Dec. 18, 1995	207	15	192	92.7
S Em±	27	23	-	-
CD (P = 0.05)	83	65	-	-
1996-97				
D_1 - Nov. 08, 1996	1475	273	1202	81.5
D_2 - Nov. 18, 1996	747	149	598	80.5
D_3 - Nov. 28, 1996	439	45	394	89.7
D_4 - Dec 08, 1996	401	00	401	100.0
D_5 - Dec. 18, 1996	215	00	215	100.0
S Em±	55	38	-	-
CD (P = 0.05)	165	110	-	-
1997-98				
D_1 - Nov. 08, 1997	1161	181	980	84.4
D_2 - Nov. 18, 1997	500	101	399	79.8
D_3 - Nov. 28, 1997	311	20	291	93.6
D_4 - Dec 08, 1997	252	09	243	96.4
D_5 - Dec. 18, 1997	175	00	175	100.0
S Em±	47	31	-	-
CD (P = 0.05)	136	90	-	-

S Em + = Standard Error of mean and CD = Critical difference

Table 4. Population dynamics of Lipaphis erysimi as influenced by different thermal environments under unprotected condition.

Sowing dates	Aphid population/ plant	Flea beetle population/ plant	Leaf damage (%)	Predator population/ plant
1995-96				
D_1 - Nov. 08, 1995	60.8 (7.8)	0.50	29.5	0.14
D_2 - Nov. 18, 1995	65.9 (8.1)	0.47	22.0	0.16
D_3 - Nov. 28, 1995	76.9 (8.8)	0.46	25.5	0.26
D_4 - Dec. 08, 1995	328.7 (18.1)	1.17	27.1	0.22
D_5 - Dec. 18, 1995	702.8 (26.5)	0.06	45.0	0.33
S Em±	1.41	-	-	-
CD (P=0.05)	4.34	-	-	-
1996-97				
D_1 - Nov. 08, 1996	98.8 (10.0)	1.36	11.0	0.10
D_2 - Nov. 18, 1996	128.2 (11.4)	1.08	18.8	0.12
D_3 - Nov. 28, 1996	147.7 (12.2)	1.89	21.3	0.17
D_4 - Dec. 08, 1996	498.8 (22.3)	0.63	20.2	0.23
D_5 - Dec. 18, 1996	915.4 (30.2)	0.21	17.6	0.29
S Em±	1.12	-	-	-
CD (P=0.05)	3.45	-	-	-
1997-98				
D_1 - Nov. 08, 1997	117.5 (10.9)	2.34	14.3	0.19
D_2 - Nov. 18, 1997	140.3 (11.9)	2.11	19.4	0.16
D_3 - Nov. 28, 1997	156.8 (12.5)	1.95	18.6	0.26
D_4 - Dec. 08, 1997	481.9 (22.0)	1.01	24.2	0.32
D_5 - Dec. 18, 1997	902.4 (30.0)	0.82	23.3	0.35
S Em±	1.44	-	-	-
CD (P=0.05)	4.43	-	-	-

Figures in parenthesis are square root transformed values
S Em + = Standard Error of mean and CD = Critical difference

Table 5. *Stepwise regression equations for estimating the influence of weather parameters on aphid population during 1997-98.*

Sowing date	Regression equations	R^2
Nov. 08,1997	$Y = 875.77-1.613x_1-21.746x_2+14.875x_3-2.421x_4-3.693x_5$	0.463
	$Y = 740.03-1.391x_1-20.964x_2+12.824x_3-4.700x_5$	0.443
	$Y = 589.16-0.358x_1-10.595x_2-3.947x_5$	0.326
	$Y = 605.91-10.015x_2-4.494x_5$	0.297
	$Y = 173.04-2.077x_5$	0.092
Nov. 18,1997	$Y = 855.54-0.862x_1-20.107x_2+11.959x_3+0.052x_4-6.671x_5$	0.499
	$Y = 858.91-0.865x_1-20.114x_2+12.000x_3-6.658x_5$	0.499
	$Y = 778.46-13.962x_2+5.199x_3-6.579x_5$	0.456
	$Y = 742.64-11.459x_2-5.881x_5$	0.417
	$Y = 278.91-3.499x_5$	0.318
Nov. 28,1997	$Y = 707.67-0.228x_1-11.659x_2+8.116x_3+0.051x_4-7.004x_5$	0.739
	$Y = 709.85-0.289x_1-11.630x_2+8.130x_3-6.984x_5$	0.739
	$Y = 658.27-8.946x_2+6.027x_3-6.870x_5$	0.735
	$Y = 315.69+4.380x_3-4.914x_5$	0.707
	$Y = 389.02-5.025x_5$	0.673
Dec. 08, 1997	$Y = -1159.10-0.610x_1+28.445x_2+49.696x_3+0.815x_4-3.428x_5$	0.966
	$Y = -1132.57-0.588x_1+28.339x_2+49.785x_3-3.286x_5$	0.966
	$Y = -1132.46+29.926x_2+47.679x_3-3.548x_5$	0.965
	$Y = -1724.69+43.758x_2+47.157x_3$	0.961
Dec. 18, 1997	$Y = -1911.69-5.177x_1+51.892x_2+111.757x_3-15.573x_4+6.933x_5$	0.962
	$Y = -937.10-4.840x_1+28.054x_2+111.095x_3-14.206x_4$	0.955
	$Y = -2406.30-3.853x_1+40.034x_2+105.943x_3$	0.945
	$Y = -2626.60+54.445x_2+92.737x_3$	0.934

X1= Rainfall (mm/week), x2 = Temperature oC (Maxi.), x3 = Temperature oC (Mini.), X4 = Relative Humidity % (morning) and x5 = Relative Humidity % (evening)

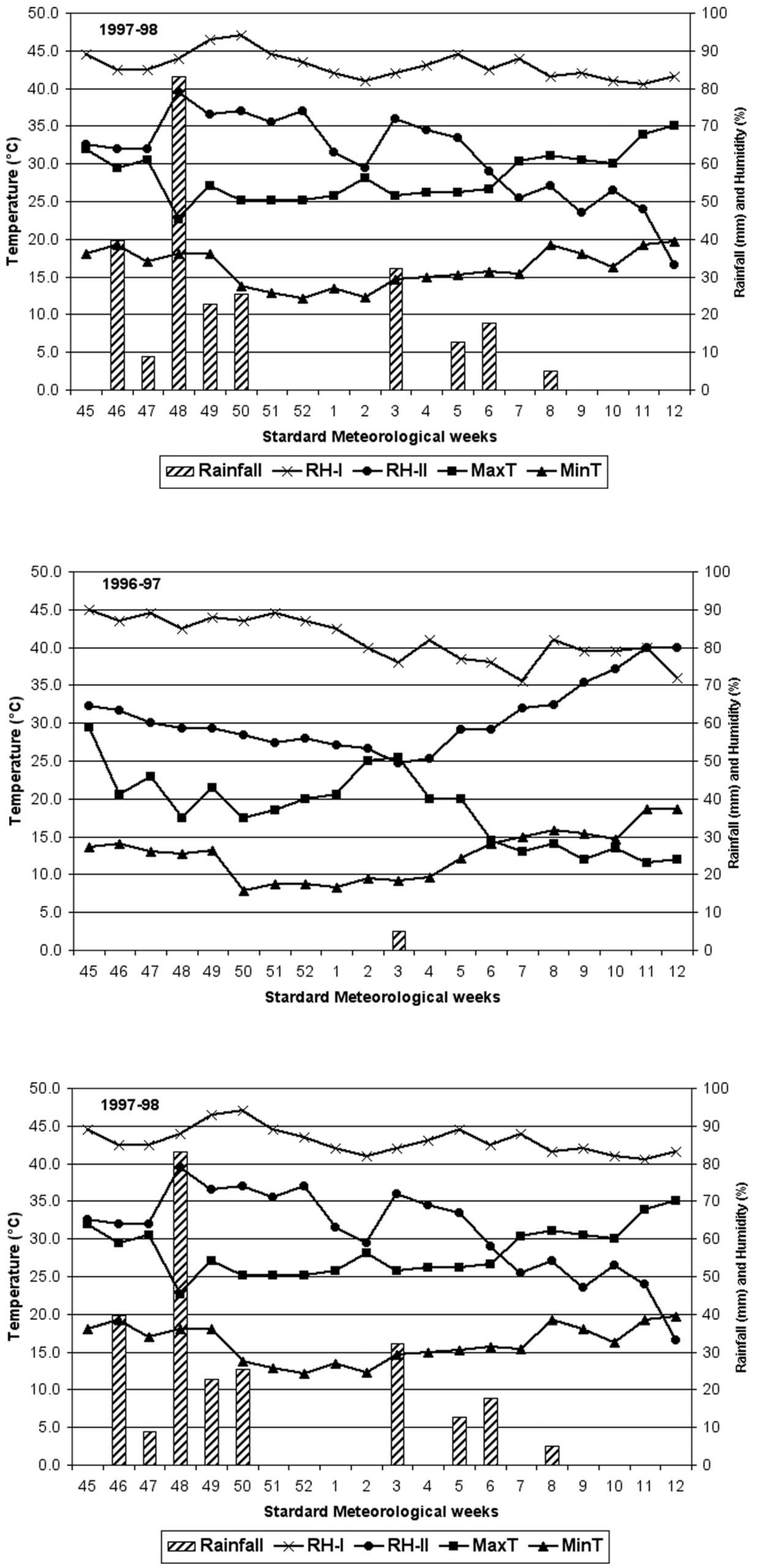

Figure 2. *Weekly pattern of temperature (°C), relative humidity (%) and rainfall (mm) during the winter seasons of 1995-96, 1996-97 and 1997-98.*

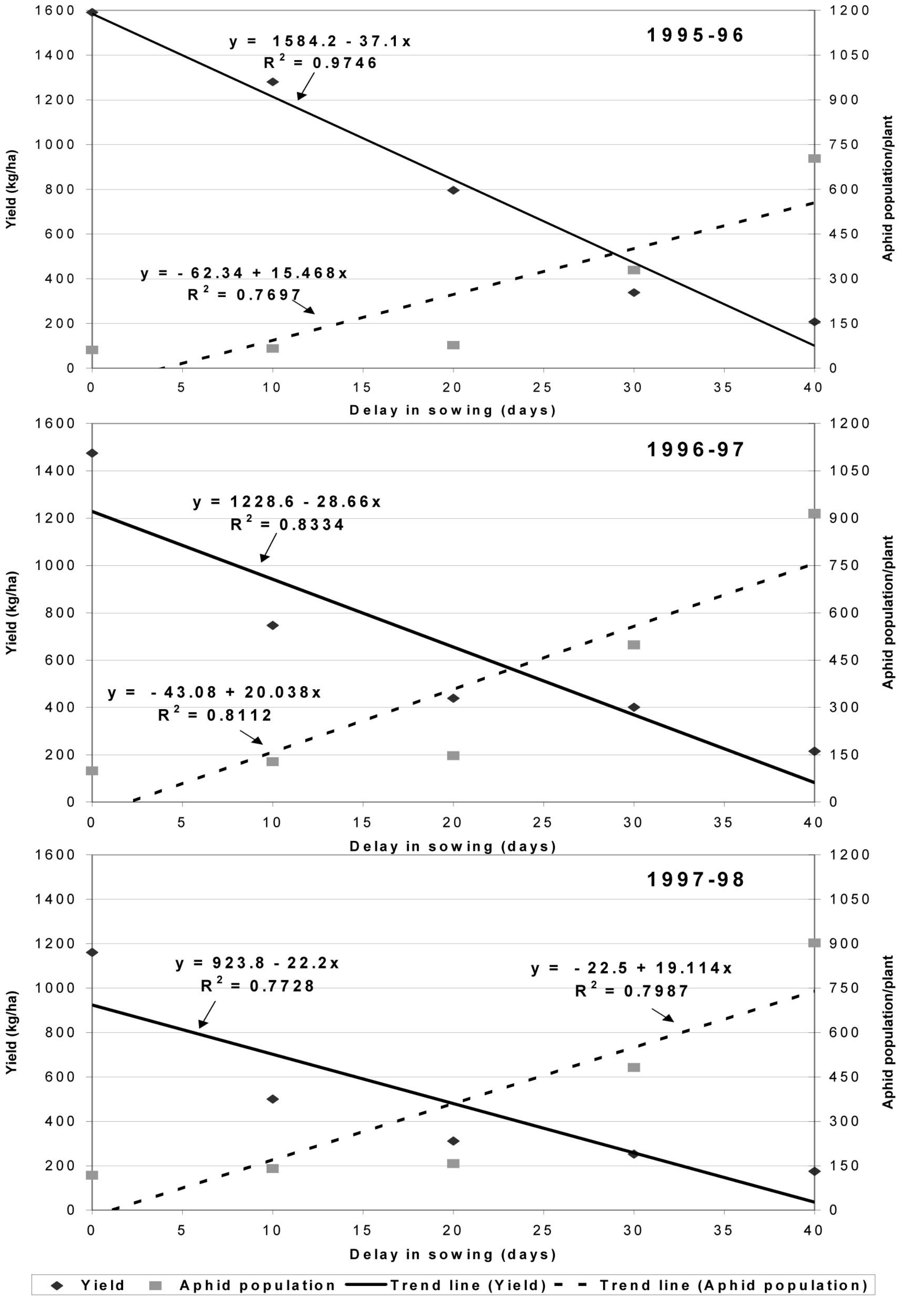

Figure 3. Relationship between delay in sowing with seed yield (protected) and aphid population (unprotected). Trend lines indicates predicted pattern of changes using regression analysis.

but the aphid population increased suddenly in 08 and 18 December sowing. Mishra et al. [10], Saha and Kanchan [15], Shrivastava [18] and Vekaria and Patel [29] also observed higher aphid population in delayed sowing. The linear relationship between aphid populations with delay in sowing showed significant relationship among them. The predicted pattern of aphid population was found to be more or less similar to the observed population with R^2 values of 0.82, 0.86 and 0.84 in 1995-96, 1996-97 and 1997-98, respectively (*Figure 3*). The population of flea beetle (*Phyllotreta cruciferae*) was observed to be lowest in delayed sowing on 18 December in all the years of experimentation. However, the activity of flea beetles was fluctuating in different thermal environments in the first two years. During 1995-96 the highest flea beetle population was observed in 08 December sown crop while in 1996-97 it was highest in 28 November sowing. In 1997-98 the highest flea beetle population was recorded in early sowing on 08 November and gradually decreased as the sowing was delayed. The percent leaf damage due to this foliage feeder ranged from 22.0 to 45%, 11.0 to 21.3% and 14.3 to 24.2% in 1995-96, 1996-97 and 1997-98, respectively.

The natural enemies observed during the cropping seasons were *Menochilus sexmaculatus* and *Coccinella septempunctata* as predominant aphid predators. The population of these predators was recorded to be low in crop sown on 08 and 18 November, and increased gradually in delayed sowing. Maximum predator population was observed in 18 December sowing. This shows that the predator population increased proportionately with the aphid population.

Regression analysis

The influence of rainfall, temperature and relative humidity on aphid population during 1997-98 was worked out through step down regression analysis.

From the step down regression analysis it was observed that the combined effect of these weather parameters had the least influence on aphid population in early sown crop on 08 November as the R^2 value was only 0.463. But, when the sowing was delayed the combined influence of this these parameters increased with R^2 value of 0.499, 0.739, 0.966 and 0.962 in 18, 28 November, 08 and 18 December sowing respectively (*Table 5*). It was observed that in early sown crop the prevailing weather conditions are not favourable for aphid multiplication, but weather conditions become favourable under delayed sowing. In 08 November the combined effect of these parameters increased with R^2 value of 0.739 and when rainfall, maximum temperature and morning relative humidity were deleted the R^2 value comes down to 0.707. This shows that these parameters have only 0.032 per cent influence on aphid multiplication. Whereas in case of 08 and 18 December sowing the value of coefficient of determination with all these parameters were 0.966 and 0.962 respectively. When the rainfall, morning and evening relative humidity were deleted the R^2 value comes down to 0.961 and 0.934 respectively. This indicates that in 08 and 18 December sowing the maximum and minimum temperature has greater influence on aphid multiplication and that the temperature alone (maximum and minimum) has more than 93% influences on aphid multiplication under late sown condition. Thus, low night temperatures are not favourable for aphid growth. The value of coefficient of determination was 0.934 when maximum and minimum temperature was considered in 18 December sowing. The next important factor besides temperature was the evening relative humidity in influencing the growth of aphids. The present findings are in complete agreement with that of Bishnoi [3], Roy and Kanchan [14] and Shrivastava et al. [19].

From the above findings it was concluded that to avoid the peak aphid population sowing of mustard should be completed before 08 November i.e. first week of November for higher yield under rice based cropping system in Chhattisgarh state in eastern central India.

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NOCTURNAL ILLUMINATION AND NIGHT FLYING INSECTS

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Abstract. The present study discusses the light trapping of insects depending on the environmental illumination, twilight polarization phenomena and the moon phases. The trapping data were taken of Hungarian national light-trap network. The important results are the followings: The Babinet-point, a polarization free spot of the sky at twilight, can be a role of orientation of insects. The height of the Moon above the horizon is in negative correlation with the number of the caught insects. The maximum individual number of species was collected at various moon phases.

Keywords: *light-trap, collecting distance, Babinet-point, moon phases*

Introduction

Great many studies in professional literature are devoted to the role of the Moon in modifying light trapping catch. The conclusions are contradictory and up to this day a good many questions have remained unclarified. True, the authors usually collected differing species at the most different geographical locations and have not even registered the Moon phase in every case.

Review of Literature

In astronomical terms, twilight means that the Sun is set just below the horizon. At the time of sunset and sunrise, the zenith distance (ZSun) is equally 90.5°. In the period of civil twilight (ZSun = 90.5°-96°), provided the sky is clear, the visible outlines of objects in the environment make appropriate orientation possible. The brightest celestial bodies which help orientation appear at the time of navigational twilight (Z Sun = 96°-102°). Complete darkness sets in at the end of astronomical twilight (ZSun = 102°-108°). From then on, provided the Moon or zodiacal light observable near the Equator does not enhance the illumination of the environment, only the brightness of the night sky is perceivable. Its mean value is $9*10^{-4}$ lux (Nielsen [116], Roach and Gordon [151]). Naturally, at dawn, the same events follow one another in reverse order.

The light of the sky at sunset and daybreak is strongly polarized. In some places, however, neutral spots can be observed in areas of a few arc-square grades where polarization is practically zero (Rozenberg [156], McCartney [101]). Babinet's point follows the Sun on its virtual trajectory by 15-25° in the evening and precedes it by the same value at daybreak, so it is observable at twilight. More recently, Hungarian researchers have also been devoting attention to the unpolarized points of the sky (Gál et al. [62], Horváth és Varjú [79]). The neutral points are presumably perceived by insects as discontinuities in a sky emitting a continuity of polarized light. Therefore we assume that these points might have a role to play in their orientation. From this point of view, Babinet's point especially may be of significance in the periods of evening and dawn twilight.

The question of the distribution of the catch by light-trap in the course of a night has been a subject of research for several decades. Williams [199] used a fractionating light-trap in four years of examining flight activity as it was changing over the night.

Tshernishev [187] claims that the flight activity of each species follows a special daily rhythm that usually corresponds to the time of flying to light. From this point of view he establishes four basic types of insects:

- Flight of short duration tied exclusively to twilight, can never be observed by night (most Ephemeroptera, Corixida, Coleoptera, Diptera and Hepialida species).
- Species of a flight of longer duration. They start their flight later, reaching the peak early in the evening. Some species fly all night (Trichoptera, Chironomida and a few east-African Ephemeroptera species).
- Intensive flight from sunset to close on sunrise, not letting up during the night (Tripuloidea and some Ephemeroptera species).
- Typical night flight with a well discernible nocturnal peak (Ophionina, Lepidoptera, especially the species of Noctuidae and *Serica brunnea* L.).

In the same work, the author lays down for a number of insect orders and for some significant species the values of illumination expressed in lux characterizing the beginning and the peak of the activity. The activity of most Lepidoptera species increases from 0.01 lux to 0.001 lux but decreases by illumination below that value.

The intensity of illumination is of outstanding importance from the point of view of the collecting area as well as regarding flight activity. For by a lower level of illumination in the environment, the light-source of the trap will be discernible from a greater distance. However, this possibility has been studied so far only in the context of the light of the Moon (Bowden and Church [23], Bowden and Morris [26]).

The fact that the polarized light of the sky has a role to play in the orientation of some insects has been known for about fifty years. Dantharanayana and Dashper [43] examined insect behaviour in response to polarized light by using three Pennsylvania type light-traps. It is quite remarkable that the result pertaining to moths contradicts an earlier finding by Kovarov and Montchadski [92] who claim that species of that order fly in masses to polarized light. We have not come across with any publication in professional literature discussing light trapping efficiency in an interrelationship with the position of neutral points.

Several researchers include moonlight in their list of factors that modify collecting, but owe us a detailed analysis of the workings of that influence (Ármai [4], Malicky [99], Harling [73], Hardwick and Lefkovich [72], Jermy [89], Lödl [96], Pedgley et al. [137]). Leinonen et al. [94] tested four different types of light-traps and bulbs in northern Finland. The 4 traps changed places every night, but on every fifth night were put back to their original places, in order to evade the influence of the Moon. Ito et al. [83] applied auto-correlation calculation to establish that collecting by light-trap has a 29-30 day periodicity. In actograph examinations, Danthanarayana and Gu [44] experienced a 27 day periodicity in the flight activity of *Epiphyas postvittana*, a period remarkably close to the length of the sideric lunar month (27.7 days). Ho and Reddy [76] have found that moonlight exerted a stronger influence on light trapping catch than on the catch by pheromon traps.

Findings by other researchers have been contradictory. Corbet [38], Hanna [70], Day and Reid [46], Chaston [34], Bidlingmayer [15], Hardwick [71], Mikkola [104], Bowden and Church [23], Bowden and Gibbs [24], Szabó and Járfás [176], Robertson [155], Pedgley [136], Holck and Meek [78] and Brinson et al. [30] either did not find adequate proof to confirm differences in insect activity during lunation, or just stated that the different species reacted to moonlight in different ways. Nabili et al. [112] hold that the lunar phase has no significant bearing on the effectiveness of light trapping useful insects, such as Coccinellidae (Coleoptera), Ophion sp. (Hymenoptera: Ichneumonidae), Chrysopa spp. (Neuroptera: Chrysopidae), Hemiptera: (Nabidae), Hemerobius spp. (Neuroptera: Hemerobiidae). A comprehensive study by Tshernishev [188] refers to several publications that contradict one another. Gregg et al. [65] did not find any difference accompanying the changing phases of the Moon when light trapping migrating noctuids (Noctuidae) and hawk-moths (Sphingidae). However, that may also be explained by the method they used. They arranged the 30 days of lunation into 6 groups, of 5 days each, and subjected them to a contingency test. However, the transformation of the optical parameters of the Moon during the lunar month is not an even process and similar optical conditions are not of identical duration, therefore the method applied does not seem to be satisfactory. Light trapping a mosquito species, *Anopheles aquasalis* Curry in Brazil, Flores-Mendoza and Lourenço-de-Oliveira [60], too, experienced no difference in the number of individuals caught in the presence or in the absence of moonlight.

Most authors, however, observed a decline in the catch under the influence of the Moon. The most fundamental studies are associated with the name of Williams [198] that devised for his specific, entomological purposes equipment to register moonlight (Williams and Emery [201]). He found that on a bright night by new moon, three times as many insects flew to light than at full moon. Under a cloudy sky, the ratio went down to 2:1, while the proportion of insects caught by new moon and full moon, respectively, was 2.7:1, cloud conditions ignored. Subsequently Williams [200] extended research to cover several orders of insects. He collected the highest number of individuals on the 20th day of the lunar month and the lowest on the first day, by full moon, that is. Williams et al. [203] offers two possible explanations:

- Moonlight reduces insect activity.
- Accompanied by moonlight, lamplight collects from a smaller area.

The past few decades did not come up with a satisfactory answer to that dilemma.

Moonlight reduces the quantity of insects trapped. This view is shared by Győrfi [67], Cleve [35], Mazochin-Pornsjakov [100], Hosny [80], Wéber [196], Barr et al. [11], Dzhafarov [55], Bréniére et al. [29], Balogh [9], Mirzayeva [105], Theowald [181], Voigt [195], Brown et al. [31], Agee et al. [1] Bowden [20], Tshernishev and Bogus [189], Schaefer [162], Persson [141], Robertson [152], [153], [154], Southwood [172], Oloy [133], Douthwaite [51], Vaishampayan and Shrivastava [192], Járfás [85], Skuhray and Zumr [170], Morton et al. [109], Herczeg and Vojnits [75], Banerjee et al. [10], Vaishampayan and Verma [193], Tucker [191], Taylor [180], Shrivastava et al. [167], Pedgley et al. [138], Dent and Pawar [47], Mészáros [103], Nag and Nath [113], Muirhead-Thomson [110], Rubio-Palis [157], Syed Nurul Alam [175], Finnamore [58], Dillon and MacKinnon [49], Steinbauer [173], Oxley [134].

Collecting in person in Madagascar, Howell [81] found that on moonless nights, the collecting sheet was covered by an uncountable multitude of insects. Various saturnid (Saturnidae) moths were light trapped by Wenzel in Venezuela (Maag [97]). He had modest catch on moonlit nights. The most favourable preconditions to successful trapping included cloudiness, warm sultriness and thunderstorm by new moon. Light trapping near full moon in Ecuador yielded very low numbers of specimens. The background illumination of the full moon makes artificial light sources practically invisible for insects. This effect is particularly strong in the tropics when the Moon is at its zenith (Brehm [28]).

According to Reinert [146], moonlight influences both light-trap effectiveness and the behaviour of mosquitoes. By full moon the light-trap will collect a smaller number of mosquitoes than by new moon. Garcia [63] collected Sphingidae species with mercury vapour light source in Venezuela. He trapped the highest number of individuals by waning Moon and the smallest number by full moon. In Burkina Faso, Constantini et al. [36] did not experience any influence of the lunar phases on the number of mosquitoes light-trapped indoors, however, out-of-doors, a smaller number of specimens were captivated at full than at new moon. According to a report by the Hock Company [77], in the light trapping of mosquitoes, there is a four-week periodicity accompanying the phases of the Moon. Collecting is successful on clouded and moonless nights. However, when 1-2 pounds of dry ice was hung up in an isolated container over the trap, there was a rise in the number of individuals caught and the influence of the Moon also diminished. In the light trapping of *Sopdoptera exampta* Walker (Lepidoptera: Noctuidae), big catches occurred much more often in the neighbourhood of a new moon than at the time of a full moon (Tucker [191]). The difference between nights with and without rain was insignificant. This refers to the fact that the relationship between rain and collecting has nothing to do with the Moon being covered by clouds. Light trapping the malaria mosquito species, *Anopheles culicifacies* Giles (Diptera: Culicidae) in India, Singh et al. [169] had a bigger catch on moonless than on moonlit nights. The difference was prevalent until midnight. Light trapping *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae), Bishop et al. [17] encountered a minimum by full moon. Whereas changing moonlight in the course of a night at the time of a full moon had no clear influence on the light-trap catch. Yela and Holyoak [204] examined with light-trap and bait trap the night-time activity of Noctuidae. The examination was going on for 2 years, encompassing 170 nights. The number of moths light-trapped diminished in the proximity of a full moon. The catch by the bait trap was not modified by full moon. The light-trap catch was, the bait trap catch was not increasing by growing cloudiness. According to Gustafson [66], the light-trap is not effective on cold nights, in rain, or when the Moon or other bright lights are visible in the area. The period from the last quarter to the new moon is the best time for light trapping. In that period, the Moon is visible little, if at all, in early evening. Butler et al. [33] found that moonlight restricted light trapping on cloudless nights. Moving from effective to less effective, he light-trap catch of *Chilo partellus* Swinhoe had the following order of success in India: new moon, first quarter, last quarter and full moon (Mahadevan and Chelliah [98]). Also in India, Rajaram et al. [145] light-trapped a higher number of specimens of 4 cotton pest species by new moon than by full moon, with differences in ratio though. Moonlight, in the first place by full moon, also slows down the activity of bats. According to Negraeff and Brigham [114], this has an explanation in the higher risk of catching a prey or the diminished number of insects.

The role of moonlight in reducing the catch is taken for granted by many researchers, so much so that they stop operating their light-traps at full moon, using it for collecting only at the time of the new moon and /or last quarter, perhaps in the period between the final and first quarter. They (Bragança et al. [27], Hall [69], Andreazze [3], Sant'Ana and Lozovei [159], Summerville and Crist [174], Toda and Kitching [183]) attempt to avoid the adverse impact of the Moon in this way. Tigar and Osborne [182], too, operated light-traps in 5 desert areas of Abu Dhabi by new moon every year. In an experiment, Csóka [40] claimed it was superfluous to operate a light-trap after moonrise, because he was convinced of the chances of a catch greatly reduced by moonlight.

Some researchers explain reduced catch by a slackening of flight activity and by a diminishing collecting area by others. The collecting area changes in line with the twilight or early morning illumination coming from the Sun, the light of the night sky and the light generated in its prevailing phase by the Moon. The light source of the trap is visible from a greater distance in weaker environmental illumination. The views found in professional writing are rather contradictory regarding the question of whether the changing light of the Moon influences the catch by modifying the collecting distance.

The collecting distance as a function of changing moonlight has been calculated by a number of researchers. Using a 125 W HPL light source, Dufay [53] determined the collecting distance as 70 meters at full moon and 830 meters at new moon. Studies by Bowden [19, 20], Bowden and Church [23] discussing in detail the fallback of light intensity from civil twilight to astronomical twilight as a function of the phase of the Moon are of fundamental importance. In these, Bowden examined with graphoanalytical method and arranged in charts the illumination generated by the Moon in its different phases in zones in the vicinity of the Equator, atmospheric light absorption also taken into account. He determined the collecting distances for his 125 W mercury vapour light source as 35 meters at full moon and 519 meters at new moon (Bowden and Morris [26]). Bowden [22] determined, by identical illumination, the collecting radius of three different lamps: 125W mercury vapour in the UV range: 57m at full moon and 736m at new moon, 160 W mercury vapour lamp with wolfram filament: 41m by full moon, 531m by new moon, 200 W wolfram lamp: 30m by full moon, 385m by new moon. Preuss and Preuss [142] established the height, direction and vertical distribution of the flight of nocturnal insects with the help of a telescope set up in the direction of the Moon. They compared their findings to their own light-trap catch data. They determined the collecting distance of the light-trap as 7m. Regarding the distance, Farrow [58] came to an identical conclusion. Observation by Rezbanyai-Reser (verbal message) confirms that light has an area of attraction of not more, perhaps less than 10-20 metres. Only those insects are flying to light that would probably have flied through the area anyway, in the absence of a lamp, too. In the case of a 100 W regular bulb, we determined these distances as 18m and 298m (Nowinszky et al. [122], Nowinszky and Tóth [124]). We also established, however, that the collecting distance had a provable impact on the quantity of the catch only in periods without moonlight when illumination was generated by the setting or rising Sun. The influence of the Moon on the catch exerts itself not only through the modification of the collecting area (Nowinszky et al. [131]).

Some important experiments have shown that insects fly into the trap only from the direct vicinity of the light source, a few meters at most. Recapturing tethered and free-flying marked imagoes of *Noctua pronuba* L. and *Agrotis (Scotia) exclamationis* L., Baker and his fellow researchers (Baker and Sadovy [7], Baker [6], Sotthibandhu and Baker [171]) found that the insects reacted to artificial light from the amazingly short distance of 3-17m, depending on the height of the light source. These authors rule out the possibility of moonlight exerting any influence on the collecting distance. They hold that the growing intensity of light slackens flight activity. The chance of recapturing insects released at different distances from the light-trap decreases in proportion to the growth of the distance (Szeőke [179], Morrison et al. [108]), while the proportion of the individuals trapped of the ones in the direct vicinity of the trap is identical (Bucher and Bracken [32]).

Other researchers are of the view that moonlight slackens the flight activity of insects. Over a period of three years, Nemec [115] collected the smallest number of *Heliothis zea* Boddie specimens at full moon, and the highest number at new moon. To find out about the reasons, he brought up the moths in total darkness in a laboratory. They became inactive as soon as illumination rose to over 0.1 lux. That observation, combined with his light-trap results, lead him to the conclusion that moonlight hindered flying activity. McGeachie [102] reached the same conclusion.

On the other hand, observations by Dufay [53] contradict the theory on the hindering impact of moonlight:

- Even in moonlight, nocturnal moths are there to be seen in the beam of car head-lights.
- The catch diminishes but does not stop altogether at a full moon.
- At the time of a lunar eclipse when the Moon is hiding, the catch is high, despite being low directly before and after the eclipse. This is a rather telling observation, as the eyes of nocturnal insect’s adept to darkness with a delay of 5-9 minutes.

Personally engaged in collecting at the time of a lunar eclipse, Rezbanyai-Reser [147] once observed stepped up insect flight activity as soon as the Moon disappeared, and again, its gradual dying away after the Moon appeared in the sky.

Bowden and Morris [26] always calculated for an identical area the volume of their catch made in the course of the lunar month in areas reduced by the effect of moonlight. The highs of the standardized data occurring in the proximity of the full moon also contradict the theory on the hindering effect of moonlight. Our own experiments (Nowinszky and Tóth [126]) have also shown, after the corrections required by the change in the area of collecting were made, a maximum catch of two pestilent species (*Scotia segetum* Schiff. and *Scotia ipsilon* Hfn.) at full moon. In a subsequent work, Bowden [22] criticizes the remark by Baker and Sadovy [7] who had claimed that the large yellow underwing (*Noctua pronuba* L.) and the heart-and-dart moth (*Scotia exclamationis* L.) fly to light only from a distance inside 3m. Were that the case, Bowden holds, a large volume of light-trap catch over a single night would entail the existence of a population too large to be true. He believes the findings of Baker and Sadovy [7] might be valid for certain forms of behaviour in the direct vicinity of a strong light source, yet argues that their method of experimentation may be subject to criticism.

Jermy's assumption [88] that the presence of moonlight reduces the catch because it helps insects by enhancing their security of orientation is remarkable, although unchecked by concrete experiments. Wehner [197] claims that nocturnal insects, guided by the light of the Moon, are capable of orientation in space, despite the fact of this being a much more complicated task than orientation by the Sun at daytime. For the Moon is not above the horizon every night, the time of its rise and set changes from night to night, and its position alters much more drastically in the course of a night than that of the Sun in the course of a day.

A number of researchers have found that intensive moonlight does not reduce, in fact, in some cases, increases the catch by light-trap (Bogus [18], Pristavko [143], Cullen [39], Johnson [90], Duviard [54], Papp and Vojnits [135], Doiron and De Oliveira [50], Bowden and Jones [25], Járfás and Viola [86], Jeffrey and Dyor [87], Cook and Perfect [37], Shrivastava et al. [166], Saroja et al. [161], Linhares and Anderson [95], Ito et al. [83], Janousek and Olson [84]). Collecting two rice pests (*Scotinophora coarctata* F. and *Scotinophora lurida* Burmeister) with a 125 W mercury vapour lamp, Balasubramani et al. [8] observed a higher catch by the full, then by the new moon. The Malayan Black Bug (*Scotinophora coarctata* Fabricius) flies to light in large quantities. It can be light-trapped in the largest masses during the five days before and after the full moon (http://pne.gsnu.ac.kr/riceipm/ scotinop.htm.) Sharma and Badan [165] observed a catch maximum both at the time of the new and the full moon and a minimum in the vicinity of the first and last quarters. Sekhar et al. [164] claims, that the catch is higher in the period from the full to the new moon than from the new to the full moon. Collecting mosquito species, Dickson and Hatch [48] encountered a catch maximum in the first or last quarter.

According to some observations, flying activity is lengthened by the stay of the Moon above the horizon (Heikkinheimo [74]) and that leads to a richer catch (Nowinszky and Tóth [124], Tóth et al. [186], Nowinszky et al. [131]). On the other hand, Siddon and Brown [168] in a suction trap experiment encountered a catch maximum 11 hours after sunset in the 7 day period preceding the full moon and 2 hours after sunset, in other words, in the moonless periods of the night in the 7 day period following the full moon.

From the point of view of clarifying the relationship between the light of the Moon and light-trap effectiveness, studies examining the moonlight-related activity of insects by use of other methods are of great significance. For these may exclude the disturbing differences in the reaction of insects to the trap stimulus. Saha and Mukhopadhyaya [158] observed a difference in the copulation activity of the species *Orthomorpha coarctata* Saussure (Polydesmida, Paradoxosomatidae) in the first quarter of lunation. In their experience, the height of activity occurred half an hour before sunset, 3-5 days before the full moon and the new moon. Kerfoot [91] reports, those nocturnal bees carried on with their collecting activity as long as the Moon stayed above the horizon. Some water insect larvae display increased liveliness of activity in the presence of moonlight (Ribbands [148]), an experience not shared by some other authors (Andersen [2], Chaston [34]). Some mosquitoes, gnats and tsetse flies become more aggressive when the Moon is visible (Vanderplank [194], Ribbands [148], Monchdadskiy [107], Muradov [111]). On the other hand, observation to the opposite effect is reported by Bhatt et al. [13]. Desert ants carry on with their daytime feeding activity on moonlit nights (Hunt [82]). Riley et al. [150] have observed in radar experiments that the presence of moonlight protracts the activity of insects flying at twilight. Sáringer [160], too, believes in the possibility of moonlight making the day longer for insects with a perception threshold of luminous intensity below that of the Moon. Therefore in the case of some species, moonlight should also be considered in any study of the photoperiodic reactions. According to Schaefer [162], the flight maximum observed by radar was not reflected in the light-trap catch in strong moonlight. Using a radar device, Drake [52] and Riley et al. [149] who also used radar as well as infrared optics found no relationship between the direction of the orientation of migratory insects and the position of the Moon, therefore they do no see the theory of orientation by the Moon confirmed. From the catch of a Jermy-type light-trap and bait trap of the same construction, Gyulai and Nádler [68] have come to the conclusion that a light-trap will catch a higher number of insect species and individuals in most parts of the year. However, at spring, in the autumn and by strong moonlight, catch results are balanced out over the year. Suction traps often demonstrate an activity peak, not indicated by light-traps, in the period of the full moon (El-Ziady [57], Bidlingmayer [14], Perfect and Cook [140]). In a subsequent suction trap examination, Bidlingmayer [15] found no difference between the collecting results in the period of the full moon on the one hand and the new moon on the other. Bidlingmayer [16] also established that the number of mosquitoes collected in the suction and bait traps from the time of the new moon to that of the full moon grew by 2-3% every day. Bowden's [21] corrected light-trap data were basically the same as those of the suction trap. Using a suction trap, Davies [45] demonstrated an activity peak in the evening and in early morning at the time of the new moon, on other nights this was modified in line with the phase of the Moon and at the time of the full moon shifted in time to coincide with the time of the rise of the Moon and the middle of the night. The light-trap catch did not confirm evening and early morning activity. In pheromone trap experiments, Sekhar et al. [163] found no difference in the number of *Helicoverpa armigera* Hbn. moths collected at the time of the full moon on the one hand and that of the new moon on the other. Mean catches of the African sweet potato weevils, *Cylas brunneus* and *Cylas puncticollis*, did not differ significantly between new and full moon caught by pheromone trap (Laboke and al. [93]). Tshernishev and Dantanarayana [190]) established in laboratory experiments that the activity of the three noctuid species (*Helicoverpa armigera* Hbn., *Helicoverpa punctigera* Wallengren and *Heliothis rubescens* Walker) studied with the help of an infrared actograph reached its peak by full moon and by new moon, and its low from the second day following the new moon to the two days preceding the full moon. Williams and Singh [202] have reported on the following suction trap catch results in the + 3 day proximity of the various Moon phases: full moon: 204, last quarter: 589, new moon: 1 259, first quarter: 562 specimens. El-Ziady [57] modifies the original question by Williams [198] on the modest catch at the time of the full moon in the following way:

- Moonlight has a direct influence on activity and reduces the number of flying insects.
- It is possible that insects stay at the shaded, darker places at the time of the full moon.
- It is equally perceivable that insects fly in the higher layers of the atmosphere in that period.

On the other hand, Danthanarayana [41] in his suction trap experiments detected a major peak in the catch at the time of the new moon and a smaller one at the time of the full moon. According to Danthanarayana [42], the flight activity of insects has a three peak lunar periodicity: in the first and last quarters and directly after the period of the full moon. The latter peak however, remains obscured in light-trap collecting as it occurs in the period characterized by the smallest collecting area. The lunar period of flight activity gets superimposed on the circadian rhythm. In his view, the three peak lunar periodicity might be related to migration. In these periods, insects fly in the higher layers of the atmosphere, thus reaching heights where they get transported by horizontally moving masses of air. In our earlier work (Nowinszky et al. [130]) we demonstrated that only in traps operated at 2 m and 10 m is the difference in the specimen number of migratory species insignificant at the time of the full moon, in the period of other lunar phases, the light-trap lying lower collects a much smaller number of individuals. This fact might support the assumption of Danthanarayana, although in the absence of a satisfactory amount of investigation in Hungary, we cannot come out with a well-founded argument on this question. And in the absence of high traps, the possibility of further research is ruled out. However, the outcome of an experiment by El-Ziady [57] might be an important contribution: using a suction trap placed at a height of about 9m (30 feet), he collected the highest number of flies (Diptera) at the time of the full moon.

Danthanarayana and Dashper [43] observed a peak in the activity of nocturnal insects at the time of the full moon and in the proximity of the first and the last quarters. The latter two maximums are related to polarized moonlight, which is of the highest intensity in the same two lunar quarters. Kovarov and Montchadski [92] found that a light-trap using polarized light was twice as effective as the one using regular light. In an earlier study (Nowinszky et al. [122]), we detected in the combined light-trap catch data of 7 species three catch maximums in the course of the lunar cycle. However, in the place of the first maximum at the time of the full moon, we found a smaller local catch maximum in the period of the new moon. The abundance of catch in the first and last quarters can be explained with the high ratio of polarized moonlight, while the catch high in the vicinity of the new moon, when there is no moonlight, might follow from the fact that this is the phase characterized by the largest collecting area. Mizutani [106] could not confirm the influence of polarized moonlight on the catch, but then he had a mere 17 nights at his disposal and there was a strong wind at the time of collecting. An experiment by Sotthibandhu and Baker [171] shows that the large yellow underwing (*Noctua pronuba* L.) finds its bearings on moonless, bright nights by the stars positioned some 95° from the North Star.

All in all, not to this day have researchers arrived at a common platform regarding the influence of the Moon on the flight activity of insects and on the light-trap catch. Therefore, making use of the enormous mass of collecting data supplied by the Hungarian national light-trap network and the hourly catch of Járfás' fractionating light-trap in Kecskemét, we examined this question in several studies (Nowinszky [119]).

Material and Methods

In our work, we used data of national light-trap network pertaining to the species listed in *Table 1.* The material of the Kecskemét fractionating light-trap, we processed data on turnip moths (*Scotia segetum* Schiff.) and heart-and-dart moths (*Scotia exclamationis* L.).

Table1. *Data of the species examined from the material of the national light-trap network.*

Species	Number of			
	Light-traps	Years	Individuals	Data
Coleoptera: Alleculidae				
Hymenalia rufipes F.	3	5	604	3602
Lepidoptera: Cossidae				
Dyspessa ulula Brkh.	33	14	2893	1351
Lepidoptera: Plutellidae				
Plutella maculipennis Curt.	26	3	3953	4821
Lepidoptera: Hhyponomeutidae				
Hyponomeuta malinellus L.	24	3	1591	994
Lepidoptera: Notodontidae				
Clostera pigra L.	2	10	1238	963
Lepidoptera: Lasiocampidae				
Odonestis pruni L.	25	17	2363	947
Lepidoptera: Lymantriidae				
Lymantria dispar L.	55	20	4721	3326
Porthesia similis Fuess	12	14	1195	786
Lepidoptera: Noctuidae				
Scotia vestigialis Schiff.	15	19	1109	3396
Amathes c-nigrum L.	7	12	4108	12401
Mamestra suasa Schiff.	28	10	6502	5447
Brachyonicha sphinx Hfn.	17	10	2142	3623
Lepidoptera: Geometridae				
Abraxas grossulariata L.	12	11	889	1105
Erannis marginaria Brkh.	10	9	1671	2077

We used our own software devised for the purpose to calculate the times of sunset and sunrise and the start of civil, navigational and astronomic twilight as well as the values of illumination in the environment expressed in log lux (Tóth et al. [185], Tóth and Nowinszky [184], Nowinszky and Tóth [125]). The same software made it possible for us to define for any given point of time the light of the twilight sky from the setting or rising Sun, illumination generated by the Moon always in correlation with the given lunar phase and the light of the starry sky which is constant (0.0009 lux). The software also considers the extent of cloudiness. Our own software also helped us to calculate the position of Babinet's point above the horizon. In the current study we have no scope to outline the theoretical bases and actual succession of calculations. However, let me refer to our earlier work (Tóth et al. [185], Nowinszky et al. [122]), where all these were described in detail, with the software in question and our results included (Nowinszky and Tóth [125], Nowinszky and Tóth [129]).

From the material of the Kecskemét fractionating light-trap we have used collecting data pertaining to the fall webworm moth (*Hyphantria cunea Drury*) and the turnip moth (*Scotia segetum* Schiff.). In the context of the position of Babinet's point, we examined light trapping efficiency concerning the catch of turnip moths (Scotia segetum Schiff.) and heart-and-dart moths (*Scotia exclamationis* L.). And with regard to the collecting distance, we processed the catch data pertaining to all three species. We computed the collecting distance of the Kecskemét fractionating light-trap for different values of environmental illumination and the ensuing probabilities of approach in a way described in an earlier study (Nowinszky et al. [122]). In what follows we give you the result without repeating the process of calculation. With the exact time of the catch within each hour unknown, the data were always calculated for the 30th minute following every full hour. By the distance of collecting we mean the radius of a circle with a circumference made up of points that receive an equal amount of illumination from the light-source and the environment. This is the formula to determine the radius:

$$r_O = \sqrt{}I/E \qquad \text{(Eq. 1)}$$

Where: ro = the collecting distance, I = the intensity of illumination by the light-trap (candela), E = the intensity of environmental illumination (lux).

If the illumination in the environment comes exclusively from the starry sky, the maximum collecting distance with a Jermy-type light-trap is this:

$$r_O = \sqrt{}80/0.0009 = 298\text{m} \qquad \text{(Eq. 2)}$$

And with the Kecskemét fractionating light-trap using F-33 light-tubes:

$$r_O = \sqrt{}255/0.0009 = 532\text{m} \qquad \text{(Eq. 3)}$$

At full moon, when the environmental illumination comes partially from the Moon, the collected distance with a Jermy-type light-trap is the following:

$$r_O = \sqrt{}80/0.25+0.0009 \approx 18\text{m} \qquad \text{(Eq. 4)}$$

And with the Kecskemét fractionating light-trap using F-33 light-tubes:

$$r_O = \sqrt{}255/0.25+0.0009 \approx 32\text{m} \qquad \text{(Eq. 5)}$$

We are to assume that the flight of an insect at distance r from the light-trap is equally probable in every direction. In that case, the probability (probability of approach) of the insect flying in the direction of the two-dimensional plane determined by the tangents to a circle with radius r_O is the following:

$$P(A) = (1/p) \arcsin (r_O/r) \qquad \text{(Eq. 6)}$$

From the catch data we calculated relative catch (RC) values by species, generations and hours. In the swarming periods of the different generations, we calculated for each night the times of sunset and sunrise as well as the start of civil, navigational and astronomic twilight in both the evening and early morning hours and the onset of night time illumination, the accompanying values of environmental illumination expressed in lux and the period in which the Moon stayed above the horizon. We arranged collecting hours into the range of illumination to which they belonged over a longer period of time, separating moonlit hours and those without moonlight. Within each range of illumination, we averaged the accompanying relative catch values. The level of significance of the difference between the catch by the same illumination before and after midnight as well as the catch belonging to consecutive ranges was checked by a t-test (Nowinszky et al. [120]).

We computed the whole collecting area for the twilight and night hours both with and without moonlight. Without considering the hour of collecting, yet making a distinction between the hours of twilight and night with and without moonlight, we arranged these areas into classes, and then drew averages (Nowinszky et al. [131]). By use of our own method, we calculated three point moving averages of the accompanying relative catch data. Then we attempted to reveal the possible connection by correlation calculations by species.

The assumed effect of Babinet's point on the orientation of insects considering both evening and daybreak collection data in a contracted form, we studied then compared with each other the modifying effect on the catch of the Moon on the one hand and Babinet's point on the other. We arranged in three classes the values of environmental illumination below -1 log lux, -2 log lux and -3 log lux. In each class, we separated two groups depending on the position of the Moon and Babinet's point above the horizon:

- The Moon and Babient's point were both above the horizon (M+Bp+).
- The Moon was not, Babinet's point was above the horizon (M-Bp+).

In all illumination classes and groups, we summed up then averaged the relative catch values of the relevant hours. Then we examined the differences, if any, between the catch data pertaining to the different species and classes of illumination. We checked the significance levels of the differences with a t-test.

Then, disregarding environmental illumination, we went on analysing those cases in which both the Moon and Babinet's point were above the horizon. Here we wanted to find out whether there is any difference in the light trapping efficiency of the different species depending on whether the Moon or Babinet's point was higher on the horizon. Since Babinet's point was always positioned below 45° above the horizon, we examined cases when the Moon, too, did not rise higher than 45°. We separated our data depending on whether the Moon or Babinet's point was positioned lower above the horizon. All in all, also distinguishing between evening and early morning hours, we studied the differences in the catch results of four different eventualities. Both in the evening and early morning hours, we compared the catch results in the following situations:

- The Moon and Babinet's point are both below 45° above the horizon, but the Moon is positioned lower.
- The Moon and Babinet's point are both below 45° above the horizon, but Babinet's point is positioned lower.

Just like before, we summed up, then averaged the relative catch values by species and checked the significance level of the differences with a t-test.

Based on a study by Austin et al. [5], Pellicori [139] and our own earlier work (Ekk et al. [56], Szabó et al. [177], Nowinszky and Tóth [123], [124], [126], Nowinszky et al. [122]), we have sketched the relative luminousness of the Moon and the ratio of its polarized light as a function of the phase angle. Based on our earlier work (Tóth and Nowinszky [184]) were calculated with the help of software of our own development (Nowinszky and Tóth [125]).

Looking at the Kecskemét collection data of the turnip moth (*Scotia segetum* Schiff.) and the heart-and-dart moth (*Scotia exclamationis* L.), we were first trying to find an answer to the question of whether the differing heights of the Moon above the horizon influenced the effectiveness of light trapping? The catch data from the fractionating light-trap provide us with the specimens caught in each full hour, within that, however, we have no knowledge of the exact time of trapping, therefore the data related to the height of the Moon above the horizon were calculated electronically, with our own software, always pertaining to the 30th minute of each hour (Nowinszky and Tóth [125]). We processed the catch of only those nights when the Moon was observable both below and above 45° on the horizon.

We arranged in classes, and then averaged the values of the height of the Moon above the horizon following Sturges' method (Odor and Iglói [132]). One by one, the relative catch values of the species examined were correlated to the values of the height of the

Moon above the horizon determined for each given hour of the catch. These were then averaged in each class. We checked with a t-test the differences between the average relative catch values in the successive classes. We made correlation calculations for both species between the height of the Moon above the horizon and the accompanying 3 point moving average of the relative catch.

We calculated the phase angle value of the Moon for the 24th hour (UT) of each night in the swarming periods of the various species. Then we formed 30 phase angle groups of the 360 degrees of the complete lunar cycle. The group of the phase values in the ± 6o vicinity of the full moon (0° or 360°) was marked 0. Starting from this, the groups through the first quarter in the direction of the new moon were marked -1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, -12, -13 and -14. And the groups from the full moon via the last quarter in the direction of the new moon are marked as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14. The phase angle group containing the new moon was ±15. Each group contains 12 phase angle values. The following phase angle groups fall in the four typical lunar quarters: full moon (-2 - +2), last quarter (3 - 9), new moon (10 - -10) and first quarter (-9 - -3). We arranged all the nights of the swarming periods of the species examined in one of the above phase angle groups, and then averaged the accompanying relative catch data of the various species. To reduce the misleading effect of other, simultaneously existing environmental factors, we performed a 10 point digital filtering of the average values (Nowinszky et al. [131], Nowinszky and Tóth [127, 128]) by use of the Hanning filter formula (Gold and Rader [64]) which contains the following filter parameters:

$$F(1) = \frac{2}{P1} - \frac{2}{P2} \quad \text{and} \quad F(k) = \sum_{k=2}^{T} \left(\sin\frac{2\pi k}{P1} - \sin\frac{2\pi k}{P2}\right) \frac{\cos\left(\frac{\pi k}{T}\right)}{2\pi k} \qquad \text{(Eq. 7 and 8)}$$

Where P1 = the lower limit of the filter, P2 = the top limit of the filter, T = P1 - P2, k = 2,... T. The filtered values and the basic data are obtained by a convolution of the above filter parameters.

On the swarming curves received after the Hanning filtering had been performed, maximum and minimum catches are found in the same groups of phase angles as on the curves drawn on the basis of the original catch data, but the maximum values lay higher and minimum values lower. So filtering had reduced the disturbing effect of other, simultaneously existing environmental factors made the curves more typical and highlighted the catch maximums and minimums. We did not perform any filtering of the Kecskemét data, as we needed the original catch values for subsequent calculation.

Results

The catch results in the context of environmental illumination of the turnip moth (*Scotia segetum* Schiff.) and the fall webworm moth (*Hyphantria cunea* Drury) are shown in *Table 2.*

Table 2. *Light-trap catches of the Scotia segetum Schiff. and the Hyphantria cunea Drury in connection with the environmental illumination, in periods with and without moonlight.*

Twilight and illumination	**Zenith distance of the Sun**	**Moonlit and without moonlight periods**	**Scotia segetum Schiff.**		**Hyphantria cunea Drury**	
			RC	**N**	**RC**	**N**
Sunset 188 lux	90.5°	Without moonlight	0.612	38	0.612	94
Civil twilight	90.5°-96°	Without moonlight	1.897	102	0.766	177
188-3.3 lux		Moonlit	0.421	37	0.905	59
Navigation twilight	96°-102°	Without moonlight	2.284	86	0.683	152
3.3-0.01 lux		Moonlit	1.074	54	1.647	103
Astronomical twilight	102°-108°	Without moonlight	1.861	87	1.001	154
0.01-0.001 lux		Moonlit	1.071	53	1.055	122
Nocturnal illumination	108°alatt	Without moonlight	1.289	292	*0.915*	553
0.0009 lux		Moonlit	1.316	221	*1.267*	479
Astronomical twilight	102°-108°	Without moonlight	0.804	78	1.050	154
0.01-0.001 lux		Moonlit	1.131	62	1.952	100
Navigation twilight	96°-102°	Without moonlight	0.516	88	1.000	171
3.3-0.01 lux		Moonlit	0.920	52	1.633	82
Civil twilight	90.5°-96°	Without moonlight	0.256	126	0.578	220
188-3.3 lux		Moonlit				
Sunrise188 lux	90.5°	Without moonlight	0.052	24	0.122	90

RC = relative catches, N = Number of observing data. Underlined and italic numbers indicate the twilight periods in which the relative catch is significantly (at least at a 99% and 95% level) higher than that of the night.

Table 3 contains the catch results by light-trap of the species examined as a function of the collecting distance, in hours without moonlight. As we could establish no relationship between the collecting distance and the catch in moonlit hours, we omit publication of the relevant results. With regard to the position of Babinet's point over the horizon, the catch results pertaining to turnip moths (*Scotia segetum* Schiff.) are seen in *Table 4* and those concerning heart-and-dart moths (*Scotia exclamationis* L.) are seen in *Table 5 Table 6* shows the results of light trapping depending on the height of the Moon above the horizon based on the material of the Kecskemét light-trap.

Table 3. *Light-trap catch of the species examined at times of civil, navigational and astronomic twilight, in hours without moonlight in the function of the collecting distance (in metres).*

Scotia segetum Schiff.			**Scotia exclamationis L.**			**Hyphantria cunea Drury**		
Distance	**RC**	**N**	**Distance**	**RC**	**N**	**Distance**	**RC**	**N**
8.9	0.856	362	1.9	0.380	319	1.5	0.974	353
33.7	0.912	54	4.3	0.371	71	34.1	1.233	56
76.5	1.036	51	11.6	0.431	59	77.3	1.566	70
126.3	1.293	33	37.7	0.736	61	129.3	1.188	31
184.3	1.306	17	72.9	0.997	59	185.4	1.402	27
228.9	1.093	22	137.0	1.180	61	241.0	1.740	30
276.7	1.033	26	242.3	1.134	60	300.8	1.341	43
334.9	1.065	66	314.4	1.134	61	359.2	1.285	13
373.0	1.124	48	348.4	1.260	59	434.1	1.610	22
416.2	1.203	27	365.1	1.174	57	492.2	2.037	13
469.5	1.404	20	425.1	0.997	67			
r = 0.631 (significance = 95%)			r = 0.783 (significance = 99%)			r = 0.688 (significance = 95%)		

Table 4. *Light-trap catch of the turnip moth (Scotia segetum Schiff.) in connection with the position of the Moon and the Babinet- point over the horizon.*

<table>
<tr><th rowspan="2">Position of the Moon and Babinet-point above the horizon</th><th colspan="2">log lux - 1.....</th><th colspan="2">log lux - 2.....</th><th colspan="2">log lux - 3.....</th></tr>
<tr><th>RC</th><th>N</th><th>RC</th><th>N</th><th>RC</th><th>N</th></tr>
<tr><td>Both the Moon and the Babinet-point are above horizon</td><td><i>1.033</i></td><td>104</td><td><i>0.686</i></td><td>89</td><td>0.246</td><td>5</td></tr>
<tr><td>The Moon is below the horizon, the Babinet-point is above the horizon</td><td><i>1.219</i></td><td>91</td><td><i>1.253</i></td><td>168</td><td>0.608</td><td>30</td></tr>
<tr><td rowspan="2">Both the Moon and the Babinet-point are lower than 45°,</td><td colspan="2"><i>Evening</i></td><td colspan="2"></td><td colspan="2"><i>At dusk</i></td></tr>
<tr><td><i>RC</i></td><td><i>N</i></td><td></td><td></td><td><i>RC</i></td><td><i>N</i></td></tr>
<tr><td>but the Babinet-point is higher,</td><td>1.008</td><td>84</td><td></td><td></td><td><i>0.614</i></td><td>49</td></tr>
<tr><td>but the Moon is higher</td><td>1.040</td><td>41</td><td></td><td></td><td><i>1.123</i></td><td>63</td></tr>
</table>

RC = relative catches, N = number of observing data. Italic numbers indicate if the differences of relative catch values one after the other are those significantly higher than 95%.

Table 5. *Light-trap catch of the turnip moth (Scotia exclamationis L.) in connection with the position of the Moon and the Babinet- point over the horizon.*

<table>
<tr><th rowspan="2">Position of the Moon and Babinet-point above the horizon</th><th colspan="2">log lux - 1.....</th><th colspan="2">log lux - 2.....</th><th colspan="2">log lux - 3.....</th></tr>
<tr><th>RC</th><th>N</th><th>RC</th><th>N</th><th>RC</th><th>N</th></tr>
<tr><td>Both the Moon and the Babinet-point are above horizon</td><td><i>0.773</i></td><td>117</td><td>1.015</td><td>85</td><td>0.777</td><td>10</td></tr>
<tr><td>The Moon is below the horizon, the Babinet-point is above the horizon</td><td><i>0.954</i></td><td>118</td><td>1.034</td><td>214</td><td>0.858</td><td>14</td></tr>
<tr><td rowspan="2">Both the Moon and the Babinet-point are lower than 45°,</td><td colspan="2"><i>Evening</i></td><td colspan="2"></td><td colspan="2"><i>At dusk</i></td></tr>
<tr><td><i>RC</i></td><td><i>N</i></td><td></td><td></td><td><i>RC</i></td><td><i>N</i></td></tr>
<tr><td>but the Babinet-point is higher,</td><td><i>0.745</i></td><td>77</td><td></td><td></td><td><i>0.811</i></td><td>78</td></tr>
<tr><td>but the Moon is higher</td><td><i>1.394</i></td><td>48</td><td></td><td></td><td><i>2.141</i></td><td>54</td></tr>
</table>

RC = relative catches, N = number of observing data. Italic numbers indicate if the differences of relative catch values one after the other are those significantly higher than 95%.

Scotia segetum Schiff.			**Scotia exclamationis L.**		
Height of the Moon above horizon (°)	**Relative catches**	**Number of data**	**Height of the Moon above horizon (°)**	**Relative catches**	**Number of data**
7.5	1.82	14	7.4	2.11	13
11.9	1.47	15	12.1	2.11	13
17.1	1.31	15	16.9	2.06	13
21.7	1.21	15	21.9	1.83	13
26.8	1.28	15	26.6	1.68	13
31.9	1.20	16	32.0	1.55	15
37.3	0.99	16	36.9	1.41	12
41.9	1.07	15	41.5	1.45	14
47.0	0.97	24	46.9	1.29	21
51.8	0.69	14	51.9	0.86	15
58.7	0.26	14	56.8	0.11	11
r = - 0.933 (Significance level is higher than 99 %)			r = - 0.867 (Significance level is higher than 99 %)		

Table 6. *Light-trap catch of the turnip moth (Scotia segetum Schiff.) and the heart-and-dart moth (Sctoia exclamationis L.) related to the height of the Moon over the horizon (in degrees) (Kecskemét, 1967-1969).*

Of the material of the national light-trap network, guided in our selection by an effort to give as wide a representation as possible to the reflection of taxonomic categories, we put forth some of our new findings concerning characteristic types of behaviour re-flecting the influence of the Moon in *Figures 1-7.*

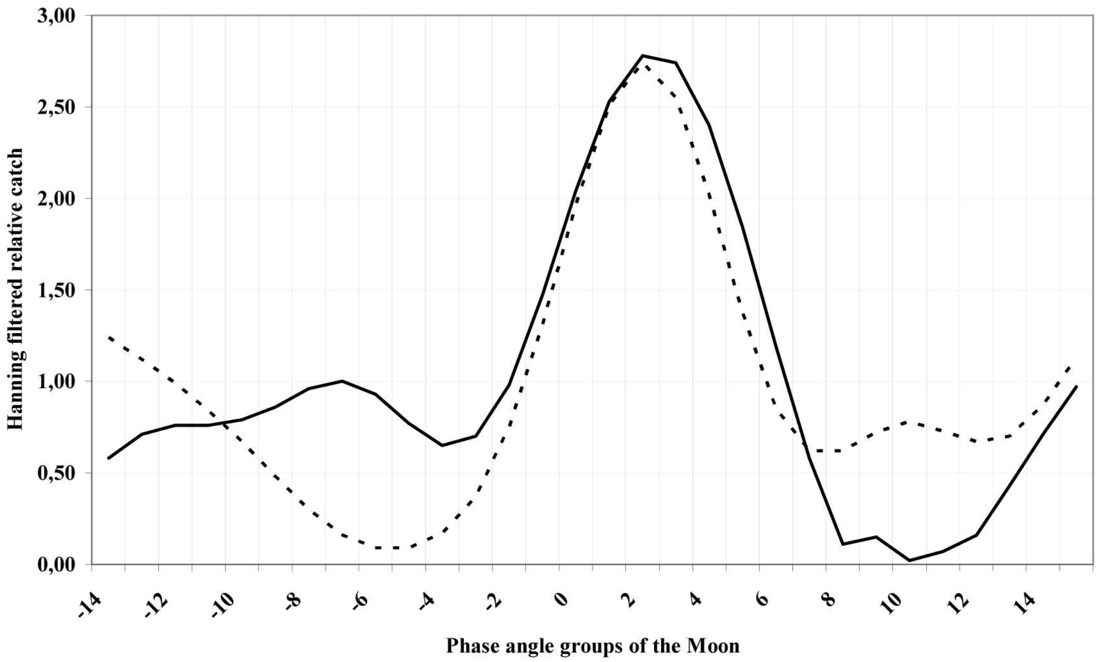
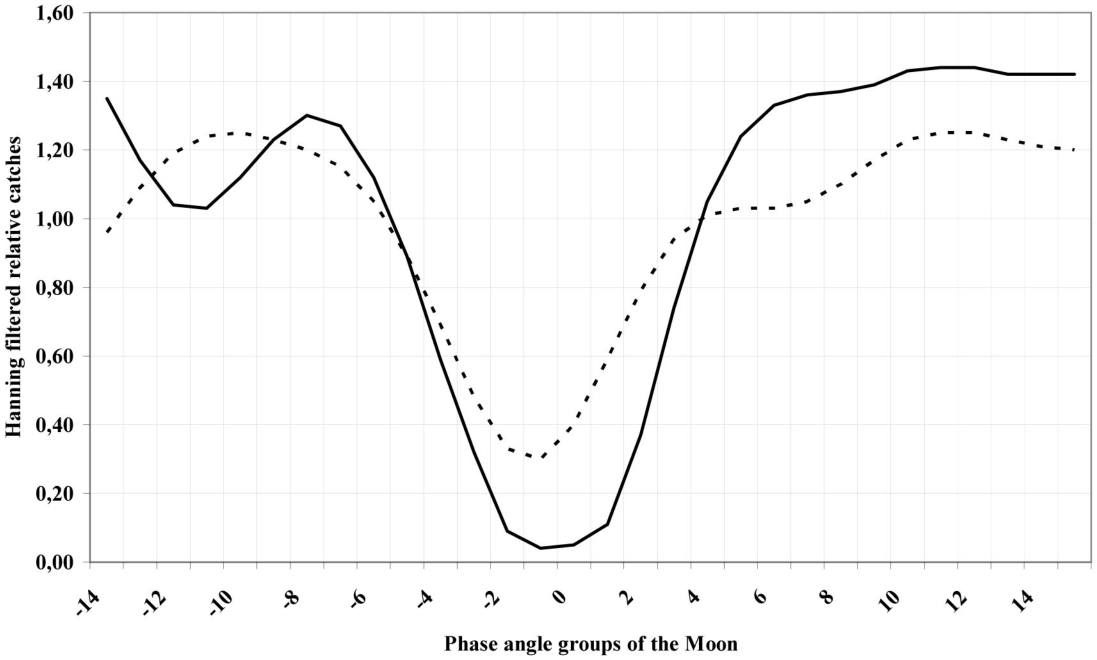
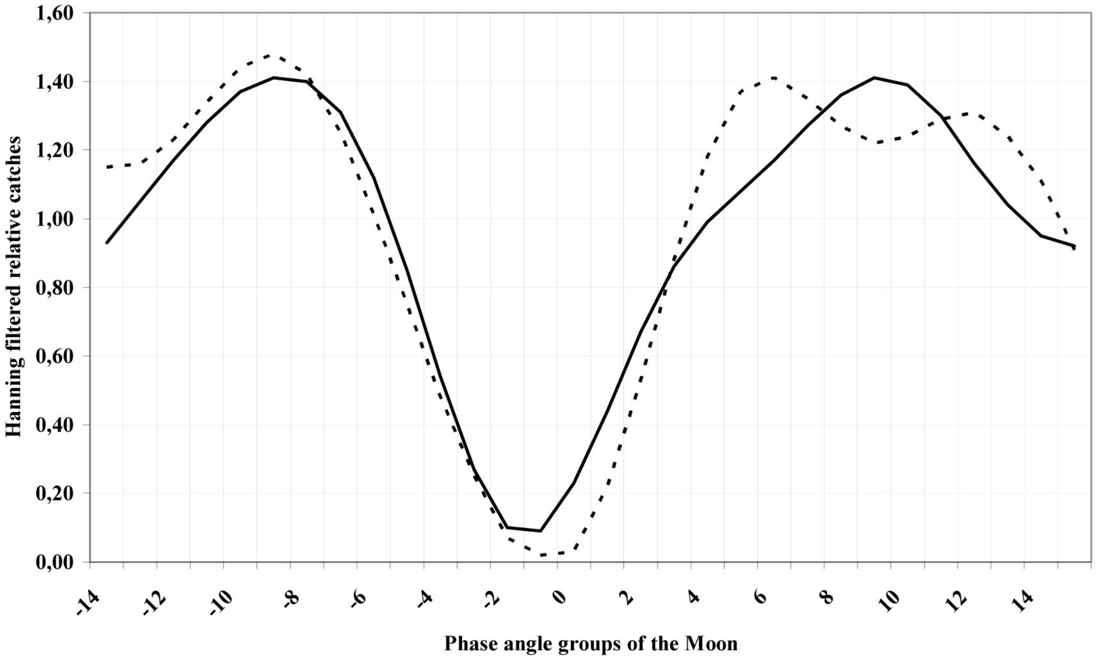


Figure 1. *Hanning filtered relative catches of the Erannis marginaria Brkh. (continuous line) and the Hymenalia rufipes F. (dotted line) depending on the phase angle groups of the Moon. (A single explicit catch maximum at full moon or directly after.)*

Figure 2. *Hanning filtered relative catches of the Hyponomeuta malinellus L.. (continuous line)*



and the Mamestra suasa Schiff. (dotted line) depending on the phase angle groups of the Moon.
(High catches from the last quarter to first one, not falling back at the new moon.)
Figure 3. *Hanning filtered relative catches of the Amathes c-nigrum L.. (continuous line) and the*



Lymantria dispar L. (dotted line) depending on the phase angle groups of the Moon.
(Two nearly identical catch maximums in the first and last quarters.)

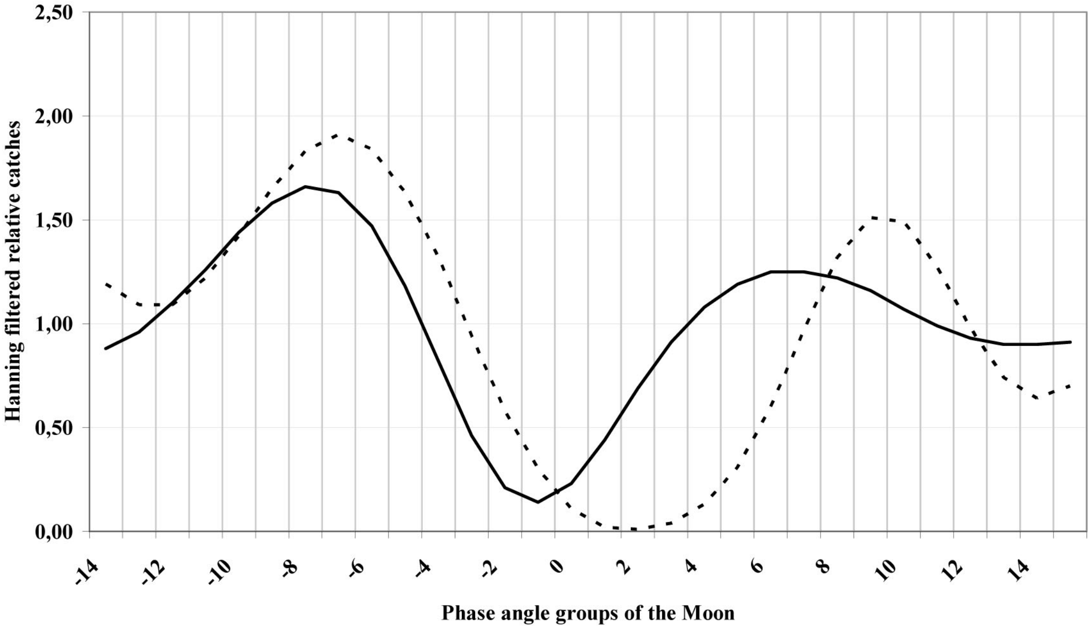


Figure 4. *Hanning filtered relative catches of the Pygaera pigra L. (continuous line) and the Brachyonica sphinx Hfn. (dotted line) depending on the phase angle groups of the Moon. (Two catch maximums, the stronger in vicinity of the first quarter.)*

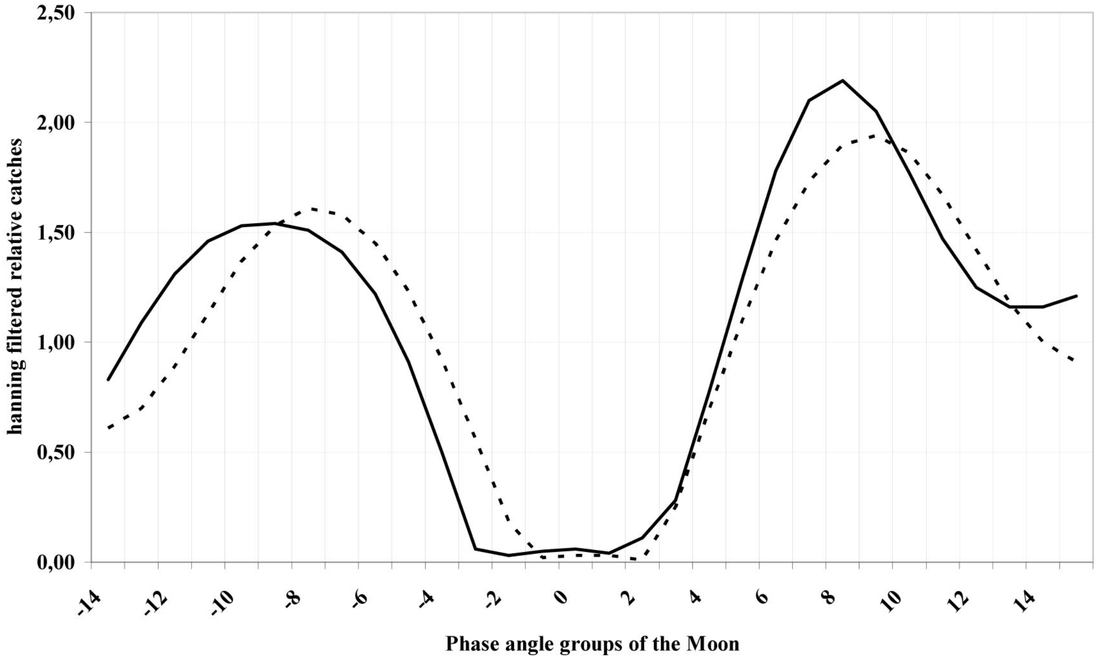


Figure 5. *Hanning filtered relative catches of the Odonestis pruni L. (continuous line) and the Porthesia similis Fuess. (dotted line) depending on the phase angle groups of the Moon. (Two catch maximums, the stronger in vicinity of the last quarter.)*

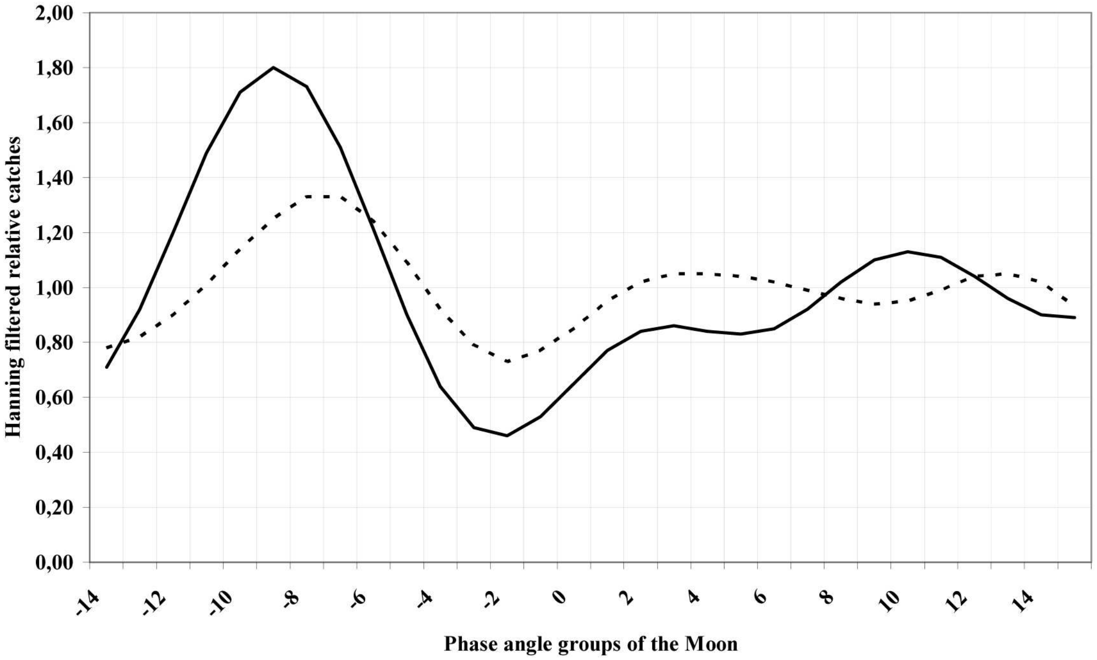
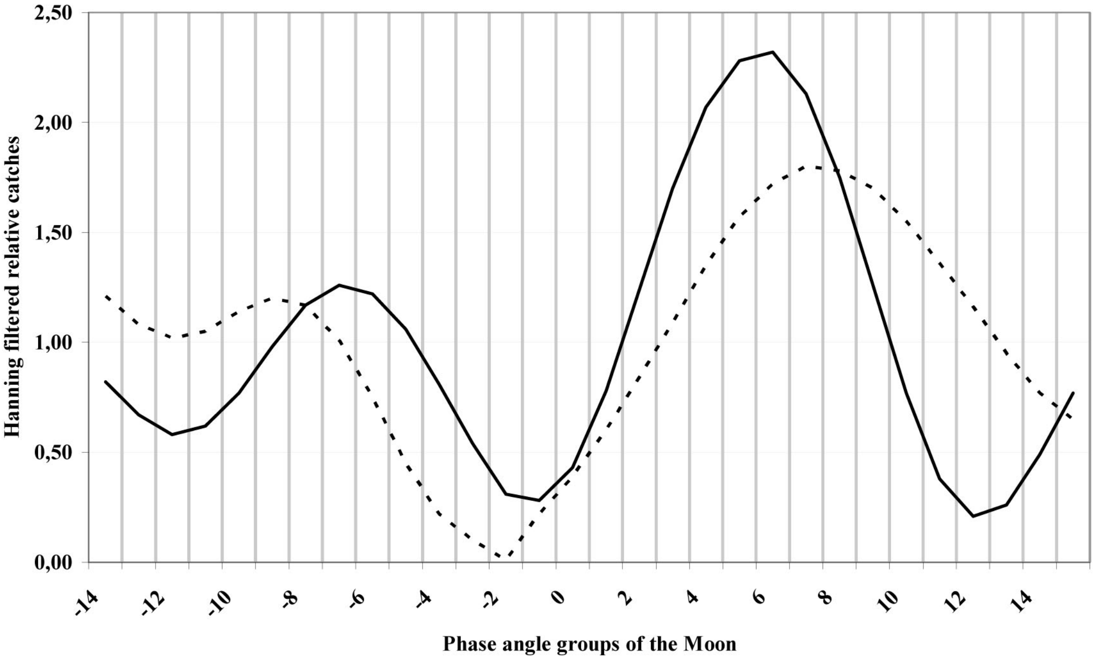


Figure 6. *Hanning filtered relative catches of the Scotia vestigialis Schiff. (continuous line) and the Plutella maculipennis Curt. (dotted line) depending on the phase angle groups of the Moon. (Single explicit catch maximum at first quarter.)*

Figure 7. *Hanning filtered relative catches of Abraxas grossulariata L. (continuous line) and Dyspessa ulula Brkh. (dotted line) depending on the phase angle groups of the Moon. (Single explicit catch maximum at last quarter.)*



Discussion

Illumination generated by the Sun and the Moon changes not only on the different days of the swarming period, but also in the course of each night, therefore it is an extremely important factor modifying collecting.

In the case of the turnip moth (*Scotia segetum* Schiff.) we find that in the periods without moonlight, every range of illumination, with the expectation of the middle hours of the night, is accompanied by significantly higher catch results before than after midnight.

In the middle of the night when the light of the nocturnal sky is the only natural source of light to ensure the illumination of the environment, the catch before and after midnight is the same, presumable because of the small time difference. Whichever of the three forms of twilight illumination may prevail, the catch after sunset is significantly higher than at the time of sunset or in the hours of illumination by night. However, the relative catch values accompanying civil, navigational and astronomic twilight show no variation. It is extremely remarkable that the number of moths caught during the successive twilight's after midnight goes on decreasing significantly (*Table 2*).

Our earlier results (Nowinszky et al. [121]), however, have also made it clear that this phenomenon is explained neither by temperature regularly dropping by dawn, nor by increasing relative humidity. Our examinations have proved that in all comparable cases by identical illumination and temperature as well as by identical illumination accompanied by relative humidity, the catch was always significantly higher before than after midnight. It is remarkable, however, that the above circadian rhythm is modified by the presence of the Moon above the horizon: the activity of flying to light does not lose from its liveliness until the navigational twilight of the early morning hours, in fact significantly more moths fly to light at dawn than under the same illumination conditions in the periods without moonlight. However, during the total solar eclipse of August 11th, 1999, the light-traps in Vas county did not catch a single insect (Puskás et al. [144]). Sudden short darkness at daytime does not disturb the circadian rhythm.

Our examination related to the fall webworm moth (*Hyphantria cunea* Drury) has produced an opposite result. In the hours of civil, navigational and astronomic twilight and by identical temperature and humidity, the imagoes of this species show greater activity in flying to light after midnight. It is remarkable, however, that in every sphere of illumination, the number of moths caught is higher in the moonlit periods.

In the hours without moonlight, the catch relevant to all three species increases as the distance of collecting grows (*Table 3*). However, this relationship cannot be proved when illumination is generated partly by the Moon. Although moonlight reduces the distance of collecting, the catch results do not go down accordingly. So the Moon exerts is influence not only through the area of collecting.

In all three spheres of illumination and regardless of the Moon being positioned above or below the horizon, the light trapping of both species will be more successful when Babinet's point is below the horizon (*Table 4* and *Table 5*). When the Moon and Babinet's point are both below 45° over the horizon, the catch will be more successful if the Moon is positioned higher. We presume that the Moon and Babinet's point both play a role in the orientation of insects, but of the two the one seen higher will be of greater importance. If Babinet's point is positioned higher, the insect may escape the light-trap, perceiving the former as a discontinuity in a sky emitting a continuity of polarized light, and it can hardly mistake that discontinuity for an artificial source of light as it is perceivably does in the case of the Moon. So, unlike the Moon, Babinet's point bolsters the security of orientation.

Gál et al. [61] observed patterns including the positions of the Arago and Babinet neutral points of the moonlit night sky and sunlit day sky are practically identical if the zenith angle of the Moon is the same as that of the Sun. The biological relevance of the polarization pattern of the moonlit night sky in the polarization vision and orientation of night-active insects is possible.

In our assumption, the Moon, when staying above the horizon, provides insects with guidance of orientation, therefore they avail of light stimuli in the first place to find their bearings in space. In that situation, light trapping is more efficient, as, provided certain conditions are given, the insect might mistake the light of the artificial sources that have been around for only a few millennia for the light of the Moon and therefore will be trapped. As shown in *Table 6*, the height of the Moon above the horizon is in negative correlation with the 3 point moving average of the relative catch. A remarkably strong and significant fallback occurs in the catch of both species when the Moon is 45° above the horizon. If the Moon is observable higher than that, the light-trap will collect few insects. We might try to interpret this observation on the basis of experiments by Baker and Sadovy [7] and Baker [6]. Insects flying on the surface of the ground will see the top of a 360-cm column of light at an angle of 45° from a distance of 3.6m. That distance fits the 3-17m determined by Baker et al. as the distance from which insects react to artificial light. If the insects fly higher than the surface of the ground, they will always see the top of the source of light from an angle less than 45°. So, provided Baker is right, we have an explanation for why the catch is high in the moonlit hours of the night only when the Moon is on the horizon at an angle smaller then 45°, as only then will insects see the top of the trap and the Moon at the same height and only when that happens can they mistake the artificial light for the light of the Moon. So, the critical point in the position of the Moon above the horizon below which we have a high catch and over which we have a low catch is 45°. It remains a problem though that the Kecskemét light-trap was equipped with fluorescent tubes instead of a point like source of light. Because if the height of the Moon above the horizon and its vertical diameter are indeed the most important factors causing confusion, it is not clear how the insect might mistake the column of light for the Moon? Another possible explanation might be this: if the Moon stays low above the horizon, moonlight will penetrate through a thicker layer of air and so its spectral composition shifts to the domain of longer wavelengths. In this case, the light source of the trap may substitute the light waves of shorter wavelengths, to which the insect eye is extremely sensitive, and the catch grows. However, this hypothesis is in sharp contradiction with all the findings we have had so far which appear to confirm that collecting is efficient when the risk of making the mistake runs high. Following from the Mie effect, moonlight gets scattered and consequently polarized much more when penetrating through a thicker layer of air than in the case of arriving in the vicinity of a right angle. This fact, well compatible with our results, may increase the effectiveness of collecting. An analysis of the catch data presented in a study by Szabóky [178] is an interesting contribution to the subject, although it cannot be regarded as decisive evidence in support of our results. The author referred to registers the exact time, with accuracy to the minute, of the landing of 57 specimens of *Anarta myrtilli* L. on the sheet as he was collecting with a 125 W mercury vapour lamp. In 43 cases of the points of time listed, the Moon was not above the horizon and 14 cases were positioned lower than 45°.

Our research into the relationship between the lunar phases and collecting by light-trap have given an answer to the question of what phase angle domains are favourable or unfavourable from the point of view of light trapping the different species. As confirmed in *Figures 1-7*, this has not been observed with any of the species. The Moon has been proved to modify collecting, while it was also established that the various spe-cies display different behaviour in the face of moonlight. Based on more recent research we have been engaged in, we have set up 7 basic types of behaviour:

1. A single explicit catch maximum at full moon or directly after.
2. High catch from the first to the last quarter, not falling back at the time of the new moon.
3. Two nearly identical catch maximums in the first and last quarters.
4. Two catch maximums, the stronger of the two observed in the vicinity of the first quarter.
5. Two catch maximums, the stronger of the two observed in the vicinity of the last quarter.
6. A single catch maximum in the first quarter.
7. A single catch maximum in the last quarter.

The influence of the Moon on light trapping varies by species, a fact that cannot be explained by either the degree of taxonomic relationship, or by the difference in the swarming periods. It is quite likely that the various species respond in different ways to the optical characteristics of the Moon, which have their maximums always in different phase angle groups. For instance, in Hungary, in the hours of light trapping (6 p. m. – 4 a. m. UT), light intensity and the duration of the stay of the Moon above the horizon is the longer in the 2nd phase angle group following the full moon, while the extent of polarization is the highest in the first and last quarters. On the other hand, the colour temperature of a regular light-trap (2900 °K) comes closest to the colour temperature of the Moon (4100 °K at full moon according to Bernolák [12]) between the first and last quarters and the new moon (-10 and 11 phase angle group) (Nowinszky et al. [131]). Although the optical characteristics presumably exert their influence in their complexity on the insects collected, individuals of the various species might react to the various features in different ways.

The sensitivity to light of the species in first type is the highest at the time of the full moon, or directly after in the +1, or +2 phase angle group. Danthanarayana [42] also reported on the catch maximums observed on such occasions. However, this group contains only a small proportion of the species studied so far. Most species examined display strongly slackening activity at the time of the full moon. Moonlight is unpolarized at the full moon, while negative polarization can be observed directly before and after, the period in which the colour temperature of the Jermy-type light-traps comes closest to that of the Moon. It is in this latter fact that can be a probable reason for the maximum observed at this time.

In the type two, catching maximum can be seen at the time of last quarter, new moon and first quarter, and deep minimum at full moon.

It is a common feature of the last five types that one or two distinct catch maximums are observed in the vicinity of the first and/or last quarters. The two maximums might be of the same size, but one might be significantly higher than the other. With these types positively polarized moonlight probably has a positive influence on activity. However, it happens in several cases that the maximum is not exactly in the phase group containing the first and the last quarters, instead, gets somewhat shifted in the direction of the new moon, non the less, in this case, too, there is a remarkable fall-back in the volume of catch at the time of the new moon.

Based on the findings of other scholars as well as our own investigation we take it as confirmed that neither the smaller size of a collecting area nor reduced flying activity are reasons of general validity in way of explaining the influence of the Moon on light-trap effectiveness.

Changes of the area of collecting cannot explain with general validity the differences in the catch results related to the lunar phases. Although, beyond doubt, the illumination generated by the Moon reduces the area of collecting, yet, this fact can have significance only exceptionally from the point of view of light-trap effectiveness. Because in this case should be observe a single catch maximum, and that at the time of the new moon. But as demonstrated the catch data, this is typical of but a few species. A catch maximum is observed with most species in the first and/or last quarter. Hourly collecting data, too, did not make any decline of the catch in relationship with the size of the collecting area apparent in moonlit hours. Therefore a smaller collecting area cannot be regarded as a general reason for the more moderate catch experiences in the vicinity of the full moon. So, regarding the majority of species, the influence of the Moon manifests itself not only through modifications of the area of collecting.

Moonlight does not reduce the flight activity of the insects either. For the catch maximum in the case of most species occurs in the vicinity of the first or the last quarter, or most often, in the neighbourhood of both. So moonlight increases instead of decreasing activity and the catch. It is quite remarkable; on the other hand, that in the vicinity of the full moon, i.e. at the time of negative polarization, there is a clearly distinguishable catch minimum with most species. The negative polarization reduces the quantity of insects collected by light-trap. Had moonlight, regardless of its phase at the time, reduced the activity of insects, there would be no catch maximum in the first and last quarters either. Collecting with other methods by the researchers quoted above do not confirm either the slackening of flight activity at the time of a full moon.

In the absence of traps in high positions, there has been no Hungarian research to confirm or refute the theory expounded by El-Ziady [57] who claims that insects fly in higher layers of the atmosphere at the time of the full moon, although this phenomenon could be detected in the case of some migratory species. Admittedly, the corrected catch results of the silver Y moth (*Autographa gamma* L.) were good also at full moon in the Jermy type traps operating at a height of 2m.

At the time of the full moon – both when there is negative polarization and when there is no polarization – the Moon steps up flight activity. The low catch results observed with most species at this time are related to the insects' security of orientation. Our own findings appear to confirm the theory of Jermy [88] who has been assuming that moonlight might increase the security of orientation, at the same time revealing the difference following from the positive and negative polarization of moonlight. We presume that at the time of positive and negative moonlight polarization, insects rely for orientation primarily on light stimuli, while in the vicinity of the full moon, probably owing to the presence of negative polarization, the difference some species perceive between moonlight and artificial light is bigger than in other lunar phases. Therefore while the Moon continues to supply them with information helping their orientation, the risk of a mix-up diminishes, in other words, the security of orientation increases and the catch falls back. This theory is confirmed by an observation made by Cleve [35] who found that insects fly from one light-trap to the other, but very rarely fall into the trap at the time of the full moon. Other species, on the other hand, can be effectively collected also at times of negative polarization. For some, so far unknown, reason the security of orientation does not increase for them (Nowinszky [118]).

Light trapping has been taking place at the most different geographical locations, under changing weather and light conditions and in different seasons. Also, researchers have been collecting different species, flying at varying hours of the night with light-traps of different types. It is possible therefore that the contradictions manifest in the findings are only apparent and further research on the most important questions may lead to new results to be used with benefit in prognostics in the foreseeable future.

At the same time, our latest findings have also supplied us with fresh evidence to prove that despite several decades of research, the influence of the Moon on light trapping has to this day remained one of the most complex and least known problems.

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OPTIMUM MOISTURE REQUIREMENT DURING VERMICOMPOSTING USING PERIONYX EXCAVATUS

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Abstract. A study was conducted to evaluate the ash content as a function of time of vermicomposting and the moisture content which plays a significant role in the process. The data revealed a linear relationship between the ash content and time in the effective range of the vermicomposting duration. The model parameters of this linear relationship also have linear correlation with the moisture content. Through substitutions, a generalized predictive model for the ash content has been evolved in terms of the duration of vermicomposting and the moistures contents. A plot of the predictive and experimentally observed values indicated a high robustness of predictive model. The study also showed that a moisture content of 80% is optimum for stabilization of waste in minimum processing time.

Keywords: *earthworm, ash content, moisture content, waste management, modeling*

Introduction

Vermiculture technology is emerging as a potential alternative for organic solid waste management. It is an attractive and economical recycling process for treatment of nontoxic organic solid wastes as little capital and energy is needed for the process. Moreover, the waste is converted into vermicasting (vermicompost), a manure rich in plant nutrient [2, 3, 13, 24, 25] to be used in agriculture and horticulture.

Advancement in vermiculture technology requires a detailed understanding of functioning of the system. There are still many fundamental aspects, which need to be studied for the success of vermicomposting process. There is abundant literature available on the response of earthworms in the stabilization of organic wastes but not much literature is available on the effect of moisture content on vermicomposting. In this paper, the effect of moisture content variation using epigeic (surface burrowing) earthworm (*Perionyx excavatus*, Perrier, 1872, family: Megascolecidae) to predict optimum moisture requirement during vermicomposting is investigated.

Review of Literature

Grant [8] reported that water constitutes 75-90% of the body weight of earthworms. Therefore, prevention of water loss is essentially required for survival of earthworms. Nevertheless, they have considerable ability to survive adverse moisture conditions; if they can not avoid dry soil they can survive with the loss of a large part of total water content of their bodies [5]. *Lumbricus terrestris* can lose 70% and *Allolobophora chlorotica* 75% of their total body water and survive [22]. Gerrard [7] reported that some species can withstand dry conditions better than others. Olson [20] reported that the largest numbers of earthworms occurred in soils containing moisture between 12% and 30%. Duweini and Ghabbour [4] reported that in soils with 5-85% gravel and sand, an increase in moisture content from 15% to 34% was usually associated with an increase in numbers, but above 34% extra moisture had no effect. Madge [16] reported that soil with a moisture content of about 23.3% appeared to be optimum for earthworms to produce casts.

Viljoen and Reinecke [26] reported that moisture level of 80% was most favorable for *Eisenia eugeuiae* in waste management. Edwards and Bater [6] reported that optimum moisture content for growth of *E. fetida*, *Dendrobaena veneta*, *E. eugeniae* and *P. excavatus* was 85% in organic waste management. Muyima et a1. [19] reported that optimum moisture content for growth and maturation of *D. veneta* in waste management was 75%.

Materials and Methods

All the experiments were performed out in truncated porous earthen pots of approximately 8-liter capacity. The pots were initially filled to a 2.5 cm height with 12.5 mm nominal size chips of stone (aggregates), which was then covered with 2.5 cm thick layer of 1-5 mm size gravel to ensure proper drainage of excess water. A layer of local soil mixed with cow dung humus in 1:1 ratio of 2.5 cm thickness was used above the gravel bed to provide natural habitat to the earthworms. The experimental pots were kept in the laboratory.

Mixed vegetable residues (organic waste) collected from hostel kitchen were vermicomposted with 40 local adult (clitellate) epigeic earthworms (*Perionyx excavatus*) for the purpose of vermicomposting. The earthworms were introduced to prepared pots one day prior to feeding of waste into the pots. This was done with a view that earthworms could acclimatize into the new environment and settle themselves in the new habitat. The organic waste (substrate) having initial moisture content 78% was then top feeded (10 cm thickness) into different experimental pots. The pots were maintained at varying moisture content of 40%, 50%, 60%, 70%, 80% and 90% respectively to ensure the effect of the moisture content variation. Assessment of maintaining stated moisture content was done by several trials before starting the actual experimentation. The amount and interval of water sprinkling required to maintain the desired moisture content were obtained thereby.

A control (of 10 cm waste thickness) was maintained without earthworms at 70% moisture level. The substrate thickness was restricted to 10 cm to ensure the maintenance of aerobic condition. All the experiments were performed in replicates. Regular water sprinkling was done in all the experimental pots including in the control pot in such a way as to ensuring the maintenance of approximately 40%, 50%, 60%, 70%, 80% and

90% moisture level, to account for the loss of water due to evaporation and drainage. Water sprinkling in different pots, except for 80% and 90%, was started after they attained the said moisture level. The experiment was carried out till 45 days. The substrate samples were drawn on 3 days interval up to 30 days and thereafter 5 days interval up to 45 days from all the experimental pots. Moisture analysis was carried out by drying the samples in a hot air oven at 105 °C for 24 hours. Ash content was determined by heating the moisture-free samples in a muffle furnace at 550 °C for 4 hours. The percent ash content on dry basis was computed and sample mean was used for analysis.

Results

The observed values of ash content (dry basis) for control of the substrate having varying moisture contents of 70% in the control (i.e., without earthworms), and 40%, 50%, 60% 70%, 80% and 90% with 40 number of initial earthworms in each test run are presented in *Table 1.*

Table 1. *Variation of Ash content (% dry basis) with different moisture contents during Vermicomposting.*

Day	**Moisture Content**						
	Control (70%)	**40%**	**50%**	**60%**	**70%**	**80%**	**90%**
0	13.2	13.2	13.2	13.2	13.2	13.2	13.2
3	14	13.8	13.8	13.9	14	14	14
6	14.7	14.7	14.7	14.9	15.1	15.2	14.8
9	16.8	16.2	16.8	17.6	17.7	17.8	17.4
12	18.9	18.5	18.7	19.4	19.8	20.2	19.4
15	22.1	19.8	20.3	22.1	22.7	23.2	21.6
18	24.8	21.3	22.4	24.9	26.2	27.2	24
21	26.1	23.2	24.6	27.8	29.8	30.7	26.2
24	29.2	25	25.8	30.6	33.5	34.6	28.6
27	31.9	26.8	27.3	33.8	36.7	38.9	33.4
30	34.2	28.2	29.7	34.9	40.2	42.8	37.2
35	36.7	30.1	31.9	38.4	44.1	46.7	40.1
40	39.6	33.1	34.9	43.6	49.6	50	43.6
45	41.8	37.1	39.2	48.7	51.2	51.6	46.7

Analysis

The ash content (α) variations with respect to time (T) of stabilization (9 to 35 days only) values are plotted as follows (*Fig. 1*).

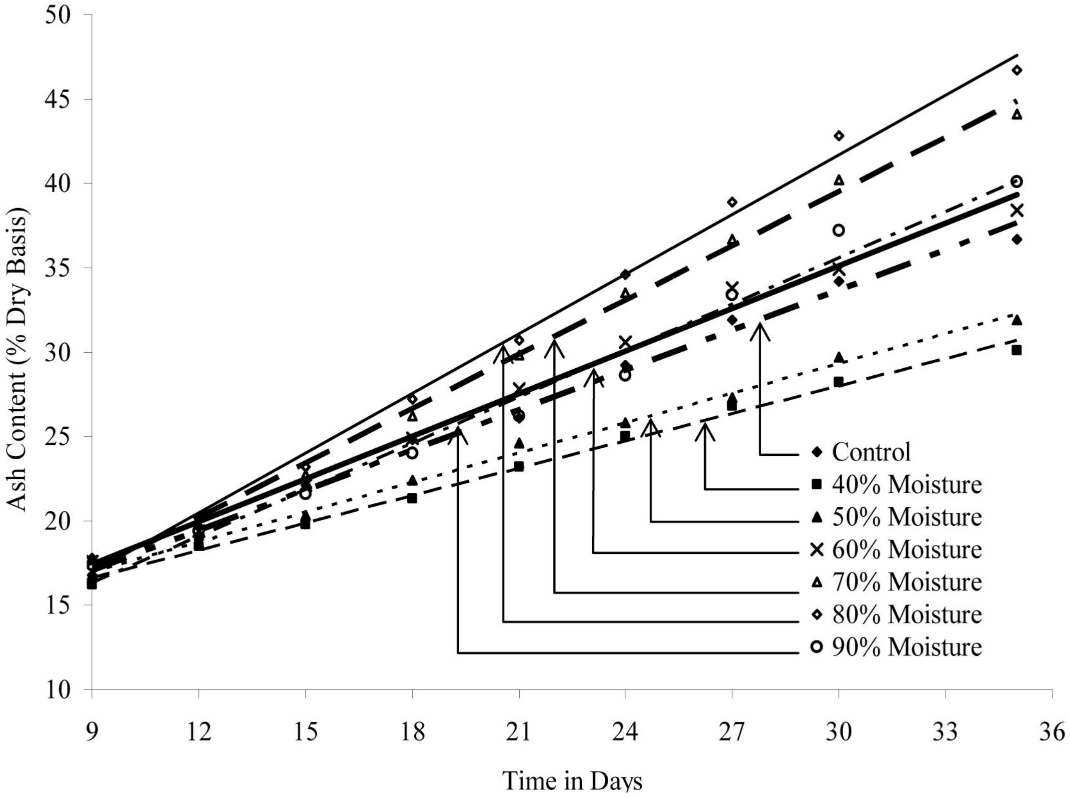

Figure 1. *Variation of ash content with different moisture contents during vermicomposting.*

It may be noted that the α values of initial phase of vermicomposting were left out as there was negligible increase in the ash content manifesting a lag phase and acclimatization stage. Similarly, α values beyond 35 days were left out as the ash content (the measure of degradation) tended to be stabilized depicting not so significant rate of increase. Therefore, data set corresponding to 9th to 35th day was used for modeling purposes, and to establish a possible correlation between α and T. The lines of best linear fits (using least square regression technique) in respect of each of the various moisture contents are also shown (*Fig. 1*). All the stated curves follow linear relationships shown in Eq. 1 having R^2 values of 0.993, 0.9946, 0.963, 0.9924, 0.9959, 0.9948 and 0.9841 respectively for the control (without earthworms at 70% moisture content), 40%, 50%, 60%, 70%, 80% and 90% moisture contents sets.

$$\alpha = mT + c \qquad \text{(Eq. 1)}$$

In Eq. 1, m and c are model parameters. The values of the model parameters m and c for all the treatments are presented in *Table 2*.

Table 2. *Model parameters m and c variation with respect to different moisture contents.*

Moisture content	**Model parameter**	
	m	**c**
Control (70%)	0.7918	9.9401
40%	0.5425	11.72
50%	0.5881	11.687
60%	0.8414	9.865
70%	1.0723	7.3205
80%	1.1788	6.3282
90%	0.9168	8.0889

The values of m and c (excluding the data for the control, 40% and 90% moisture content runs), when regressed (using least square regression technique) with respect to the moisture content (w as percentage) also followed linear trends as shown in following figures (*Fig. 2 and Fig. 3*).

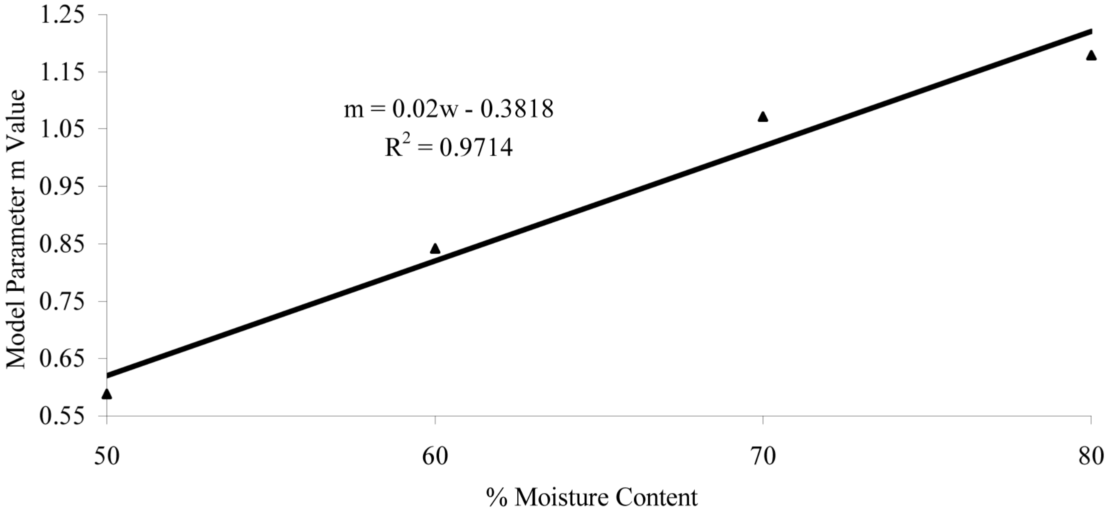

Figure 2. *Model parameter m variation with respect to moisture content.*

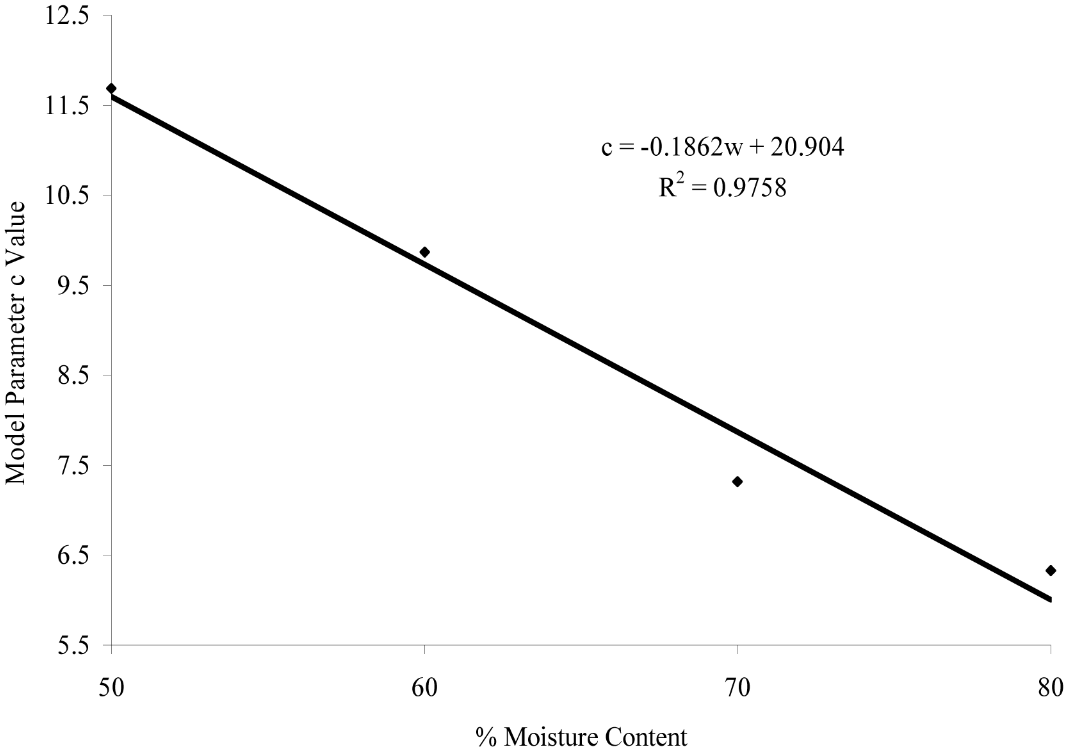

Figure 3. *Model parameter c variation with respect to moisture content.*

The reasons for excluding the control, 40% and 90% values were that the control run was without any earthworms and the 40% moisture content experimental set showed almost negligible earthworm activity while the substrate having 90% moisture content turned anaerobic. The respective expressions are shown in Eq. 2 and Eq. 3:

$$m = 0.02\ w - 0.3818\ (R^2 = 0.9714) \qquad \text{(Eq. 2)}$$

$$c = -\ 0.1862\ w + 20.904\ (R^2 = 0.9758) \qquad \text{(Eq. 3)}$$

By substitution of the m and c values shown respectively in Eq. 2 and 3 in Eq. 1, a generalized model for α as a function of time and moisture content is obtained as shown in Eq. 4:

$$\alpha = (0.02w - 0.3818)\ T - 0.1862w + 20.904 \qquad \text{(Eq. 4)}$$

The proposed model for predicting α (using Eq 4) at different times of vermicomposting and for various values of the moisture content can be useful in predicting the ash content or the degree of stabilization (at around 45% ash content, almost complete degradation was achieved).

On the basis of the above stated generalized and predictive model, the ash content values were computed for comparison with the observed experimental values. The α computed deviated by about 3% from the experimentally observed values. α (experimental) and α (computed) values are plotted as a scatter diagram (*Fig. 4*).

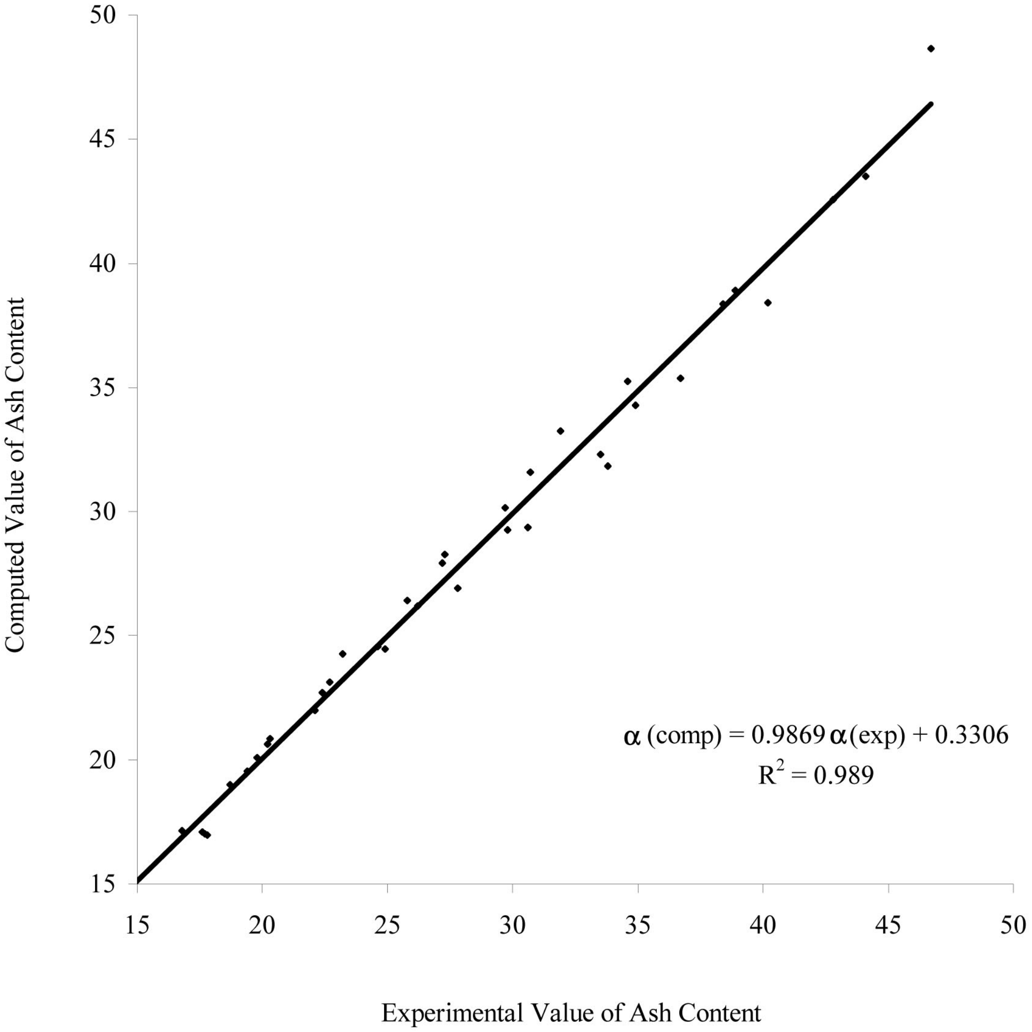

Figure 4. *Comparison of computed and observed data of ash content with different moisture contents.*

Using the least square regression technique, it yielded into a linear fit having a R^2 value of 0.989, which not only validated the proposed universalized model, but manifested the robustness of the presented predictive model shown in Eq. 4.

Discussion

The ash content of the substrate during vermicomposting was measured with a view to assess the degree of substrate stabilization as has been reported to be a good indicator of degradation and mineralization [12, 14, 17, 25]. Ash content increased slightly up to 6 days of vermicomposting in all the above stated runs. This is probably due to the acclimatizing effect. The increase in the ash content was observed to be faster thereafter, which can be ascribed to increased microbial activity after a lag phase (representing the acclimatizing phase) of the microbial growth [18]. The faster rate of increase in ash content can also be the result of faster consumption of substrate by earthworms due to an increased palatability of waste after initial decomposition [5]. Faster rate of increase in ash content indicated the higher rate of volatilization, which is a good measure of degradation of the organic waste. The experimental set having 90% moisture content turned anaerobic on 12th day of vermicomposting. The probable reason of anaerobicity can be the presence of excess water which restricted the air movement through the available pore spaces in the substrate. The development of anaerobic conditions [15, 23] and leachate production [11] in high moisture levels during aerobic composting have also been reported earlier. The ash content increased with the increasing moisture content (40% to 80% range). However, the rate of ashing in the substrate having 90% moisture was observed to be slower as compared to the substrates having 60%, 70% and 80% moisture contents. This lower rate of increase in ash content however, is attributed to negligible earthworm activity due to anaerobicity. The observation does not therefore match with the findings of Edwards and Bater [6] who have recommended moisture content up to 90% for use in waste management using *E. fetida.*

Similarly, the substrate having 40% moisture content showed minimum increase is ash content, which can be the result of less earthworm and microbial activity due to lesser moisture content as the earthworms also restricted their activity and kept themselves within the bedding. They were not seen inside the substrate, processing the waste during entire period of observations, while in other experimental sets they were seen inside the substrate, processing the waste during sampling and water sprinkling. The earthworms require sufficient level of moisture for normal activities as also, the water constitutes 75 to 90% of their body weight [8]. This may be the reason for keeping themselves in the bedding. Decreased microbial activity in lesser moisture content has also been reported during aerobic composting [11, 23].

The substrate having 40% and 50% moisture content showed very slow decomposition (as depicted from the values of ash content at different periods). Even after 45 days of vermicomposting, the degradation process of the waste continued in them. In contrast, the control (i.e. without earthworms at 70% moisture content) showed faster rate of mineralization (as manifested from the ash content) when compared to substrates having 40% and 50% moisture contents. The lesser increase in ash content and simultaneously, lesser decomposition can be ascribed to lesser assimilation by microbial population as well as due to lesser palatability of the substrate to earthworms.

The substrates having 70% and 80% moisture content showed faster increase in ash content and depicted the higher rate of volatilization/degradation. However, substrate having 80% moisture content showed fastest rate of volatilization and simultaneous four fold (approximately) increase in ash content from 13.2% to 51.6%. Six folds increase [10]; three and half folds increase [25]; two folds increase [14]; and two folds increase [1] have already been reported during vermicomposting. Further, almost complete degradation was observed in 35 days of vermicomposting resulting in 44.1% and 46.7% ash content in the substrates having 70% and 80% moisture contents respectively. This indicates the need of maintaining sufficient level of moisture content during vermicomposting. It is thus depicted that approximately 80% moisture is optimum for quicker stabilization of the organic solid wastes using *P. excavatus.* Similar results have been reported by different workers using different epigeic earthworm species in waste management [6, 9, 19, 21, 26, 27].

The study reveals that the ash content is greatly influenced by the moisture content of the substrate and varies with time. The predictive model expressing the ash content as a function of the moisture content and the time of vermicomposting can be used for determining the processing time to achieve the desired level of ash content at specific moisture content. The model's predictive values of the ash content are within about 3% of the observed values. The model thus fully justifies it robustness.

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IDENTIFICATION AND CHARACTERIZATION OF RUBBER DEGRADING ACTINOBACTERIA

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Abstract. Of a great concern, the huge amount of waste rubber materials can cause environmental problems. Various methods have been proposed to solve this proplem. One of those is the biodegradation of it by microorganisms. Bacteria able to degrade and use natural rubber latex as the sole source of carbon and energy were isolated from different ecosystems. 42 rubber-degrading bacteria were isolated. Out of these isolates, 31 were identified as Streptomyces, 5 as Micromonospora, 3 as Actinoplanes, 2 as Gordona and 1 as Nocardia species. All rubber-degrading isolates were identified as members of Actinobacteria which is a large group of mycelium forming Gram-positive bacteria. Interestingly no Gram-negative bacteria could be isolated.

Keywords: *Streptomyces, Micromonospora, rubber degradation, isolation*

Introduction

Waste rubber is becoming a world wide waste disposal problem [13]. One particular concern is used it because of the huge number of natural rubber product produced and discarded annually and of potential environmental hazard should a NR stock pile catch on fire [14]. Consequently, it is very important and worth trying to develop a microbial process for waste NR disposal [6]. Natural rubber is consisting mainly of cis-1,4-polyisoprene and it is synthesized by more than 2000 plant species belonging mostly to the Euphorbiaceae. NR is still produced in large amounts (~ 10^7 tons / year) from the rubber tree *Hevea brasiliensis.* The cis-1,4-polyisoprene, with an average molecular mass about 10^6 Da, is the main constituent (> 90% of dry weight) of NR. NR is relatively resistant to microbial decomposition compared with many other natural polymers. Since the study by Sohngen and Fol, many reports have been published on the biodegradation of natural rubber by microorganisms [15, 10, 19, 18, 12 and 20]. NR contains a minimum of 90% rubber hydrocarbon together with small amounts of proteins, resins, fatty acids, sugars, and minerals [23]. Organic impurities in the rubber can support microbial growth [2 and 22]. Although many studies have been issued during the last decades on microbial degradation of rubber, only little is known about the occurrence of NR-degrading bacteria. Actinomycetes were almost the only organisms able to considerably decompose NR and use the hydrocarbon as a sole source of carbon and energy [4].

The present study was initiated to isolate and characterize a number of NR-degrading bacteria from various ecosystems in Egypt. It also suggests that rubber-degrading bacteria might be useful for the disposal of discarded rubber products. Identification and development of rubber metabolizing microorganisms potentially could provide a biotechnological solution to this problem.

Materials and Methods

Sampling sites

This study has concentrated on isolation of NR degrading actinobacteria from some localites in Egypt (*Fig 1*). The sampling sites were collected from various ecosystems (soils and fresh water from the River Nile as well as its bottom sediments).

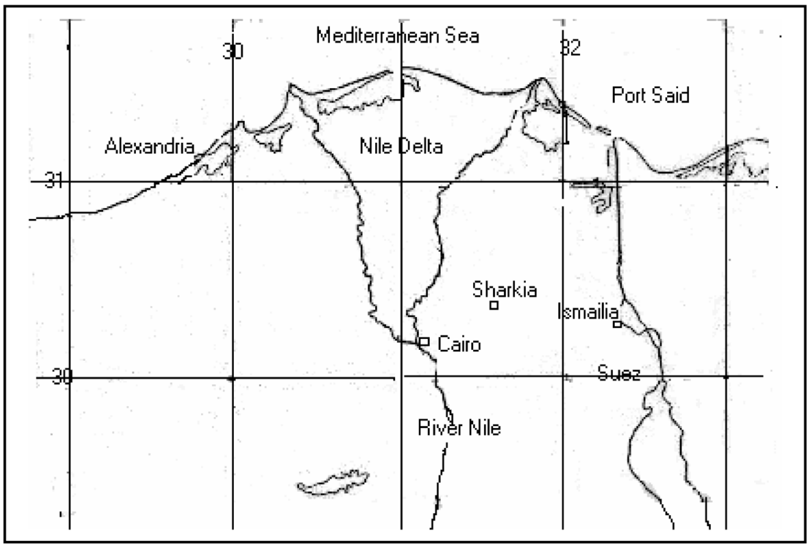

Figure 1. *Location map*

Natural rubber source

Latex of *Hevea brasiliensis* was obtained from Weber and Schaer (Hamburg, Germany).

Isolation and Identification of NR-degrading bacteria

Microorganisms were isolated on mineral salts medium {8.0 g K_2HPO_4, 1.0 g KH_2PO_4, 0.5 g $(NH_4)_2$ SO_4, 0.2 g Mg SO_4x$7H_2O$, 0.1g NaCl, 0.1g Ca $(NO_3)2$, 20 mg $CaCl_2$x$2H_2O$, 20 mg of $FeSO_4$x$7H_2O$, 0.5 mg Na_2MoO_4xH_2O and 0.5 mg $MnSO_4$ / L of deionised water} containing 25 to 100 mg of yeast extract and 20 g of agar / L that had been surface coated with a thin film (20-30 mg) of natural rubber. A hexane solution was also applied at the same medium and it was allowed to evaporate under a microbiological hood. Samples from different localities of Egypt from various ecosystems (soils, fresh water and their bottom sediments) were collected. The samples were serially diluted with sterile mineral medium and spread onto mineral plates coated with rubber and hexane and incubated for several weeks at 28 °C. The observed colonies were streaked onto the same rubber-coated plates until pure cultures were obtained.

Taxonomic characterisation of isolates

The isolates were characterised according to [1, 21 and 17] schemes based on their macro- and micromorphological properties. Adequate phenotypical tests set and chemotaxonomical investigations were used for the identification of strains including colony and micromorphological characteristics, pigment production tests, whole cell sugar pattern, cell wall chemotype, lecithinase, lipolysis, proteolysis, hydrolysis of pectin, chitin, hippurate, casein, esculin, gelatine, degradation of xanthine, elastein, arbutin, utilization of sucrose, m-inositol, mannitol, L-rhamnose, raffinose, d-ribose, salicin, glucose, arabinose, fructose, xylose, galactose, nitrate reduction and H_2S production. The SPSS for Window release 6.0 statistical software has been used for clustering of the isolates, similarity calculations were based on simple matching coefficient (S_{SM}; [16]). The results obtained were further evaluated using the above mentioned different systematic and determinative bacteriological manuals.

Qualitative assay method

Sugars (glucose, fructose, arabinose, sucrose, xylose, inositol, mannitol, rhamnose and raffinose) were sterilised by membrane filtration. 5% of each sugar was supplemented to the same mineral medium. Development or absence of the clearing zone formation was recorded.

Results and discussion

Isolation of NR-degrading bacteria

Ten samples from different ecosystems were screened for the presence of NR-degrading bacteria. 42 NR- degrading bacteria were isolated. They were identified as indicated by (i) size of the colonies developed on solid medium with purified NR latex as the sole source of carbon and energy in comparison to control a plate without NR and (ii) the appearance of translucent halos around the colonies. Interestingly all NR-degrading isolates belonged to the Actinobacteria and no Gram negative bacteria were isolated. Such results are in accordance with those reported by [4 and 20]. The first example of a Gram-negative rubber degrading bacterium, a Xanthomonas species, was reported by [19]. Linos et al. reported a new Gram- negative bacterium species namely *Pseudomonas aeruginosa* for NR-degradation [11]. However, our results cannot exclude NR-degradation capabilities encoded by Gram-negative bacteria in general. Potential Gram-negative NR-degraders might just require additional growth factors or degrade NR by co-metabolism.

Characterisation of NR-degrading bacteria

The isolates were divided into two main groups based on their colony morphology: the polysporic “streptomycetes” and the monosporic or non sporulating “other actinobacteria”. The members of the first group (31 strains) the isolates produced a yellow to grey coloured aerial mycelium with rectusflexiblis or spiral spore chains. The streptomycete isolates were identified as *Str. griseus*, *Str. rochei*, *Str. coelicolor* and *Str. Halstedii* according to the identification schemes of [21 and 17] and taking into consideration the scheme of Bergey’s Manual. Only 5 isolates were Streptomyces sp. due to low similarity indices (*Table 1*).

Members of the “other actinobacteria” group could be divided into 4 genera. The analysis of the first clusters (5 strains) showed morphological features such as well-developed, branched, septate mycelium with a diameter about 0.5 µm, and non-motile single spores, which were characteristic to the genus Micromonospora according to [8]. The members of this cluster showed an orange (young cells) to black–coloured (old cells) substrate mycelium phenotype, and no aerial mycelium was formed. After subse-

Table 1. *Phenotypic and metabolic properties of NR-degrading actinobacteri.*

Strain No.	Source	Species	NR	Hexadecane	Glucose	Fructose	Arabinose	Sucrose	Xylose
NR1	S	Str. griseus	+	-	+R	+	-	-	±
NR2	S	Str. griseus	+	-	+R	+	-	-	±
NR3	S	Str. griseus	+	-	+R	+	-	-	±
NR4	S	Str. griseus	+	-	+R	+	-	-	±
NR5	S	Str. griseus	+	-	+R	+	-	-	±
NR6	S	Str. griseus	+	-	+R	+	-	-	±
NR7	FW	Str. griseus	+	-	+R	+	-	-	±
NR8	FW	Str. griseus	+	-	+R	+	-	-	±
NR9	FW	Str. griseus	+	-	+R	+	-	-	±
NR10	FW	Str. griseus	+	-	+R	+	-	-	±
NR11	FWD	Str. griseus	+	-	+R	+	-	±	±
NR12	S	Str. rochei	+	-	+R	+	+	-	+
NR13	S	Str. rochei	+	-	+R	+	+	-	+
NR14	S	Str. rochei	+	-	+R	+	+	-	+
NR15	FW	Str. rochei	+	-	+R	+	+	-	+
NR16	FW	Str. rochei	+	-	+R	+	+	±	+
NR17	FWD	Str. rochei	+	-	+R	+	+	-	+
NR18	FWD	Str. rochei	+	-	+R	+	+	-	±
NR19	S	Str. coelicolor	+	-	+R	+	+	-	-
NR20	S	Str. coelicolor	+	-	+R	+	+	-	-
NR21	S	Str. coelicolor	+	-	+R	+	+	-	-
NR22	S	Str. coelicolor	+	-	+R	+	+	±	-
NR23	FW	Str. coelicolor	+	-	+R	+	+	-	-
NR24	FW	Str. halstedii	+	-	+R	+	+	±	-
NR25	FWD	Str. halstedii	+	-	+R	+	+	-	-
NR26	FWD	Str. halstedii	+	-	+R	+	+	-	±
NR27	S	Streptomyces sp.	+	-	+R	+	+	-	+
NR28	S	Streptomyces sp.	+	-	+R	+	+	-	+
NR29	S	Streptomyces sp.	+	-	+R	+	+	-	+
NR30	FW	Streptomyces sp.	+	-	+R	+	+	-	+
NR31	FWD	Streptomyces sp.	+	-	+R	+	+	-	+
NR32	S	Micromonospora aurantiaca	+	-	±R	-	+	-	-
NR33	FW	Micromonospora aurantiaca	+	-	±R	±	+	±	±
NR34	FWD	Micromonospora aurantiaca	+	-	+R	±	+	±	±
NR35	FWD	Micromonospora aurantiaca	+	-	+R	±	+	±	±
NR36	FWD	Micromonospora aurantiaca	+	-	+R	±	+	±	±
NR37	FW	Actinoplanes italicus	+	-	±R	-	+	±	±
NR38	FWD	Actinoplanes italicus	+	-	+R	+	+	+	±
NR39	FWD	Actinoplanes italicus	+	-	+R	+	+	+	±
NR40	S	Gordona sp.	+	-	+R	±	+	+	-
NR41	FW	Gordona sp.	+	-	+R	±	+	+	-
NR42	S	Nocardia sp.	+	-	+R	±	+	-	-

S: Soil, FW: Fresh water, FWD: Fresh water sediment +: good growth/halo formation
±: poor growth/halo formation -: same growth as on mineral medium without carbon source/no halo
RF: spore chain rectusflexibilies SP: spiral spore chain R: repression of natural rubber degrading activity

Cont.

Strain No.	Source	Species	Inositol	Mannitol	Rhamnose	Raffinose	Aerial mycelium	Substrate mycelium	Spore chain
NR1	S	Str. griseus	-	+	-	-	yellow	light brown	RF
NR2	S	Str. griseus	-	+	-	-	yellow	light brown	RF
NR3	S	Str. griseus	-	+	-	-	yellow	light brown	RF
NR4	S	Str. griseus	-	+	-	-	yellow	light brown	RF
NR5	S	Str. griseus	-	+	-	-	yellow	light brown	RF
NR6	S	Str. griseus	-	+	-	-	yellow	light brown	RF
NR7	FW	Str. griseus	-	+	-	-	yellow	light brown	RF
NR8	FW	Str. griseus	-	+	-	-	yellow	light brown	RF
NR9	FW	Str. griseus	-	+	-	-	yellow	light brown	RF
NR10	FW	Str. griseus	-	+	-	-	grey	light brown	RF
NR11	FWD	Str. griseus	-	+	-	-	grey	light brown	RF
NR12	S	Str. rochei	+	+	+	-	grey	dark brown	SP
NR13	S	Str. rochei	+	+	+	-	grey	dark brown	SP
NR14	S	Str. rochei	+	+	+	-	grey	dark brown	SP
NR15	FW	Str. rochei	+	+	+	-	grey	dark brown	SP
NR16	FW	Str. rochei	+	+	+	-	grey	dark brown	SP
NR17	FWD	Str. rochei	+	+	+	-	grey	dark brown	SP
NR18	FWD	Str. rochei	+	+	-	-	grey	dark brown	SP
NR19	S	Str. coelicolor	-	+	-	-	yellow	dark brown	RF
NR20	S	Str. coelicolor	-	+	-	-	yellow	dark brown	RF
NR21	S	Str. coelicolor	-	+	-	-	yellow	dark brown	RF
NR22	S	Str. coelicolor	-	+	-	-	yellow	dark brown	RF
NR23	FW	Str. coelicolor	-	+	-	-	yellow	dark brown	RF
NR24	FW	Str. halstedii	+	+	-	-	grey	dark brown	RF
NR25	FWD	Str. halstedii	-	+	+	-	grey	dark brown	RF
NR26	FWD	Str. halstedii	-	+	+	-	grey	dark brown	SP
NR27	S	Streptomyces sp.	+	+	+	-	grey	light brown	SP
NR28	S	Streptomyces sp.	+	+	+	-	grey	light brown	SP
NR29	S	Streptomyces sp.	+	+	+	-	grey	light brown	SP
NR30	FW	Streptomyces sp.	+	+	+	-	grey	dark brown	RF
NR31	FWD	Streptomyces sp.	+	-	+	-	grey	dark brown	RF
NR32	S	Micromonospora aurantiaca	-	-	-	+	-	orange	mono-sporic
NR33	FW	Micromonospora aurantiaca	-	-	-	+	-	orange	mono-sporic
NR34	FWD	Micromonospora aurantiaca	-	-	-	+	-	orange-black	mono-sporic
NR35	FWD	Micromonospora aurantiaca	-	-	-	+	-	orange-black	mono-sporic
NR36	FWD	Micromonospora aurantiaca	-	-	-	+	-	orange-black	mono-sporic
NR37	FW	Actinoplanes italicus	-	-	-	+	-	orange	mono-sporic
NR38	FWD	Actinoplanes italicus	-	-	-	+	-	orange	mono-sporic
NR39	FWD	Actinoplanes italicus	-	-	-	+	-	orange	mono-sporic
NR40	S	Gordona sp.	-	-	-	+	-	orange to red	mono-sporic
NR41	FW	Gordona sp.	-	-	-	+	-	orange to red	mono-sporic
NR42	S	Nocardia sp.	-	-	-	+	-	orange	mono-sporic

quent analysis of this cluster, it could be identified as *M. aurantiaca*. Koch et al. described this bacterium as a new species [9].

The second cluster was identified as Actinoplanes (3 isolates). Colonies had orange colour and produced pink to cherry coloured pigments diffusing into the medium. According to the Bergey's Manual of Systematic Bacteriology, this cluster is *Actinoplanes italicus*.

The third cluster was identified as Gordona (2 isolates). These isolates had rod shaped or coccoid cells. The genus Gordona is assigned to the suprageneric but phylogenetically coherent group of mycolic acid containing Actinobacteria [7]. These two isolates need more molecular analysis to reach the species level.

The last cluster (one isolate) had mycelia that eventually fragmented into rod shaped or coccoid cells. These characteristics class this isolate into the genus Nocardia. The present strain seems to be a newtype strain of the genus Nocardia and it needs more investigation.

This study demonstrates that certain bacteria can use the hydrocarbon of NR as a sole source of carbon and energy. These microorganisms may play an ecological role in the environment by mineralising NR latexes. Although no attempt was made to isolate the Actinobacteria selectively, all of the isolated rubber metabolising microorganisms were identified as Streptomyces, Micromonospora, Gordona and Nocardia. It suggests that degradation of NR is the privilege of mycelium forming microorganisms. Our results are consistent with those indicate that rubber degrading species of these genera are widely distributed in soils, water and fresh and marine sediments [5, 23 and 3]. Some of these isolates may have enough degrading potential that enables biotechnological use particularly in rubber products.

Regulation of NR degradation

The ability of NR-degrading bacteria to use low molecular mass monomers and high molecular mass polymer as carbon sources was tested (*Table 1*). When clearing zone formation was studied on NR plates containing one additional soluble carbon source, evidence for inhibition of NR degrading enzyme expression was obtained. Carbon sources that allow good growth e.g. glucose repressed the NR-degrading enzyme in most strains (*Table 1*). The extent of inhibition varied with strains and substrates. The biochemical mechanism of NR degradation has not been investigated. Since NR is a high molecular mass compound, which is too large to be taken by bacteria, as a first step the polymer has to be cleaved extracellularly. The extracellular nature of such enzyme system was shown by the appearance of translucent halos on latex containing solid media.

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REGULATION OF PHOSPHATASE ACTIVITY IN CHROOCOCCIDIOPSIS ISOLATES FROM TWO DIVERSE HABITATS: EFFECT OF LIGHT, PH AND TEMPERATURE.

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Abstract: Phosphomonoeaterase (PMEase) and phosphodiesterase (PDEase) activity was studied in the cyanobacterial cultures of Chroococcidiopsis isolated from two diverse cryptoendolithic habitats of Antarctic and Arizona. Because this organism is found within the rocks it appears that phosphorus metabolism by alkaline phosphatase activity is a key factor to sustain growth of these organisms in that state, there being no other source of external P except in bound form in the rocks. The main findings in this paper show that specific pH and temperature regulate the PMEase and PDEase activities studied with different substrates in isolated of Chroococcidiopsis 1 and 2 although values were vastly different. The pH and temperature optima for phosphatase activity (PMEase and PDEase) of Chroococcidiopsis 1 and 2 were 9.5, 20 °C and 8.5, 40 °C respectively. It needs mentioning here that although the pH optimum for the enzyme activities in the Antarctic rock samples was the same i.e. 9.5 there was a striking difference in the temperature optimum in which maximum activity of both enzymes were recorded at 5 °C. A very crucial role of light and dark conditions were important for the enzyme activity and differed to a significant extent when compared with naturally occurring organisms in the Antarctic rocks. Low light fluxes of about 8 µmol photon $m^{-2}s^{-1}$ showed higher PMEase and PDEase activity then total dark conditions in the Chroococcidiopsis -1 culture. However under natural conditions when this organism is found within the rocks 8-µmol photon $m^{-2}s^{-1}$ was found to be inhibitory and dark conditions gave higher PMEase and PDEase activity. Arizona rock samples containing Chroococcidiopsis -2 however did not show dark stimulation. Increase in light intensity from 8 µmol photon $m^{-2}s^{-1}$ to 60 µmol photon $m^{-2}s^{-1}$ maintained in the culture room increased the PMEase and PDEase activity of both cultures. The unique light and temperature responses for PMEase and PDEase activities in Chroococcidiopsis-1 found within the Antarctic rocks are unique. It points to some change in the cells probably by producing some cryoprotectant which protects the enzymes from becoming non functional at low temperatures. It also indicates that exposure to light fluxes as low as 8 µmol photon $m^{-2}s^{-1}$ can probably alter the properties of the enzymes thus reducing its activity compared to total darkness.

Keywords: *endolithic, cyanobacteria, phosphomonoesterase, Antarctic, Arizona*

Introduction

The Ross desert of the McMurdo dry valley, Antarctica is a place of extremes with the highest coldest and windiest environments and with very little snowfall most of the continent is technically a desert [23, 26, 25]. Areas of extensive rock exposure represent one of the harshest environments of our planet. Examples of other such extreme habitats are the hot deserts like Arizona USA. The surface of these hot and cold deserts is practically abiotic and communities of indigenous life forms if present are hidden within the cracks and depressions as cryptoendoliths. Microorganisms that occupy a sharply defined zone colonize the communities existing under the surface of the rocks. The dominant organism in these habitats are the cyanobacteria [20, 22, 12, 25]. Among the cyanobacterial species the most dominant form is the unicellular cyanobacterium Chroococcidiopsis, and is found to grow where all other organisms fail to grow and is the most primitive organism [2, 9, 10, 11].

Microbial life in the deserts is poised at the limits of survival. Water is the primary limiting factor in both hot and cold deserts and melting snow is the only source of water in the Antarctic. There are many other physical, chemical and ecological factors, which are influential in maintaining the delicate balance swinging from survival to their establishment or extinction. They survive because they are able to interact within their niche and either exploit its attributes or modify it to make it more suitable [1, 2, 4, 25, 26, 12, 20, 7].

Most studies on nutrient uptake and cycling in endolithic communities of hot and cold deserts have dealt with carbon (3, 19] and there are no reports on phosphorus and nitrogen metabolism. Results on whole communities (rock samples) of the Antarctic [1, 2] showed for the first time low temperature and low light adaptations for the phosphatase enzyme and high rate of P uptake by Chroococcidiopsis, the dominant flora within these rocks. There are however no reports on phosphatase activity of cyanobacterial isolates from endoliths of either the Antarctic or any hot desert. There is almost complete lack of knowledge regarding the ecology, survival mechanisms, physiology, metabolic pathways and applications of these permanently immobilized cells within the rocks. Such microorganisms living inside the rocks share several features with the immobilized cells in those laboratory systems where limited metabolic activity is required [6, 7, 10, 11, 25]

We therefore decided to study the PMEase and PDEase activity of Chroococcidiopsis isolated from two extreme habitats of hot and cold deserts of Antarctic and Arizona using different substrates. Also an attempt has been made to see the regulation of the enzyme activities with differing pH, temperature and light regimes under laboratory conditions and a comparison has been made with natural habitats, which is the author's own work.

Materials And Method

Test Organism and Sampling Sites

Axenic cultures of the endolithic cyanobacterium Chroococcidiopsis the only phototroph present in the rocks of both Antarctic (from Barwick boulder) and Arizona, USA were isolated from the rocks (Courtesy Late Dr. D.D. Wynn Williams and Charles Cockles of British Antarctic Survey and Dr .Bukhard Büdel, Universitat Kaiserslautern, Germany) in the laboratory of Algal Biotechnology, Bioscience Department ,Barkatullah University Bhopal by standard microbiological techniques. The Antarctic sample came from Beacon sandstone boulder in the Linneaus Terrace (McMurdo Dry valley) on the NNE flank of the Apocalypse peak. The hot desert sample came from a sandstone endolith from Arizona Coconino, 20 Km from the Grand Canyon National Park USA. Other details of the rocks relevant to the study are given in *Table 1.*

Table 1. *Some features of the rocks from where the microbial communities were sampled.*

Variable	Antarctic	Arizona
1.Dominant phototroph	*Chroococcidiopsis*	*Chroococcidiopsis*
2.pH	9.2	9.0
3. FRP (μg g^{-1})*	nd	11.36
4. TP (μg g^{-1})**	nd	883
5. TOP (μg g^{-1})***	nd	871
6. Chlorophyll (μg g^{-1})	7.56	9.22

**FRP= Filterable reactive phosphorus*
*** =Total phosphorus*
****= Total organic phosphorus*

Table 2. *Comparison of rates of PMEase and PDEase activities of Chroococcidiopsis 1 using 100 mM of different substrates. Rates are expressed as product (mmol pNP, MU, bis-pNP) released per hour ±SD (n=3). Experiments have been carried out at 20 °C and 275 Lux light intensity. (0 hour activity was 0.21±0.002)*

Time in hours	PNPP	MUP	Bis-pNPP
2 L	7.53±0.48	4.06±0.68	2.86±0.77
2D	3.22±0.12	1.93±0.12	1.86±0.31
4L	15.85±1.13	8.86±1.0	6.88±1.21
4D	7.12±1.11	3.26±0.78	2.79±0.22
8L	22.1 ±3.2	11.98±2.3	9.78±1.67
8D	4.3 ±0.78	2.33±0.88	2.03±0.55
12L	13.66±2.6	13.66±2.6	11.60± 2.01
12D	1.23±0.33	1.23±0.33	0.98±0.02

Culture conditions

The cultures were maintained in modified CHU-10 (CHU10-D) medium as given by, [5] and modified by [13]; P was reduced to $1 mgl^{-1}$, EDTA used as a chelator , and pH buffered to 7.6 with HEPES. Ammonium -N ($1 mgl^{-1}$) was used as the nitrogen source in place of nitrate. Cultures were maintained at 20±2 °C and a light flux of 60-70 µmol photons $m^{-2}s^{-1}$ and a light and dark cycle of 14:10 hrs. The material used for experimental studies was grown for six months under the above-mentioned conditions to acclimatize the organism to its new environment in the laboratory before starting the experiments. Also as these are extremely slow growing organisms this time period was needed to generate the cell mass required for the experiments. One set of cultures was kept at 5 °C and 8 µmol photon $m^{-2}s^{-1}$ in Orbital Shaking Incubator, representing conditions likely to occur in the Antarctic and the other set from Arizona was kept at 40 °C, and 10 µmol photon $m^{-2}s^{-1}$ to ensure that both the isolated organisms could still grow in culture under the probable natural conditions in which they are found. These data have been obtained from scientists who have collected the rock samples. The light source for low light conditions was a 15 W fluorescent Phillips bulb fitted inside the Orbital shaker.

Phosphatase Assay

Phosphomonoesterase (PMEase) activity was assayed routinely using the fluorometric method with the fluorogenic substrate 4-methylumbelliferyl phosphate (MUP) and the calorimetric method using para-nitro-phenyl phosphate (pNPP). Phosphodiestrase activity was assayed using Bis pNPP calorimetrically. The practical details are given in [24, 2].

As the cultures were grown in very low concentrations of P in the medium ($1mgl^{-1}$) initially they were transferred to a P minus medium before the assay for two weeks to deplete the cells of P. Assays were carried out in a P free version of the CHU-10 D assay medium described in [17]. The effect of pH on PMEase activity was carried out in a medium that was buffered to give a range of pH values using 100mM final concentration of DMG (3,3-dimethyl-glutaric acid) for (pH 4.5 -5.5), HEPES (N-(2 hydroxyethyl) piperazine -N’-(2 ethanesulphonic acid) for (pH 6.0-7.5) and glycine for (pH 8.0 -10) for effect of pH on PMEase activity. After buffering, each specific pH from acidic to alkaline range was maintained and checked with a Systronics m pH meter system 361. Different temperature ranges were maintained in a Remi Instrument, temperature controlled Orbital Shaking Incubator and other incubators. Wrapping the universal tubes with aluminum foil created dark conditions. Assays were conducted in a water bath with gentle shaking. Temperature was measured at the beginning and end of the experiments to ensure that there was no difference between light and dark especially for the Arizona samples where high temperatures were involved. A 0.1 mL aliquot of substrate from a stock solution of 600 mmol was added to the universal bottles containing the required cultures to give a final concentration of 100 µM for routine assays. Two controls with assay medium were sampled at each time interval along with the experimental ones: substrate but no alga, and alga but no substrate to ensure the presence of no other source of fluorescence/ colour development. After the assay the samples were passed through a GF/C filter and activity ended by using 10%(v/v) of the correct base/acid terminator. Using excitation at 356 nm, fluorescence emission was measured at 444nm. For spectrophotometric analysis absorbance was measured by recording the optical density in a

Systronic spectrophotometer model-169 at 405 nm The filter paper with the alga was dried for 24 hours in a vacuum oven at 105 °C. The results are expressed as product (MU/pNP,/Bis pNP) formed g^{-1} d.wt h^{-1}. (MU = 4 methylumbelliferone, pNP= paranitrophenol, Bis-pNP=Bis para nitrophenol). All results are mean ± standard deviation of three independent replicates. Individual experiments have been conducted with MUP as the substrate as it is the most sensitive method of phosphatase activity measurement while comparative studies have been conducted with all three substrates.

Phosphate Analysis

FRP (Filterable reactive phosphate-P) was measured using the method in [8]. Total P was also measured after extraction from the rock samples using the digestion mixture as in [8].

Results

Table 1 shows some of the properties of the rock samples from where the two cultures of Chroococcidiopsis were obtained. For ease of description and tabulation the Antarctic culture has been referred as Chroococcidiopsis-1 and the Arizona culture has been referred as Chroococcidiopsis-2 in the rest of the text.

Table 2 shows the comparative time course studies on PMEase and PDEase activity of Chroococcidiopsis -1 using MUP and pNPP as PMEase substrate and Bis-pNPP as PDEase substrate at light low light intensities of 8 mmol photon $m^{-2}s^{-1}$ as probably found in the Antarctic endoliths. Unlike the rocks samples it was found that even this low light intensity was stimulatory for both enzymes compared to dark conditions ($p = < 0.01$). With increase in time the light driven activity increased steadily, while the dark activity decreased drastically after the fourth day. The substrate pNPP gave higher activity of PMEase compared to MUP and PDEase activity was nearly half that of the PMEase activity. *Table 3* shows the comparative PMEase activity and PDEase activity of Chroococcidiopsis 2 using low light intensities as the probable Arizona endolithic conditions. Here similar to the rock samples higher PMEase and PDEase activity was obtained with cultures in low light compared to dark conditions ($p = < 0.05$). The substrate pNPP gave higher activity of PMEase compared to MUP and PDEase activity was nearly half that of the PMEase activity. Experiments were also conducted in normal culture room light (60 mmol photon $m^{-2}s^{-1}$) to study the PMEase and PDEase activities of isolate 1 to see the variations if any in higher light intensities. It was found that the activity increased to a great extent under these conditions in all three substrates use (*Table 4*). Very similar observations were obtained in isolate 2 but here the rates were higher than in isolate 1 (*Table 5*). *Table 6* shows the percent increase of PMEase activity in light and dark of Antarctic and Arizona rocks and cultures of Chroococcidiopsis 1 and 2. Antarctic rocks showed 51.1 percent increase in dark over low light in PMEase activity ($p = < 0.01$). Cultures of Chroococcidipsis 1 however showed higher PMEase activity in low light (53.2 percent) and dark conditions were found to be inhibitory ($p = < 0.01$). For the Arizona rocks and Chroococcidiopsis 2 the percent increase was nearly the same (49.2 and 50.2 percent) in low light conditions compared to dark and dark stimulation effect in rock samples was not documented. *Figure 1* shows the percent increase in PMEase and PDEase activity of Chroococcidiopsis 1 and 2 compared to low light intensities (8 and 60 mmol photon $m^{-2}s^{-1}$) using MUP as a substrate. There was considerable increase in PMEase ($p = < 0.01$) and PDEase ($p = < 0.05$) activity in both isolates with increase in light intensity but it was higher in isolate 2. *Figure 2* shows the percent increase in PMEase and PDEase activity of Chroococcidiopsis 1 and 2 when high light intensities (60 mmol photon $m^{-2}s^{-1}$) are compared with dark conditions using MUP as a substrate. In this case there was greater increase in PDEase activity in both organisms compared to PMEase ($p = < 0.05$).

Figure 3 shows the effect of different pH values on PMEase activity of both cultures using MUP as the substrate. The pH optima were 9.5 and 8.5 for Chroococcidiopsis 1 and 2 respectively ($p = < 0.01$) compared to the acidic range. These values were recorded for the rock samples of the two habitats. *Figure 4* shows the temperature optima of PMEase activity of the two cultures using MUP as substrate. It was 20 °C for Chroococcidiopsis 1 and 40 °C for Chroococcidiopsis 2. *Table 7* shows the comparison for maximum PMEase activity in Antarctic and Arizona rock samples dominated by Chroococcidiopsis values of which are available, Banerjee et al (2000a) and the respective cultures isolated from them with apparent values of K_m and V_{max}. When the pH optima for the enzyme in both the Antarctic and Arizona rocks were compared with Chroococcidiopsis 1 and 2 , the result in organism 1 was nearly the same i.e. 9.5 but it differed slightly in the Arizona rock and organism 2. The temperature optima for maximum PMEase activity of Antarctic rock and culture were however found to be very different with rocks at 5 °C and cultures at 20 °C ($p = < 0.01$). In contrast the values for Arizona rocks and samples were quite similar. From the K_m and V_{max} values it appears that PMEase activity of Antarctic rocks had a very high affinity for substrate while the isolate from it showed a low affinity in spite of the fact that the cultures were grown in very low concentrations of P in the medium in initial stages and then transferred to P minus medium before the assay. Arizona rock and culture showed significant and similar affinity for the substrate.

Discussion

The differing response obtained in cultured Chroococcidiopsis-1 is a very significant observation but it seems unlikely that such marked changes as observed for Antarctic whole cells and cultures in response to temperature and light could occur due to genetic drift. It seems more likely that it is an environmental response occurring within a few cell generations of the organism being removed from the rock and exposed to more favourable conditions. Substantial portions of total P present in rocks or soil system are in organic form and are derived from decaying or lysed microorganisms and comprise large portion of P uptake in such endolithic systems apart from the organic P released from the rocks. Therefore phosphatase activity is the key to metabolism of P for this organism inside the rocks and the same holds good for their cultures. The higher activity of pNPP compared to MUP as a substrate in the present study is probably because it has been shown that hydrolysis of pNPP can be brought about by a range of enzymes that are present in the cells and not just PMEase [16]. The significantly less PDEase activity compared to PMEase activity in this study was similar to results obtained for cultured cyanobacteria [15]. This is probably because the PDEase releases about 25% of the pi from Bis-pNPP compared to that released by pNPP. The monoesterases are capa-

Table 3. *Comparison of rates of PMEase and PDEase activities of Chroococcidiopsis 2 using 100 mM of different substrates. Rates are expressed as product (mmol pNP, MU, bis-pNP) released per hour ±SD(n=3). Experiments have been carried out at 40 °C and 275 Lux light intensity. (0 hour activity was 0.25±0.001)*

Time in hours	**pNPP**	**MUP**	**Bis-pNPP**
2 L	9.12±1.48	3.10±0.65	4.62±0.71
2D	2.26±0.72	1.8±0.17	1.88±0.34
4L	16.12±1.23	6.2±1.05	7.51±1.01
4D	4.98±1.01	2.93±0.87	2.33±0.25
8L	20.06 ±2.9	8.37±1.3	10.24±1.17
8D	3.24 ±0.88	1.06±0.18	1.56±0.45
12L	22.42±3.6	11.35±2.7	11.11± 2.13
12D	1.63±0.35	0.80±0.03	0.94±0.02

Table 4. *Comparison of rates of PMEase and PDEase activities of Chroococcidiopsis 1 using 100 mM of different substrates. Rates are expressed as product (mmol pNP, MU, bis-pNP) released per hour ±SD (n=3). Experiments have been carried out at 20 °C and 2,500 Lux light intensity. (0 hour activity was 0.21±0.002)*

Time in hours	**pNPP**	**MUP**	**Bis-pNPP**
2 L	10.68±1.37	8.9±0.95	4.96±0.91
4L	17.32±1.56	13.3±1.05	8.22±0.99
8L	23.06 ±3.2	18.1±1.3	11.59±1.17
12L	26.22±3.6	20.6±2.6	14.06±2.13

ble of providing the necessary levels of phosphorus to the cells as the substrates are easily available and energetic related to the reactions less compared to diesterases and their activity therefore occurs at higher rates as observed.

The higher percent increase in PDEase activity when effect of high light intensities was compared to dark is probably due to the general observation that PDEase activity was greatly reduced in the dark compared to low as well as high light intensities.

The dark effect and other characteristic such as low temperature adaptation of phosphatase seem to be unique features of Antarctic rocks and are by no means universal. The observation that a flux value as low as 8 µmol photons $m^{-2}s^{-1}$ is inhibitory suggests that even this low value maybe above the optimum not only for PMEase activity of endolithic communities. These organisms might be possessing special accessory pigments for harvesting PAR or the chloroplasts and/or thylakoids of the cryptoendoliths may be morphologically adapted to scavenge the lowest limit of available photons. It is also possible that dark exposure may lead to some change in the wall or enzyme structure which renders the active sites more accessible to the substrate. The precise reason for the difference thermal response of the Chroococcidiopsis –1 and the Antarctic rocks containing the same organism may be due to the ability of naturally immobilized cells in the rocks to undergo a change which protects the enzyme from becoming non functional at low temperatures.

Low temperature may also initiate de novo synthesis of certain proteins that are cryoprotective in nature which safe guards the enzyme from damage caused by freezing or sub freezing temperatures. A suggestion similar to this was given by Guy [14]. The observation with hot desert culture showing high phosphatase activity at temperatures as high as 42 °C focus on the significance of adaptation of these enzymes to higher temperature and reflects the importance of flexibility of extreme values which could denature enzymes in nature.

Table 5. *Comparison of rates of PMEase and PDEase activities of Chroococcidiopsis 2using100 mM of different substrates. Rates are expressed as product (m mol pNP, MU,bis-pNP) released per hour ±SD (n=3). Experiments have been carried out at 40 °C and 2,500 Lux light intensity. (0 hour activity was 0.25±0.001)*

Time in hours	**pNPP**	**MUP**	**Bis-pNPP**
2 L	12.06±1.77	9.38±1.95	6.73±1.91
4L	19.19±2.06	15.19±1.25	9.75±1.59
8L	24.16 ±3.4	19.1±2.3	12.09±1.67
12L	27.28±3.67	20.6±2.8	13.66±2.73

Table 6. *Percent increase in PMEase activity of Antarctic and Arizona rocks and cultures of Chroococcidiopsis isolated from them in two light conditions and dark at 96h. Percent increase has been calculated with light compared to dark and dark compared to light as the case may be and as indicated in the conditions.*

Sample	**Condition**	**Percent increase**
1.Antarctic culture*	Light	53.2
2 Arizona cultures*	Light	50.2
3. Antarctic rock*	Dark	51.1
4. Arizona rocks*	Light	49.2

Table 7. *Comparison of optima for maximum PMEase activity of Antarctic and Arizona rocks and cultures of Chroococcidiopsis isolated from them together with their apparent K_m and V_{max} values. Assay condition 8µ mol photon $m^{-2}s^{-1}$, substrate concentration 100 µM MUP buffered at pH 9.5 by glycine for Antarctic and 10 µ mol photon $m^{-2}s^{-1}$, substrate concentration 100 µM MUP buffered at pH 9.5 by glycine for Arizona.*

Sample	**pH**	**Temperature**	**Km (µM)**	**V_{max} (µ mol MUg^{-1} h^{-1})**
Antarctic rocks	9.2	5 ° C	230.8	0.053
Chroococcidiopsis -1	9.5	20° C	164.1	7.60
Arizona rocks	9.0	42° C	136.0	0.025
Chroococcidiopsis -2	8.5	40° C	132.8	0.021

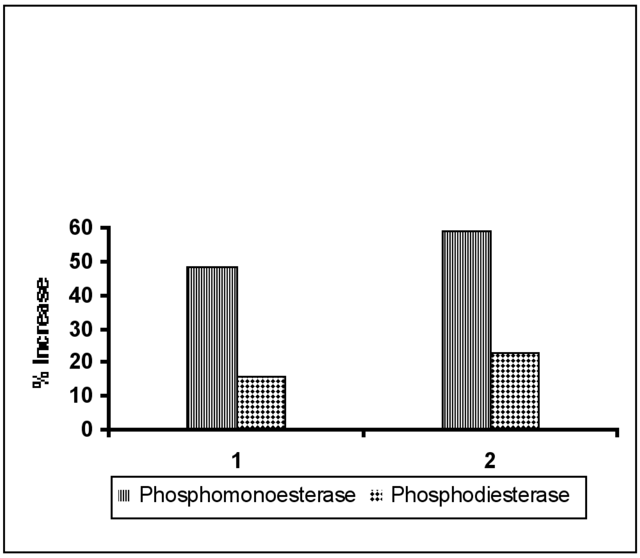

Figure 1. *Percent increase in PMEase and PDEase activity of Chroococcidiopsis 1 and Chrococcidiopsis 2 when high Light intensity are compared to low light intensity (60 & 8 mmol photon* $m^{-2}s\text{-}^{1}$*).*

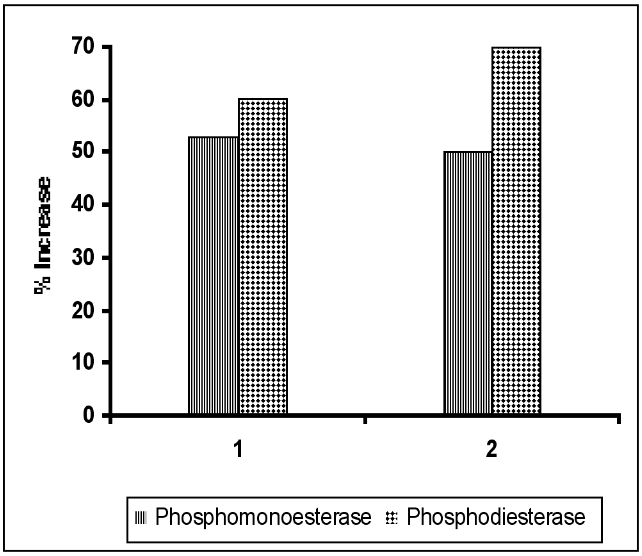

Figure 2. *Percent increase in PMEase and PDEase activity of Chroococcidiopsis 1 and Chrococcidiopsis 2 when high light intensity (60 mmol photon* $m^{-2}s^{-1}$*) are compared to dark conditions.*

Accumulation of disaccharide sugars is common in desert cyanobacteria like Chroococcidiopsis sp. [21] and it is probably this ability that allows not only the naturally occurring organism but also the culture to survive under dark conditions. This phenomenon needs more detailed explanation, not only because of its intrinsic interest but also for understanding the properties of organisms in the Antarctic dry desert so near the limits of life and should prove to be a fertile area for research in the future.

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GEOGRAPHIC INFORMATION SYSTEMS (GIS) AND REMOTE SENSING IN THE MANAGEMENT OF SHALLOW TROPICAL LAKES

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Abstract. This paper reviews applications of remote sensing and geographic information systems (GIS) techniques to the assessment of tropical waters. These applications are discussed in the context of specific management objectives and sensors used. The need to monitor the spreading patterns of weeds in the tropical waters, land-use changes in the areas surrounding them, change detection, disappearance of wetlands, productivity and nutrient status, in order to establish trends and subsequently develop predictive models to facilitate effective management, is highlighted. GIS capability can be used to link ecological information with the management decisions of these waters. Remote sensing provides useful information in the form of satellite images and aerial photographs that can be integrated and analyzed in a GIS to provide useful spatial information and temporal changes over large geographic areas affecting the structure and function of tropical waters.

Keywords: *GIS, remote sensing, sustainable management*

Introduction

Shallow tropical waters play a vital role in many people's lives and contain remarkable communities of plants and animals. Shallow tropical waters are profoundly affected by their locality and by changes taking place on land, even at great distances from them. They are often faced with a number of threats as a result of socio-economic activities, taking place within them and their catchments. Rapid population growth in catchment zones has resulted in intensive use of land for farming, deforestation and growth of urban centers. Consequently, there is accelerated runoff leading to increased silt and nutrients discharge into the shallow tropical waters.

Currently most tropical lakes are being choked by water hyacinth (*Eichhornia crassipes*), which continues to cause severe hardship and immense economic difficulties to most countries. The pressure on tropical waters from the weed requires intensive research in order to come up with useful suggestions towards their management. It is at this level that availability of automated, real time data becomes imperative. Remote sensing has developed rapidly to address these needs. The advantage of remote sensing as documented by Richards [64] and Sabins [69] is its ability to capture and record land details instantaneously. Its spatial resolution and aerial coverage provide the researcher with a synoptic view of a land surface. Such data derived from remotely sensed images may be stored efficiently and analysed effectively in a GIS (Risser and Treworgy, [67]).

This facilitates analysis of local, regional and inter-regional patterns. Because of its nature, processing of remote sensing data can also be part of the interpretation process [35, 64, 69, 80].

Before the launch of satellites, remote-sensing activities mainly revolved around the use of aerial photography. The introduction of digital satellite technology in the1970s has since stimulated a lot of application researches for the simple reason that digital data are collected uniformly in time and space, and over large areas. Twenty-seven years have gone since the establishment of this. The principal aim of this paper is to discuss the applications and, to some extent, limitations of remote sensing and GIS technologies in the overall monitoring and management of tropical waters. The paper starts with a brief discussion of what is meant by remote sensing, and GIS. Fundamental facts about these technologies are discussed. Regular management applications of these techniques are also highlighted. The degree, to which the problem of invasion of tropical waters by weeds such as the water hyacinth could be investigated using remote sensing and GIS capability, is also examined.

Definitions and fundamental facts

Remote sensing is the acquisition of information about an object, area or event, on the basis of measurements taken at some distance from it. For the purpose of this discussion, the term is taken to describe the collection and analysis of data made by instruments carried in or above the earth’s atmosphere. Such data are obtained as a result of interactions of electromagnetic radiation with the earth’s surface, measured by airborne or spaceborne sensors. The term sensor here refers to a device that detects and measures a radiation as a physical parameter and converts it into a form that can be stored or transmitted to a receiving system. When electromagnetic radiation falls upon a surface some of the energy is absorbed. Some is transmitted through the surface, and some is reflected. Surfaces also naturally emit radiation, mostly in the form of heat (infrared radiation). It is reflected and emitted radiation that is recorded on either photographic film or a digital sensor. Since the intensity and wavelengths or this radiation are a function of the surface in question, each surface is described as possessing a characteristic “spectral signature”. If an instrument can identify and distinguish different spectral signatures, it is possible to map the extent of such surfaces using remote sensing.

The sensors commonly used in tropical areas include passive sensors that collect electromagnetic radiation (visible 0.38-0.75 μm) and infrared (0.76-1,000 μm) which is reflected and emitted from the earth. Since the sun is the source for such radiation data collection is restricted to daylight hours with clear skies. In contrast active sensors generate their own radiation (e.g. from radar and laser) and measure that which is reflected back to the sensor. Radar radiation (0.1-10cm) will penetrate cloud cover and is unaffected by nightfall. Most satellite are sun-synchronous which means that for any given point on the earth the satellite will pass overhead at the same time of day.

Satellite data can be obtained from international distributors such as the National Remote Sensing Centre (NRSC) in the United Kingdom or SPOT IMAGE in France. The potential buyer of remotely sensed data simply has to supply the relevant coordinates of an area of interest and request that the image catalog be searched. Airborne remote sensing is far more flexible. Given sufficiently good weather, the user can specify precisely when and where data are to be collected.

The following are terms commonly used in remote sensing:

1. Spatial resolution is a measure of the area on the ground covered by each sampling unit (pixel) and is dependent on altitude and sensor design. A whole image may be made up of millions of pixels, which partly accounts for the large data volume often encountered in remote sensing.

2. Swath width is the total width of the area on the earth's surface covered by the scanner. Together, spatial resolution and swath width determine the degree of detail that is revealed by the sensor and how large an area is covered.

3. Temporal resolution is the time interval between consecutive overpasses of a fixed point by a satellite. Generally, it is the maximum frequency at which imagery of any area covered by the satellite's orbit can be obtained.

4. Spectral resolution many sensors collect electromagnetic radiation in several distinct bands and are thus call "multispectral". Spectral resolution refers to the number and width of bands. For example, Landsat Thematic Mapper has seven bands, one of which (band1) records energy between 0.45 and 0.52um (blue light). A sensor with high spectral resolution has numerous bands of narrow range.

5. Radiometric resolution refers to the number of digital levels used to express the data collected by the sensor. The radiometric resolution of Landsat TM is such that the level of light intensity recorded for each pixel in each wave band can have a value between 0 (no reflectance) and 255 (100% reflectance), each value being referred to as a digital number (DN).

The Use of Different Sensors

Before the individual management applications of remote sensing are discussed in further detail, it is useful to make some general comparisons between each of the sensors.

Landsat Multispectral Scanner. The most widely used sensor employed for coastal applications is Landsat Multispectral Scanner (MSS). This does not necessarily suggest, however, that MSS has been found to be the most desirable sensor. Landsat MSS has been available since 1972 and has supported much fundamental remote sensing work. Although the specifications of Landsat TM and SPOT sensors are superior to that of MSS for most applications, MSS retains the advantages of offering a long time series and data that are considerably less expensive than those from either Landsat TM or SPOT.

Landsat Thematic Mapper and SPOT XS. Both Landsat TM and SPOT XS offer good spatial and spectral resolution and have been applied to a wide variety of objectives.

SPOT Panchromatic and Airborne Multispectral Scanner. Surprisingly the digital sensors with the highest spatial resolution, SPOT Panchromatic (SPOT Pan) and Airborne MSS, have not been adopted widely for tropical area applications. To help explain this apparent anomaly, the problems of inadequate spectral resolution and the high cost of airborne data will be addressed briefly, although a full discussion of the major constraints to remote sensing appears at the end of this paper. SPOT Pan Data is limited to a single monochrome band spanning 0.55-0.75 µm. High spatial resolution (10 m) does not enable different habitats to be distinguished if their spectral signatures overlap in a single broad band and are therefore effectively inseparable.

MODIS, one of the most advanced remote sensing instruments built to date, is a moderate resolution multispectral imager designed to measure biological and physical processes globally. The ground swath and resolution provide images of every point on earth in a 48 hour cycle. MODIS collects information on surface temperature, concentration of chlorophyll, vegetative conditions – including leaf area index, cloud cover and cloud properties, and fire occurrence, size, and temperature. MODIS gathers data frequently, measures radiation in 36 spectral bands – the largest range ever, be "on" all the time, and have long-term calibration stability. In contrast to MODIS both Landsat 7 and ASTER are high resolution multi-spectral imagers. Data from these instruments generally resembles the high resolution data available from Landsats 4 and 5 France's SPOT, and India's IRS-1 satellite.

The sea-viewing Wide Field – of-view Sensor (Sea WiFS) instrument launched in August 1997 on the OrbView-2 platform is providing fast, repeated global coverage of marine phytoplankton, ocean surface currents, and global climate change, its primary application is commercial and sports fishing, fisheries management, and coastal zone management by state, county and regional planners and managers.

Tropical Rainfall Measuring (TRMM), launched in November 1997, obtains daily global estimates of tropical and subtropical rainfall. TRMM includes the first rain radar in the ESE constellation satellites. TRMM gathers data from the tropics and subtropics (33 degrees north latitude to 35 degrees south latitude).

Aerial photography. It seems that among the available remote sensing technologies producing high spatial resolution data, aerial photography has been superior to space-borne data, despite the higher spectral resolution of the latter. However, digital air-borne multispectral imagery such as the compact air-borne spectrographic imager (CASI) is at least as accurate as aerial photography for the same purpose and it is less expensive to acquire and therefore more cost-effective [57]. It is also important to proceed in the evaluation of new scientific applications of more common imaging techniques such as video and photography from low-flying aircrafts [27, 36].

In space-borne remote sensing, the IKONOS satellite, launched in September 1999, was the first one to challenge the very high spatial resolution data obtained from air-borne remote sensing technology. Compared to aerial photography, which may have a spatial resolution as low as 20 cm, and CASI (resolution: 1 m), IKONOS has a spatial resolution of 1 m for panchromatic imagery and 4 m for multi-spectral imagery, and its future successors are reported to generate images with a spatial resolution of approximately 50 cm [65]. The EROS satellite, launched in December 2000, has a spatial resolution of 1.8 m but no multi-spectral capability. However, its future successors are reported to generate multi-spectral imagery combined with a spatial resolution of 0.82 m [25]. In the mean time, the QUICKBIRD satellite, launched in October 2001, leads the quality list of optical remote sensing with panchromatic imagery of 70 cm spatial resolution, and multi-spectral imagery of 3 m spatial resolution. Yet, it is not unthinkable that too much spatial detail (particularly if combined with a high spectral resolution) may obscure image analysis, as single image objects such as tree crowns will be characterized by a large array of pixels featuring internal variation, for instance crown side (sun/shade), leaf age (fresh/senescent), water content, etc. Therefore, it is as important to explore the construction of both manual identification keys and programmed identification algorithms that integrate spectral data (cf. 'tonality' above), 'texture' and 'structure' analysis. With respect to the spectral resolution, future research should continue to assess the applications of remote sensing sensors. On one hand, this should be done specifically for the identification of a larger range of organisms; on the other hand, it should concentrate on a larger range of wavelengths. Vegetation is easier to study because of its immobility, and, specifically in remote sensing, because of its characteristic reflectance in the infrared wavelengths due to its photosynthetic pigments.

Geographic Information System (GIS)

Geographic Information System (GIS) refers to a system used for storing, manipulating, and retrieving spatially referenced data. This definition also includes systems designed to capture spatial information and to process it. Data in a GIS are its database, usually composed of data planes derived from different data sources. The combination of data sets allows data interpretation [95]. A data plane is composed of one data type, for example, digitised elevation data. Digital data may either be in form of written text, maps, tables or photographs.

In order to manage tropical waters effectively it is inevitable that a large amount of data is handled. Those involved in the general management of these resources require rapid access to statistical data and thematic maps. Manual interpretation only allows integrating of relatively small amounts of field data, maps and aerial imagery. A GIS brings together spatially referenced statistics and remotely sensed imagery into one integrated system. GIS can also be useful in improving information extraction capabilities from remotely sensed data as outlined by Strahler [86]. The integration of remote-sensing into GIS has provided environmental studies with a genuine investigation power [68]. Nevertheless, it is only a potential source of data among others whose use finds its justification in the aim to be reached. At the spatial scales at which satellites observe the Earth, one cannot seriously envision to use satellite imagery to monitor the dynamics of small environments on short time-scale, e.g. every 5 years. To detect space changes in these areas, data-acquisition and-analysis scales must be greater than 1:5 000, with a measurement precision of 1 meter. Today, numerical orthophotographies or aerial remote-sensing (CASI) can punctually overcome the too low resolution of satellite sensors; so, one can use them to monitor tropical shallow lakes.

A GIS must be able to present information to users in a language and format that is not only accurate, but also graphic and comprehensible to all users. To facilitate urgent response from decision-makers in matters related to tropical water management, it is prudent to have a high ratio of maps and diagrams in written text. Such documents constitute a visual help essential for field staff, an aid for drawing up inventory as well as a mean of information and communication. In a short access time GIS allows one to store data from various origins, facilitates the design of maps meeting specific needs, e.g. scale, typology, and enables one to spare time in the production information through a possible automation of design. All these characteristics not only increase map production, but also improve their quality by a better adequacy with the objectives to be reached.

To secure such information, there are six prerequisite stages in a GIS to be followed as detailed by Tomlinson et al., [81] and Jackson [37]: Data acquisition, input and storage, processing, output and use.

Management applications

The work of Graetz et al., [33] illustrates the existence of two-way flow of information between remote sensing and GIS, meaning that both can be used in monitoring physical and chemical variables important in the sustainable management of tropical waters. Given the extent of current threats facing tropical waters such as pollution, land-use changes in the areas surrounding them and the disappearance of wetlands, GIS is an essential tool for the process of assessing and monitoring the impact, as well as for manipulating and displaying information essential for their management. In most cases, much of the required data can be made available by remote sensing. Of particular importance is access to remotely sensed images in digital form, which can allow rapid integration of the results of remote sensing analysis into a GIS. The same technologies can also be used to study productivity and nutrient status of tropical waters in order to develop predictive models to facilitate effective management.

The following is a discussion on the main management applications of the said technologies.

Ecological information

Remote sensing can be used to transit the gap between pure science and sustainable planning and management of any natural ecosystem [42]. GIS capability is used to link such ecological information with the management decisions for ecosystems. Remote sensing can provide useful information in the form of satellite images [40] and aerial photographs [59, 94] that can be integrated and analysed in a GIS environment. Pereira and Itami [60] have indicated that remote sensing data provide useful spatial information and temporal changes over large geographic areas. For instance, remote sensing and GIS have emerged to be important tools in the management and inventory of aquatic macrophyte distributions [1, 11, 13, 16, 17, 29, 38, 91, 92]. These technologies provide resource managers with an efficient method for monitoring plant distributions over large geographic areas. GIS analysis procedures permit managers to determine changes in macrophyte distributions over time and to identify critical environmental parameters influencing their growth. Models can be created that describe existing relationships among landscape components, predict future plant distributions and assist in making ecologically sound management decisions. Ecological models are generally defined as representations or abstractions of reality. They are used to represent interactions among ecosystem components in order to describe and ultimately understand systems as a whole. Such models can enhance the management of aquatic environments, because system behaviour can be quantified, analyzed and predicted [78, 84, 58]. An understanding of changes occurring over space as well as time is necessary for models to be useful for management purposes [67]. Such models can provide managers with information on both spatial and temporal responses of systems to proposed management scenarios [20, 74].

GIS can assist in modelling procedures by storing and organizing geocoded data at the landscape level [8]. They can also be utilized to create a specific type of spatial model known as a cartographic model [82]. Cartographic models are defined as the logical sequencing of map processes, conceptualized as an algebraic solution of equations which variables are replaced by data layers and fundamental operations can be combined to perform complex analyses [9, 10, 14, 83]. For example, a cartographic model might be created to determine sub-emergent and emergent macrophyte changes between two different dates in any given tropical shallow lake.

Therefore, the use of GIS and remote sensing techniques in the management of tropical waters facilitates the linking of ecological information with decision making in a quick and efficient manner. Since environmental degradation is an issue of concern to many governmental authorities, research institutes, commercial and private enterprises; objective, reliable and comparable information about the status of the environment is invaluable. Use of GIS and remote sensing technologies will facilitate information flow and thus, help to foster sustainable management of tropical waters without compromising the needs of future generations. Other authors [19, 39, 48, 51] have explained eloquently how remote sensing can be used as a tool for natural resource management.

Water plants

There is increased recognition of GIS capability in integrating remote sensing data and database development to monitor natural resources. This is illustrated by an excellent example of a database constructed from aerial photographs, maps, and statistical information, and subsequent development of a lake management information system (LMIS) for monitoring aquatic macrophytes and water quality in the large inland reservoirs of South Carolina [91]. The construction of this database was aimed at understanding whether changes in water quality will cause an increase in an already extensive aquatic plant population of Upper Lake Marion, and promote the spread of undesirable macrophytes to other parts of the system. The database was used to determine changes over time and relate these to water quality, bathymetry and sedimentation.

Using this same approach, it is possible, for instance, to create an integrated database to address the issue of rapid spread of water hyacinth in Lake Victoria, Kenya. To achieve this, it is imperative that various data sets are structured. These should include information on bathymetry of the lakes, data on water quality, depth, aspect, suspended sediments, water temperature, wave action and a base map. Maps of vegetation representing changes over years, derived from aerial photographs or satellite imagery, are also part of this database. Jensen et al. [38] in most of their work have used Landsat Thematic Mapper to map aquatic vegetation.

Once these data sets are ready, the next step is to construct a database in order to answer the question of ecological relationships between water hyacinth, water quality and other environmental factors. The data sets should be compatible with each other for easy correlation, and this can be achieved through the use of software PMAP, available from spatial information systems. At this level, using GIS's analytical capability, it is possible to measure the actual aerial coverage of water hyacinth over a tropical water surface, and identify growth and spreading patterns by performing change detection analysis [91] using multi-date imagery. Such information is invaluable in assessing the success of control methods or in establishing environmental impacts of the weed. This ability to make change studies can be used as long as the ecology of the target species is known, and is understood to predict the future distribution of such weeds in invaded tropical waters. Several researchers [11, 13, 29, 38, 91] have documented the theory behind the use of these techniques for monitoring aquatic weeds.

This process is, however, likely to face the problem of obtaining cloud-free image coverage in all seasons. High spectral resolution airborne multi-spectral scanner data are an option, notwithstanding their acute geometric distortions.

The following is a discussion of a case study by Welch and Remillard [90], demonstrating where remote sensing and GIS technologies have been used to good effect in guiding management of aquatic systems.

GIS technologies for aquatic macrophyte studies

Database development and changes in the aquatic environment

A case in point is the management of Lake Marion, South Carolina. The Lake experiences excessive growth of exotic aquatic macrophytes such as hydrilla (*Hydrilla verticillata*), native species such as Brazillian egeria (*Egeria densa*) and Water primose (*Uduigia uruguagensis).* These plants often cover extensive shallow water areas of the lake, block the passage of boats, and prohibit swimming and dock access along shorelines.

In order to control these weeds, the South Carolina Water Resources Commission (WRC) in conjunction with South Carolina Aquatic Plant Management Council and the South Carolina Department of Health and Environmental Control (DHEC), have applied herbicides to problem areas of the Lake. However, this effort has proved to be labor intensive and expensive. More cost-effective approaches such as the release of sterile triploid grass carp have been put in place.

It is important that ecological impacts of these practices are monitored, and the management potential of herbicides and grass carp on aquatic macrophytes be evaluated. It is against such a background that Lake Marion Project was formed with an aim of developing a database and GIS analyses approach to determine spatial and temporal changes in aquatic plant distributions related to herbicide applications. The GIS database for Lake Marion included macrophyte distributions for 1972-88, bathymetry, sedimentation and water chemistry. The aim of the database and GIS analyses was to assess changes in this ecosystem related to the management practices.

Methods

To facilitate GIS analysis of the relatively large study area, Lake Marion was divided into 2- by 2.5-km map segments, tied to the Universal Transverse Mercator (UTM) Coordinate system. Color infra-red aerial photographs in film transparency format were interpreted under magnification with Bausch and Lamb Zoom 70 and SIS 95 instruments. Polygons representing the different types of aquatic vegetation were delineated on clear polyester overlays registered to the photographs. Based on the interpreted aerial photos, aquatic macrophyte distribution maps of Lake Marion were produced as 1:10,000 and 1:24,000 scale for 72 and 76. These analog maps (72-85) were converted to digital data compatible with ARC/INFO using the software package, CAPTURE.

The digitized areas were edited and topology developed to create ARC/INFO data layers. Attribute information for plant species and structural type (emergent and submergent) was input to the relational database, INFO. Herbicide application maps for 83, 84 and 85 were also digitized, converted to ARC/INFO format and entered into the Lake Marion database. The herbicide data layers permitted comparisons with vegetation change over time, for evaluation of herbicide effectiveness in aquatic plant management.

Another important component of L. Marion GIS's database was water quality. Information of levels of nitrogen, phosphorus and dissolved oxygen, sampled at the top and bottom of the water column, and of absolute light and percentage light at I meter depth, were included in the database because of their potential influence on aquatic plant growth. The ARC/INFO Triangulated Irregular Network (TIN) software was used to spatially interpolate water quality values by sampling stations. Information on environmental factors potentially influencing the growth of aquatic vegetation included water depth and sedimentation. Water depths at 1.2 m intervals were digitized and entered into the database.

Analysis

Data sets for macrophyte distributions (72, 76, 83, 84, 85, 88) and herbicide applications (83, 84 and 85) within the Lake were analysed using PC ARC/INFO overlay procedures. To determine trends in aquatic plant growth, the vegetation coverages of different dates were coregistered and the ARC/INFO overlay command UNION used to produce maps depicting changes.

Results

In this study, observation of changes indicated that herbicides act as a disturbance to normal wetland succession. In the absence of herbicide spraying, the normal sequence of aquatic plant succession was evident (i.e. open water areas being invaded by submergents which in turn succeeded to emergent dominance). These ecological finding was very useful to the managers of Lake Marion in the evaluation of herbicide for aquatic plant control. The study also demonstrated the effectiveness of herbicides in controlling nuisance macrophyte growth. However, it was found out that the spraying must be repeated annually to maintain clear open water areas in the reservoir. In the absence of herbicide applications in a single season, the study demonstrated that aquatic plants quickly reinvaded and proceeded in normal patterns of wetland succession.

Significance

This way remote sensing and GIS database technologies have greatly assisted the managers and ecologists in Lake Marion in assessing ecological impacts of aquatic plant growth control on the lake. Further analyses include modeling macrophyte distributions to predict future plant growth and projecting vegetative responses to proposed management practices. The GIS procedures have assisted in constructing management plans that balance multipurpose uses of the reservoir with ecological stability of the aquatic environment.

Cost effectiveness

This procedure was conducted on a suitably equipped IBM PC/AT and inexpensive GIS software designed to work with databases in raster format. It is an alternative to the costly minicomputer based GIS systems for inventorying tasks (*Table 1, 2*).

Table 1. *Software and prices.*

SOFTWARE	PRICE
Aoutodesk 1.0 world	$2,395
AutoCAD Map 2.0	$4,250
Arcview Image	$2,500
Arcview	$2,000
ERDAS IMAGINE	$3,000
Geoconcept	$2,000
Geomedia Professional	£6,910
Geomedia webmap	£6,915
WinGIS Crypto HP	£6,900
Datamap	£9,000
Strumap	£9,000

Source: GEOEUROPE, issue 7, July 1999

Table 2. *Peripherals and hardware.*

PERIPHERAL AND HARDWARE	PRICE
Intergraph TDZ's	£2,500
Intergraph TD PC's	£500

Source: GEOEUROPE, issue 7, July 1999

Conclusion

This case study demonstrates an exciting prospect in the use of remote sensing and GIS capabilities to link theoretically oriented ecological studies with practical applications in resource planning and management. Scientists in all tropical countries need to assess the impact of management practices on the aquatic environment and can utilize the PC-based procedures developed by this case study.

Change detection

Intuitively, remote sensing is ideally suited to the task of assessing changes in tropical waters (e.g. changes in water quality, aerial coverage and productivity changes) and in areas surrounding them. For instance, Landsat TM imagery is available for all tropical areas at intervals of 18 days. This means that changes in tropical water areas can be monitored twice monthly, or that historic changes can be assessed by reference to archived imagery. This ability to detect change is therefore crucial for an efficient management for it allows one to establish surveys, analyze the change-inducing factors and predict potential changes. However, use of remote sensing for change detection is faced by the following constraints:

a) The high costs of obtaining several sets of satellite imagery, for instance, a full scene of Landsat TM image which costs USD 4500.00.
b) The specified temporal resolution of the satellite data is rarely the same as the practical frequency at which useful data are collected, a case in point is 18 days for Landsat TM.

c) Most satellite data are not duly available for countries that are not actively involved in image acquisition, for example, the East African States. Bureaucracy, image cataloguing, ordering, pre-processing and delivery occasion these delays.

Field surveys

Detailed field studies are vital in establishing databases on tropical waters. These data are in most cases missing and wherever they exist, they are outdated, scattered and incomplete. Remote sensing can play a critical role in the planning of such detailed field studies. For instance, as detailed by Link and Long [50] remotely sensed data has been used to plan detailed aerial surveys of aquatic plants.

GIS can be used to settle and implement field monitoring schemes, to detect and model ecological changes and simulate impacts taking place within the tropical lakes catchments. GIS technologies can be used to localize the sites to be inventoried prior to the field stage on combining specific data stored in the database to determine the ecological variables to be sampled. After the mission, the integration of the geographical coordinates of study plots permits one to better manage experimental sites. Within this framework, and thanks to the technological improvements of GPS, the localization as near as one meter of study plots is made possible, which reduces the risks of mistakes in the recording of spatial information. Within this operational context GIS can also help the piloting of experimentation plan through the search for the sites meeting the criteria required to set the operation or focus actions onto tropical shallow lakes either vulnerable or with a high heritage value.

One can also utilize GIS to elaborate a field-sampling or -operation scheme. For example, relief, nature of soils and vegetation often constitute the basic data used to define the place where study plots will be made to get a representation of the ecological diversity at a given site. This functionality is used above all by conservation institutions like the National Parks [12]. Another use of GIS technologies is the elaboration of intervention plan from a selection of sites meeting specific criteria.

Studies on suspended sedimentation and agricultural non-point pollution

Understanding the eutrophication process has improved the ability to manage water as a multi-use resource. Understanding variability of indicators of eutrophication endpoints will improve the ability to monitor water bodies and detect significant changes due to management practices. Such restoration projects such as Medical Lake, Washington [73, 75], Lake Washington [26] and Shagawa Lake, Minnesota [46] have emphasized the benefits gained from a thorough understanding of the eutrophication process. There is a continuing need therefore to determine effective indicators which will aid in the monitoring of the eutrophication process in most of the tropical shallow lakes.

For the purpose of management, it is useful to identify the pathways of land-based runoff and regions of suspended sediments since, these directly affect aquatic environments. Dissolved organic compounds, thus nutrient runoff from agricultural farms needs to be monitored and mapped. In all these cases, remote sensing provides qualitative information. Ritchie et al., [66] determined that Landsat Multispectral Scanner data can be used to effectively estimate suspended sediments in aquatic systems. Lathrop and Lillesand, [47] found significant relationships between Thematic Mapper (TM) data and sechhi disk depth, chrolophyll-a concentrations, turbidity, and surface temperature in

Green Bay and Central Michigan. Lathrop and Lillesand, [47] also found significant correlations of SPOT-1 Multispectral data with the same water quality parameters, with the exception of temperature, not measured by the SPOT-1 system. Both Lillesand et al. [49], using Landsat Multispectral Scanner data, and Wezernak et al., [93], using low altitude mulispectral scanner data, determined that remotely sensed data could be used to effectively predict the trophic status of inland water bodies.

Agricultural non-point source pollution is currently one of the primary concerns in tropical water quality. Pollutants generated from agricultural activities are diffuse, stochastic and dynamic in nature [5]. In order to protect tropical waters from such pollution, there is need to:

i) Identify the critical areas generating this kind of pollution.
ii) Monitor spread patterns, determine the physical and chemical characteristics facilitating spread.
iii) Determine the best management practices, and
iv) Construct a comprehensive pollution control plan.

This will definitely require a lot of information, which in the long run will pose data quantity problems. However, studies by Vieux et al., [89] illustrate how different spatial data sets are combined with such hydrological non-point pollution source data to reduce time and effort required for data input and manipulation. Arnold et al., [3], Srinivassan and Engel]71] and Mohite et al., [55], give an in-depth description of this approach. Review of this literature reveals that through the integration of simulation models and GIS databases, we should be able to use our tropical waters more efficiently and effectively for informed decision making.

Productivity measurement

In studies of this type, remote sensing can only be used to map the extent of the habitat. In turn this can be related to other aspects like biomass or population size.

Limitations of remote sensing

The use of remote sensing technology in the monitoring and management of tropical waters is likely to face some limitations as outlined below:

- Practical limitations, which are usually inherent in the technology itself, for instance, the limited ability of light to penetrate in water and atmospheric attenuation.
- User limitations referring to difficulties of assessing suitability of certain sensors. For example, remote sensing tends to provide geomorphological rather than ecological information on reef structure. This is due to limited spectral and spatial resolution of the sensors, and factors that confound image interpretation such as turbidity and variation in water depth. A more pronounced limitation in the tropics is cloud cover which significantly reduces the number of suitable images [7] to be available at all seasons. This situation has serious implications on change detection studies and efforts to relate field data to images.

Recommendations

Tropical countries should be encouraged to establish computerized databases to address the need to integrate data of numerous and complex forms essential for better management of shallow tropical waters. There is need to carry out tropical water management oriented research using remote sensing and GIS technologies for informed decision making.

Currently, most researchers that intend to use satellite remote sensing technique in their works will find it unappealing because it does not provide enough information at a fine scale, for example, data on a single tree species in the catchment area (see the case study below). There is need to address this through further research in order to understand the correlation between image data and ecological systems. Once this is achieved, researchers will be able to make meaningful interpretations of remotely sensed data as soon as they are available.

On the other hand the use of GIS technology in ecological studies is a recent phenomenon, and therefore, lacks enough literature [21]. More studies at the ecosystem level should be carried out in an attempt to understand the complex interrelationships occurring over large geographic areas. This can be done through use of GIS to aid management decisions.

The following is a case study by Lo and Lee [52], to demonstrate where remote sensing and GIS technologies fail to capture the fine details ecologists are interested in.

OKefenokee swamp vegetation mapping with landsat thematic mapper data

OKefenokee swamp covers about 167,00 ha in Ware, Charlton, and Clinch counties; Georgia and Baker County. Its climate is humid sub-tropical. The OKefenokee is a fresh water swamp.

In 1980, McCaffrey and Hamiltom mapped the vegetation communities in this fresh water swamp at a scale of 1:63,360 based on an interpretation of 1:30,000 scale color infra-red aerial photographs obtained in 1977, as well as field observations. McCaffrey and Hamilton developed a wetland vegetation classification scheme for the Okefenokee swamp based on a hierarchy of classes, sub-classes and dominance types. The mapping units defined from aerial photos closely correspond for classes, sub-classes or dominant types in this classification system.

In 1994, another research was done to produce a wetland vegetation map covering an area within the Okefenokee Swamp, using Landsat TM data and a digital approach, at a level of detail comparable to that of the map produced by McCaffrey and Hamilton from aerial photography and field observations. To equitably assess the ability of Landsat TM data and digital image processing to map the vegetation communities within the swamp, two small study areas were carefully selected to represent the full diversity and spatial complexity of the vegetation as a whole. An extensive survey of vegetation communities found in these study areas was done.

Method

Color infrared aerial photos were interpreted and fieldwork done to produce a vegetation map of the area. This formed the basis on which the computer-assisted wetland vegetation mapping capability of TM data was evaluated using different sampling schemes and a GIS approach.

Findings

This research revealed that despite the high spatial resolution of the Landsat TM data, an accurate 21-class vegetation map of Okefenokee swamp could not be produced from computer-assisted digital image classification. The primary reason was the large number of vegetation classes, which tended to overlap in spectral signatures. This was particularly severe among heterogeneous classes, which exhibited very similar spectral response patterns. It should be noted that the accuracy of such vegetation map could be improved only by aggregating the original categories of the vegetation map into few, closely-related vegetation classes, however, with a considerable loss of detail on the ecological characteristics of the vegetation communities. Therefore, digital remote sensing data can only be fruitfully employed to produce generalized vegetation map of a diverse wetland environment.

Integration of past, present and future remote sensing studies

The current challenge to remote sensing and GIS-based research on tropical shallow lakes is to combine data from the past and present in order to predict the future. On one hand, data from the past that were never ground-truthed require a calibration to current data, preferably (but not always possible), within the same remote sensing technology [23]. On the other hand it is likely that a long-term or integrative study will combine data from different sources. This requires a calibration between technology sensing technologies Discrepancies in post-launch calibrations of certain remote sensing devices may cause artefacts such as surface area change [30], and so may the shift from remote sensing one source to another [61, 62]. However, it is possible to integrate cartographic and multi-source remote sensing data into a homogeneous time series.

An important challenge to the sustainable management of ecosystems, whether or not tropical, is the use of GIS in the integration of data originating from ecology, geography, sociology, and other disciplines. For instance, biocomplexity, ethnobiology, demography, sociology and economics, research fields that are not directly associated with the use of remote sensing and GIS, can nonetheless often be integrated into such a spatial database [41]. A GIS-based combination of such fields to understand the biocomplexity of certain systems is not common. However, more applied possibilities such as GIS-based (disaster) management now becomes more and more widespread [2, 31, 70, 86]. At the level of organising new satellites for improved remote sensing, an emphasis should be put on international collaboration in order to reduce the cost of the space-borne missions [5].

Conclusion

It is apparent that there is considerable progress in the use of remote sensing and GIS in studies related to shallow waters in general. Because of its multi-spectral capabilities, remote sensing seems to be the only method available in presenting a unique perspective for observation and measurement of bio-physical characteristics of large tropical areas, uniformly in space and time, and repeatedly. However, there is a need for more comprehensive approaches that deal with new remote sensing technologies and analysis in a GIS-environment, and that integrate findings collected over longer periods with the aim of prediction. It is also imperative to collect and integrate data from different disciplines. These are essential in the spirit of sustainable development and management, particularly in developing countries. Not only do these countries hold a large part of the world's biodiversity (particularly from tropical aquatic ecosystems), but also they are the most vulnerable to environmental degradation. Several authors [18, 22, 23, 24, 45, 79, 88] provided remote sensing studies relevant to the field of sustainable development in tropical developing countries. It should be emphasized that next to technological innovation and multidisciplinary integration there is also a need for fundamental understanding of the biocomplexity (including human factors) of tropical shallow lakes.

In spite of the advantages of remote sensing and GIS technologies in the management of tropical waters, there are several limitations that have to be overcome by most countries in order to realize maximum use of these technologies. For instance, many countries have no adequate materials, analytical equipment, computers (software and hardware) and imagery. The high cost of materials associated with these technologies is another problem (*Table 1 and 2*).

On the other hand, lack of suitably trained staff continues to be one of the most important factors hampering the development of remote sensing and GIS technologies in tropical countries. The situation is aggravated by many countries in the affected regions giving priority to other activities other than these technologies. Thus, a direct effect of conflicting priorities continues and will in future divert attention from these technologies.

In the long run, these technologies hold the key to the solution of many problems facing tropical waters, and are likely to become the accepted methods in the future. They are mainly limited today because of their high operating costs.

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A SECOND LOOK ON BIOGEOGRAPHICAL PROVINCE OF MIANKALEH BIOSPHERE RESERVE

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Abstract. In Udvardy classification of introducing geographical biosphere provinces, the Miankaleh biosphere reserve of Iran, located in the southern coast of the Caspian Sea, has been expressed as mixed mountainous biome system. As the Udvardy classification, in large scale, is for national and regional use, it may not be able to separate the various kinds of existing habitats in a Biogeographical Province. That is why to prove the Udvardy classification theory and to investigate the probable disorder in it, by recognition of flora, the vegetation of the reserve has been studied, considering the ecological points of view. In the present research, the cluster analysis for classification of plant community and the ordination method for analysis of environmental (water table level and electrical conductivity of soil and water) relationships with plant communities have been used. The floras of the region, with over 200 species, with the origin of Euro- Siberian and Irano- Touranian, have been recognized and the presences of plant communities have been related to water table level and salinity of soil and water. In relation to Udvardy theory it seems that based on the existing and paleoecological documents of 1905, before fragmentation of Miankaleh winter and Alborz mountainous habitats, because of the urban developments, the place of the biosphere reserve of Miankaleh, as a unit, was a mixed mountainous biome.

Keywords: *Iran, biosphere reserve, Udvardy, Miankaleh, geographical province, Caspian Sea.*

Introduction

UNESCO, in its Man and Biosphere (M & B) program, tried to determine the main regions of the world and provide a classification of Biogeographical Province. Professor Micholos Udvardy made the classification in 1975. This system of classification determined 14 kinds of the biome types in one or eight bioclimate (realm) found throughout the world and separated them. Each climate is divided into geographical provinces, recognized by biome types, and all these units will be 193 Biogeographical Provinces all over the world.

In the above-mentioned classification system, the bioclimate of Iran has been determined as paleoarctic climate, the biome type as mixed mountainous system, and Miankaleh biosphere reserve, Golestan national park and Arasbaran protected region as indices for introducing Iran in mixed mountainous biome.

As the Udvardy classification system, in large scale, is for use in national and regional levels and is not able to separate types of existing habitats in Biogeographical Province and can not distinguish them, the present study is thought to be necessary to combine the regional ecological studies with the large scale worldwide standards in local units.

Paleoecology of the region, considering the manuscripts of the king Zellolsoltan in 1905 [7] and native knowledge of old people showed that some animal species like red deer (*Ceruus elaphus*), tiger (*Panthera tigris*) and even Persian Wild Ass (*Equus hemionus* VU) were present. Presence of these animals shows the special ecological characteristics in that time and continuity of Miankaleh plain regions with Alborz mountainous high lands and steppe regions. At the present time, the presence of animal species in Miankaleh, due to fragmentation made up of urban development and fragmentation of ecosystems, is unbelievable.

Farrukh [8] classified and made a multi-variable analysis of vegetation in Swabi region. In his research, he found 3 main plant communities and their relationships with soil physical and chemical characteristics have been analyzed. He has concluded that elements like phosphorus, calcium and acidity of the soil were the most important factors controlling the vegetation.

Carnevale [5], by investigation of vegetation distribution along salinity gradient, showed that there is a special relationship between soil salt concentration and rehabilitation of plant species.

Roo-Zielinska [15] in 1996 has evaluated the role of soil in plant communities around the city of Pitcho. He found the acidity, moisture and soil nitrogen roles in rehabilitation of plant communities effective. Based on Frey and others studies [9, 10], four halophytic communities habitats were recognized in Iran and the region under investigation has been expressed as the halophytic community of southern Caspian Sea Coasts.

In this research, in order to prove the Udvardy theory, introducing the characteristics of Miankaleh Biogeography with vegetation analysis and understanding the causes of plant communities of the region, have been carried out. This biosphere reserve with mean sea level of -30 m, mean annual precipitation of 717.21 mm, warm semi - humid to temperate climates (16) latitude of 36° 50' North and longitude of 53° 17' East, is located in northern Iran and southern Caspian Sea Coasts (*Figure 1*).

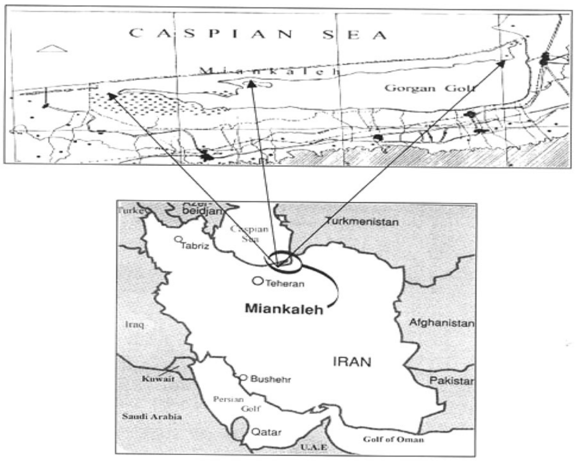

Figure 1. *The geografical position of Mienkaleh in Iran.*

Materials and Methods

Considering the role of vegetation in introducing and separation of Biogeographical Provinces, the emphasis in this research was on studying the flora and plant ecology and recognition of environmental factors constituting the Miankaleh plant communities. With this in mind, to investigate the vegetation, using a map with a scale of 1:25000, and aerial photograph with a scale of 1:20000 along the transects and in the direction of sea to land in the West, Center and East of Miankaleh, inside the representative stand, by using plots whose area was determined by least square method, the relative vegetation cover has been evaluated. The sampling method was systematic -stochastic and the number of samples was determined using statistical method [13]. For recognition of plants the Flora of Turkey [6], Flora of USSR (11) and Flora Iranica [14] references were used. In each plant community, by digging a profile and taking samples from 0-30 cm and 30-60 cm, the soil texture has been determined. Water table levels and piezometric surfaces were determined, using auger-hole and piezometrs, respectively. The soil and water salinity was evaluated in each sample unit [1].

The classification of the sample units were performed by cluster least mean analysis and Euclidean dissimilarity criterion by statistical software of MVSP Ver. 3.2 [12].

n investigation of ecological similarity of sampling units with each other and correlation of sampling units (plant masses) with soil and water factors by PCO analysis and using the software MVSP Ver. 3.2 [12] were carried out.

Results and Discussions

Floristics

Flora of the region constitutes of 207 tree, shrub and herbaceous species. Out of these, 17 are tree and shrub species like: *Acre velutinum, Ulmus minor, Quercus castaneifoli*, and *Alnus glutinosa* are found in the western part of Miankaleh Penisula (*Table 1*). Among perennial and annual species, the halophytes such as *Aeuropus litoralis*, *Salicornia herbacea* and *Frankenia histrusta*, the hydrophyte species like *Lemna minor*, *Utricularia vulgaris*, *Myriophyllum spicatum* and *Ceratophyllum demersum* are observed (*Table 1*). The species (Juncus sp) is almost in all over the region and Rubus is in a vast part of the area.

The plant communities

The classification of vegetation by cluster analysis, showed the following plant communities:

Juncus Community

The dendrogram (*Figure 2*) shows that the masses of 10, 18, 24, 1 and 8, 16, 14, 21 are very similar to each other. These masses are combined with the masses of 27, 31, 29, and constitute the Juncus community. As it can be observed from *Table 1*, the Juncus is dominant in all above masses. This community with some variations of species composition is observed all over the southern Caspian Sea Coasts and north of Gorgan Gulf (before halophytes).

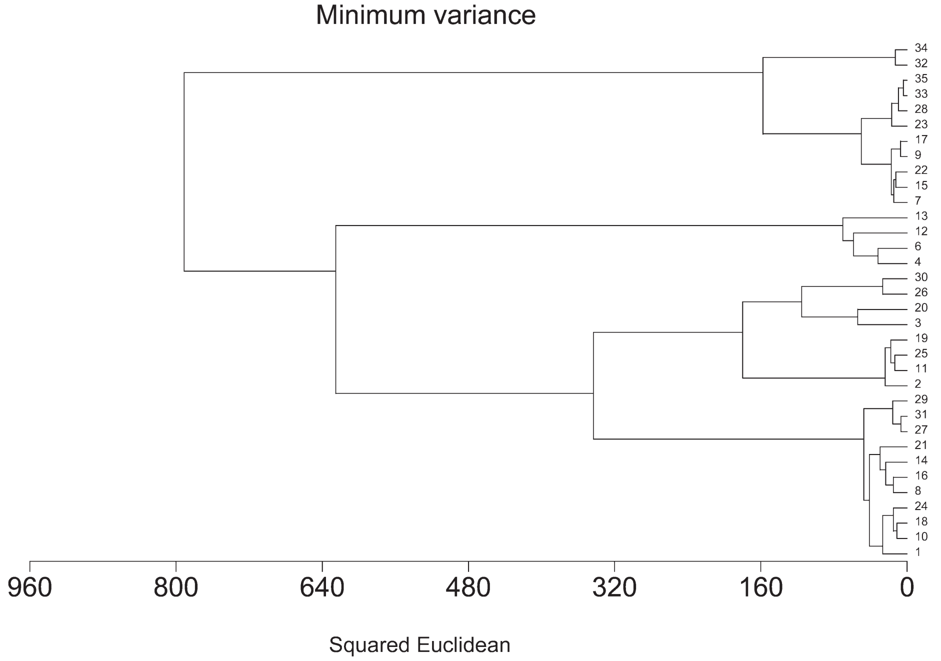

Figure 2. *Dendrogram from cluster analysis of representative stand of Miankaleh vegetation (34 Releves, Tab. 1)*

Rubus Community

The above dendrogram (*Figure 2*) shows that the masses of 3, 20, and 26, 30 are very similar (two by two). These masses are combined and constitute the Rubus community. This community with a change in floristic composition is scattered in all Miankaleh Penisula from West to East.

Punica Community

The above dendrogram (*Figure 2*) shows that the masses of 4, 6 with high similarity combines with masses of 12, 13 and constitutes Punica community. This community has been observed mainly in Western part of Miankaleh Penisula and at interface of the sea and the Gulf (in the most remote distance from the sea and the Gulf).

Sand Dune

The above dendrogram (*Figure 2*) shows that the masses 11, 25, 19, with high similarity to each other, combine with 2, and constitutes the Sand Dune. This community is observed as old and newly established Sand Dunes throughout the coast of the sea between Juncus and Rubus communities. In this community, based on performed evaluations, the mean non-vegetation is 87%; the canopy cover and litter area is 23%. The dominant species is Artemisia spp.

Halophyte Community

The above dendrogram (*Figure 2*) shows that the masses 7, 15, 22, 9, 17 and 23, 28, 33, 35 with high similarity to each other combine with masses 32, 34 and constitutes the Halophyte Community.

This community is located mainly in the northern, western, southern and eastern coasts of Gorgan Gulf. In this community the sub- community Schoenoplectus is observed.

Table 1. *Floristic composition of Miankaleh Reserve Biosphere*

Releve no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Acer velutinum				1*		1		1				1	1							1															
Achillea millefolium				1		2		1				1	1							1															
Aegilops tauchi	1	1	2	1		1		2		1			2	2		1				1						1									
Aeloropus spp.							6		8					1	9		8					8	4				2	8			3	3	6	7	8
Alhagi pseudalhagi																								1	1	3	1		2	3					
Alnus glutinosa			1	2		2		1				2	2	1						1						1									
Amaranthus blitoides	2	1	2	1		2		1				2	2							2	1				1	2									
Amaranthus retroflexus	1		1	2								2	1							1															
Ammi majus			1	2		1						1																							
Anagalis arvensis	1		1	2		1							2																						
Anthemis cotula	1			1																2															
Arabis nova			1			1							2																						
Arnebia decumbens	1			1																	2					2									
Artemisia annua		1		1				1																		1									
Artemisia kulbadica		4								1	3							1	2						2	2			1	2					
Artemisia turanica																		2	3	1					1	2	1		3	2					
Artemisia vulgaris	1									1	1	1																							
Arundo donax				2	3	1		1				2															2		2	2	2				
Asperula sp.				1		1						2	1																						
Aster tripolium	1						2	1	1					1	2	1	1				1						1								
Avena fatua			1	1							1	1			1				1					1	1										
Bolboscho maritimus					1																														
Brachypodium sylvaticum			2	2		1						1	1							1															
Briza minor			1	1		1							1							1															
Bromus tectorum		1	2	2		1						2	1							1						1									
Butamus umbellatus					1																														
Calamagrostis epigeios			1	1								1	1													1									
Calystegia sepium	1		2	1		3						2	1							1															
Campanula ranunculus			1	1		2						1																							
Capsella bursa-pastoris	1		1	1		1						1	1					1		1															
Cardamine hirsuta			1	1								1																							
Carex maritima					1	1						1		1							1														
Carpinus betulus			2	2		2						3	1							1						1									
Carthamus turkestanicus											1							1	2	1						2	1								
Catapodium rigidum	1		2	2		1	1						1																						
Celtis australis			1	2		2						1																							
Centaurea iberica	1		1										1								2					2				1					
Centaurea solstitialis								1						1		1					1														
Centaurium spicatum	1					1								1							1	1					1			1					

Table 1. (Continued)

Releve no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Ceratophyllum sp.					2																														
Chara sp.					4																														
Chelidonium majus			1	4		2						1																							
Chenopodium album	1	1						1				1				1			1																
Chenopodium murale			1										1							1															
Chrozophora tinctoria											1							1	1					1	1										
Cichorium intybus			1										1																						
Cirsium arvense		1	1								1	1								1															
Convolvulus arvensis	1	1	2	1		1						1	2							1															
Conyza canadensis			1			1							1							1															
Cornus australis				1		1							1																						
Crepis sp.	1		1			1						1	1																						
Crypsis aculeata			1							1				1																					
Cutandia memphitica			2										1	1																					
Cynanchium acutum			2										3							3						1									
Cynodon dactylon	2			2						3								2						2											
Cynoglossum creticum			1			1						1	1	1						1															
Cyperus spp.		2	1	1	1	1				1	1	1	1	1				1	1													2		2	
Dactylis glomerata			1	1		1						1	1	1						1															
Daucus littoralis	1	2	1	1		1					1	1	1						1	1															
Descurainia sophia	1			1						1			1							1				1											
Dittrichia graveolens		1	1								1	1	1							1															
Equisetum arvense	1			1		1						1	1																						
Eragrostis cilianensis			1	1		1						1																							
Eremopyrom bonaeopartis							1	1	4						2		4					1	2					1					1		
Erodium cicutarium		1	1	1		1					1	1	1							1															
Eryngium caucasicum				1		1						1								1															
Euphorbia chamasyce	1		1							1	1							1	1	1					1	1									
Euphorbia helioscopia	1	1				1					1							1	1						1										
Euphorbia prostrata		1	1							1								1	1																
Ficus carica			2											2		1										2				1					
Filago spp.		1	1									1							1																
Frankenia spp.							2		2						2		1					2	3					2				2	3	2	2
Fumaria parviflora			1	1		1						1	1																						
Galium spp.			1			1							1																						
Gastridium sp.				1		1						1																							
Geranium spp.			1	2		3						2	3																						
Glycyrrhiza echinata	1							2					1																						
Halocnemum strobilaceum															1							3	3					3					3	3	3

Table 1. *(Continued)*

Releve no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Halostachys belangeriana																																	1		
Heliotropium spp.			1								1									2					1	2				2					
Hibiscus trionum		1	1										1																						
Holosteum glutinosum			1	2		2						2	2							1															
Hordeum spp.		1	1	1							1	1							1	1	1														
Hypericum cabrum			1	1		1						1																							
Imperata cylindrica	1	1						1			1								1	1															
Inula britanica			1	2		2						2	1							2				1											
Iris pseudacorus					2																														
Juncus spp.	8		2	1	1	1		8		8		1	1	7		7		8		1	7	1		6		3	6		7	2	6	2		2	
Kichxia spuria	1	1	1								1	1							1	1															
Lactuca sp.																			2	1					2	1			1	1					
Lamium spp.			1	2		2						1	2							1															
Lappula spp.			1										1						1								1								
Lathyrus sphaericum		1	2	1		1						1								1										1					
Lemna minor					3																														
Limonium spp.							3			2					2		2					2	3				1	3					2		1
Lippa nodiflora				1	2	1						1	1								1											1		1	
Lolium spp.			1			1							1								1														
Lonicera floribunda			1	1		1							1																						
Lotus corniculatus		1	1			1															1														
Lycopus europaeus			2									1																							
Lythrum salicaria				1	2	1																													
Malcolmia turkestanica														1						1	1					1									
Malva spp.			1	2		3						2	2								2					1									
Marrubium vulgare			1											1						1	1					1									
Medicago lupulina	1		2	3		4						2	2						1	1						1									
Medicago minima		1	1	1		2					1	1	1						1	1															
Medicago polymorpha				1		1																													
Medicago sativa			1			1						1								1						1									
Melia azadrach				1		1						1																							
Melilotus officinalis		1										1	1							1	1					1									
Mentha spp.				1		1						1																							
Mespilus germanica																																			
Myosotis propinqua			1											1						1															
Myriophyllum					3																														
Nitella sp					2																														
Onopordon sp.			1										1							1						1									
Oxalis corniculata		1	1	1		1					1		1	1					1																

Table 1. *(Continued)*

Releve no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Paliurus spina-christi			1											1						1						1									
Papaver chelidonifolium			1	1									1							1															
Parapholis incurva																										1	1			1	1				
Parentucellia spp.				1									1							1															
Parietaria officinalis		1	1											1						1															
Parrotia persica				1		1							1																						
Paspalum paspaloides				1	3	1							1																						
Peganum harmala			1											1						1						1	1								
Phalaris paradoxa			1	1		1							1							1															
Phleum paniculatum				1		1						1								1															
Phragmites australis	1			1	4	2						1				2					2						3		2		3	3		3	
Pimpinella affinis			1										1							1															
Plantago psylium		1	3	2		2					2	4	2						2	2	1				1										
Plantago cronopus							1								3		1					1	2					2					2		2
Plantago lanceolata				1		1						1	1							1															
Poa bulbosa			3	1		1							2							2															
Polygonum aviculare			1	1		1						1																							
Polygonum hydropiper				1	1	1																													
Polypogon monspeliens				1		1						1																							
Populus caspica				1		1																													
Portulaca oleraceae			1									1	1							1															
Potamogeton crispus					1																														
Potamogeton pectinatus					1																											2		2	
Potentilla repens			1	2		2							2							1															
Psylotostacys spicatum							1		1						2		1					2	2				1	2					1		1
Pterocarya fraxinifolia				1		1																													
Pterohagia velutina							2		2						2	1	2				1	2	2					1							
Punica granatum			1	5		7		2				7	6	1						4						2									
Pyrus boissieri				1		1								1																					
Quercus castaneifolia						1																													
Ranunculus muricatus		1	1	1								1		1					1	1															
Ranunculus scleratus			1			1							1								1														
Ranunculs trichophylos					3																														
Rapistrum rugosum	1		1			1							1						1	1															
Rhamnus pallasii			1	7		6						6	7					1																	
Rhaphanus raphanistru			1								1		1						1																
Rhynchocorys elephas				1		1																													
Rubus spp.		1	7	2		2		2			1	5	4			1		1	1	7					1	8				7					
Rumex spp.			1	2		1						1								1															

Table 1. *(Continued)*

Releve no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Ruppia maritima																																2		2	
Saccharum spontareum	1	1								1	1							1	1																
Salicornia spp.							2		2					1	3		2				1	2	3				1	3			1		2		3
Salix alba			1	1		1						1								1															
Salsola baryosma															1		2						2					2							
Salsola kali	2	1						1		1						1			1	1							1								
Sambucus ebulus				1		1																													
Schoenoplectus spp.					2																		1					2				6		7	
Scrophularia vernalis				1		1																													
Sedum spp.			1										1							1															
Senecio vernalis	1	1	1									1				1				1						1									
Setaria viridis			1					1					1							1															
Silene spp.			1	1		1				1	1					1		1	1	1															
Silybium marinum			1								1		1							1	1														
Smilax excelsa				1		1						1																							
Solanum nigrum			1								1	1									1						1								
Sonchus palustris				1		1						1																							
Sorghum halepense		1	1								1									1															
Sparganium erectum					2																														
Spergularia spp.							1		2						2		2					2	2					1				2	1	2	1
Stachys byzantina			1										1							1															
Stellaria media				2		2						1	1																						
Suaeda maritima																							1					2					2		2
Sysimbrium irio		1						1					1						1	1															
Tamarix ramosissima							3		4						2		2					4						1					1		1
Taraxacum sp.			1	1		1						1								1															
Torilis arvensis				1									1						1	1															
Tournefortia sibirica																		1		1				2		3	2		1	2	1				
Tribulus terresteris			1																	1				1											
Trifolium arvense			1																	2						2				1					
Trifolium angustifolium												1							1	2						1									
Trifolium campestre				1		1							1																						
Trifolium fragiferum							2							2		1					1					2									
Trifolium subterraneum				3		3						2	3							2															
Typha spp.				1	4	1																													
Ulmus minor				1		2							2	1							2														
Urtica dioica				1		1							1	1							1														
Urticularia vulgaris					1																														
Vaccaria pyramidata			2							1								1								1									

Table 1. *(Continued)*

Releve no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Verbascum songaricum																																			
Veronica spp.			1												1					1						1									
Vicia odorata			1			1							1							1															
Viscum album				1		1						1	1																						
Vitis sylvestris				1		1																													
Vulpia myuros			1									1						1																	
Xanthium spinosum		1	1								1	1							1	1					1	1									
Xanthium strumarius			1									1	1	1																					
Zanichellia palustris																																1		1	
Zelkova carpinifolia				1		1																													
Zostera noltii																																1		1	
EC(Soil)mmohs/cm(0-30cm)	7.5	3.1	3.2	1.8	0.7	0.9	23	8.3	23	8.5	4.5	1.9	0.9	9.8	22	8.2	24	7.3	5.1	1.1	9.6	25	27	9.8	7.1	6.5	9.8	32	8.5	6.8	12	21	40	19	32
pH (Soil)	8.2	7.8	7.9	7.3		7.2	8.4	7.9	8.4	7.3	7.6	7.1	7.1	8	8.3	8.1	8.4	7.9	7.9	7.4	8.2	8.4	8.4	8.3	7.9	7.4	8.4	8.4	8.3	8.1	8.4	8.4	8.4	8.4	8.4
Water table level (cm)	89	65	75	120		203	84	57	49	58	85	245	195	58	84	52	50	58	88	112	62	81	57	59	88	120	72	61	58	98	57	10	57	5	56
Soil texture *	S	S	SL	LS		LS	S	S	S	S	S	LS	LS	S	S	S	S	S	S	LS	S	S	S	S	S	LS	S	S	S	LS	S		S		S
Vegetation cover (%)*	63	19	75	85		89	42	59	43	57	14	87	86	50	40	46	36	52	14	79	51	41	25	37	11	71	45	38	41	65	44	42	39	54	59

* Vegetation cover %, Vander Mareel Scale
** S = Sand, L=Loam
Date: Spring 2002

Schoenoplectus Sub- Community

This sub-community is located distinguishably in the eastern and southern coasts of Gorgan Gulf in a relatively narrow strip.

Hydrophytes Community

The mass 5 does not combine with any other masses. This mass belongs to the water ecosystem located in farthest western part of Gorgan Gulf. The following aquatic species are observed:

Ceratophyllum demersum, Myriophyllum spicatum, Potamogeton crispus, Potamogeton pectinantus, Ranunculus trichophyos, and *Lemna minor.*

Vegetation analysis

- The results of dissimilarity matrix analysis are shown in *Figure 3.*

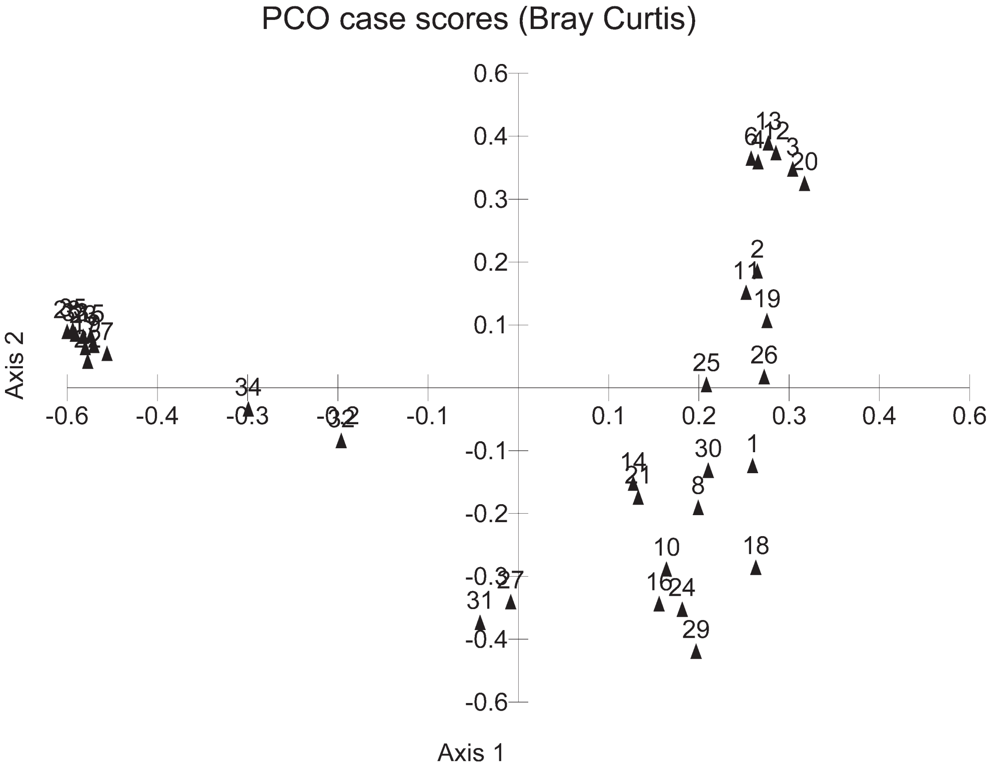
PCO case scores (Bray Curtis)

Figure 3. *Ordination of 34 representative stand of Miankaleh vegetation (based the data of Tab. 1)*

The percentage of dependence or the determining role of axis in the location of masses is depicted in *Table 2.* As it can be seen, the axes of 1 and 2 have the most determining roles.

- The masses related to Halophytes are in the farthest left end of axis 2.
- Masses related to Punica Communities are in the farthest right end of axis 2.

Considering the salinity of the soil of the above 2 communities (*Table 1*), the distribution of masses on axis 2 is affected by salinity.

- The masses related to Punica are in the uppermost of axis 1 and the masses belonging to Juncus communities are in the lowest section of axis 1. Considering the water tables in the above two communities (*Table 1*), the distribution of masses are on axis 1 and are affected by water table level.

In general the locations of masses on 2 axes (*Figure 3*) indicate the relative correlation of masses and environmental factors. Correlation of these masses with environmental factors is in two gradients of salinity (axis 2) and water table (axis 1). The soil texture is generally sandy and sandy loam and as a result did not have any effect on distribution of plant communities.

Table 2. *The percentage of dependence of axis on the location of communities.*

	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6	Axis 7
Eigenvalues	4.122	2.264	1.334	0.914	0.794	0.56	0.405
Percentage	33.403	18.347	10.809	7.406	6.435	4.538	3.279
Cum. Percentage	33.403	51.75	62.56	69.966	76.401	80.939	84.218

Conclusions

The flora of the region, with over 200 species has the origin of Euro-Siberian and Irano-Touranian [16]. Out of these, over 90% of annual and perennial herbaceous species is specifically of a non-mountainous region, and the formation of the plant communities is more affected by water table and the salinity of soil and water, which as overall indicates coastal regions.

Udvardy has introduced Golestan National Park and Arasbaran protected region, in addition to Miankaleh biosphere reserve, in the mixed mountainous systems biome.

Based on the studies on Golestan national park [2] and protected Arasbaran region [3, 4], the two regions can be located in the Udvardy suggested system.

To express the relationship between upper mountainous region of Miankaleh in old times based on the results of this research and also paleoecologic investigation of the reserve, one can conclude:

Existence of red deer and Persian wild ass in winter seasons of very old days in Miankaleh shows a continuity of this region in the south with high lands of Alborz Mountains and in the East with steppe regions.

At present time, due to urban developments and road networks, there is not continuity between upper mountainous regions with Caspian Sea plains and coasts and from East between steppes with Miankaleh. That is why; nothing is heard about red deer, tiger and Persian Wild Ass in Miankaleh any more. The Udvardy classification in incarnation with landscape ecology in that time can be coincided, so if total Tajan river catchment (from -30 m to over 3000m mean sea levels) is considered as one unit, concerning the ideal form of a biosphere reserve in each geographical province, the Udvardy classification is acceptable. But it is better that at present time the Miankaleh reserve can be in the Littoral Classification system.

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BIOMETRICAL APPROACHES FOR MANDARIN LEAF IMAGE ANALYSIS

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Abstract. A study examining correlations between fruit trees physiological and visual properties collected by remote sensing technology started at Budapest University of Economic Sciences and Public Administration, Faculty of Horticultural Science, together with Kyoto University and Wakayama Research Center of Agriculture, Forestry and Fisheries, Fruit Tree Experiment Station (Japan). Experimental plant was Satsuma mandarin (*Citrus unshiu* MARC. var. satsuma), the physiological property to examine was leaf water potential, which correlates with the sugar content of mandarin fruit according to preceding studies. Large amount of visual information had been recorded at ground level and was processed by the methods of image analysis and biometry. Under field conditions images of given leaves showed different type of reflectance frequency distribution. These distributions often could be identified as normal distribution, however left skewed, right skewed and bimodal distributions also occurred even partially shifted out of the perceptive range of the measuring device. Comparison of the applied biometric methods was analyzed to adjust with the natural field conditions, hardware and software features.
Results showed that normal distribution model could be applied for analyzing data obtained at optimal conditions, however these phenomena under natural field conditions could often be described better when weighted averaging methods were applied on frequency distributions.

Keywords: *image analysis, satsumas, mandarin, multispectral, NDVI*

Introduction

A study began at Budapest University of Economic Sciences and Public Administration Faculty of Horticultural Sciences (former SZIU Faculty of Horticultural Sciences) together with Kyoto University and Wakayama Research Center of Agriculture, Forestry and Fisheries, Fruit Tree Experiment Station (Japan) on examining the correlation between fruit tree physiological and visual properties collected by remote sensing technology.

Like most taxons, drought stress effects many plant physiological properties of citruses including photosynthesis [1], sugar accumulation [2, 3] fruit growth [4], stomatal closure [5] and abscisic acid content [6].

Experimental plant was Satsuma mandarin (*Citrus unshiu* MARC. var. satsuma), the examined physiological property was leaf water potential, which is correlated to the sugar content of mandarin fruit in peel, locular membrane, and juice sac [7]. Related to the topic, Láng et al. [8, 9, 10] conducted successful researches to reveal correlations between fruit tree leaf color spectra and nutrition supply. Working on „Aoshima" Satsuma mandarin, Iwagaki [11] reported higher sugar content, improved rind color, ans increased citric acid content.

'Field spectroscopy' term was introduced in 1974 by Longshaw [12] and its usage is supported by several researchers. As Milton writes: "Field spectroscopy involves the study of the interrelationships between the spectral characteristics of objects and their biophysical attributes in field environment" [13]. In this case spectral characteristics mean reflectance at some spectral channels, and objects mean individual leaves or full canopy of either one tree or a whole orchard. A simpler method of measuring the above mentioned objects' reflectance is to compare the radiance of those objects to that of the radiance of a portable reflectance target having known characteristics. Palmer [14] lists desired characteristics of such a reference target, and suggests the Kodak Gray Card (previously also called as Kodak Neutral Card) to be the standard reflectance target. Milton [15] evaluated spectral reflectances of Kodak Gray Card over the range 400-1100 nm. His results show good consistency between cards selected from a single batch.

The method of visual data collection and numerical data extraction had already been developed [16]. Large amount of obtained data was analyzed by statistical methods, in a way to be possible to interpret by optics, image analysis, and plant production.

Objective

Fruit leaf objects were investigated by image analysis, remote sensing and field spectroscopy methods resulting frequency tables. Analyzing frequency tables, a suitable data calculation method became necessary to reduce reflectance information into a single value. Biometrical analysis seemed to be difficult, because the type of objects' empirical density function was not uniform. Development of a uniform, algorithmizable statistical method was required to be able to analyze several thousand frames (images) that showed several different distribution types.

Materials and methods

Hardware and software tools

For image recording, "Silvacam" digital false color 3 channel CCD video camera was used, made by VTT. This camera was built on JVC GY-DV500 body and was equipped with DV output. For image analysis the Aphelion software package was used made by Acdis SA and AAI Inc. Data was analyzed statistically with SPSS for Windows 10.0 Server version software package, which is the property of SPSS Inc.

Table 1. *Silvacam videocamera Spectral Channel Perceptional Specifications.*

Color Channel Name on Digital Output	**Spectral Channel Wavelength Range (nm)**	**Spectral Channel Name**
Red (R)	760-900	NIR
Green (G)	580-680	Red
Blue (B)	490-580	Green

Reference objects

For calibration purpose two kind of reference objects were used.

- Kodak Gray Card (hereafter: GC as Gray Card) with known characteristics,
- a specially made calibration paper with 4 different grayscale level plates (hereafter: CP as Calibration Paper) characteristics measured by GC. (*Figure 1*)

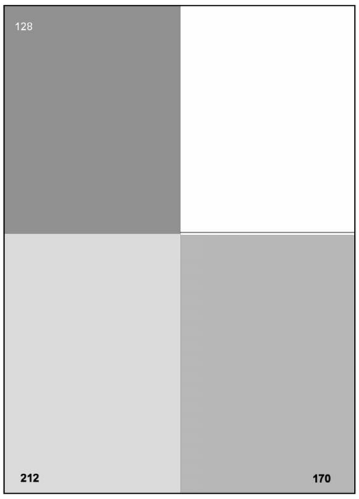


Figure 1. *Image of Calibration Paper with 4 different grayscale level plates.*

CP specifications:

- Paper material: Recycled Paper G70 A4 size, made by Toppan Forms Co. Ltd., Japan.
- Printer: Hewlett-Packard LaserJet 5000 Series PCL 6 was used to print the gray rectangle plates 212, 170, 128. The panel 255 was the pure paper surface.

Image layout

Image composition requires consideration of many factors. At first, flat field must be set to include foreground objects: leaf and reference plate. It is practical to adjust videocamera zoom setting to get the greatest virtual size of these objects on flat field, thereby maximizing objects' pixel number. During the research process image composition method had been improved empirically. The process is shown on *Figure 2-4*, as NIR channel image components in favor of higher contrast.

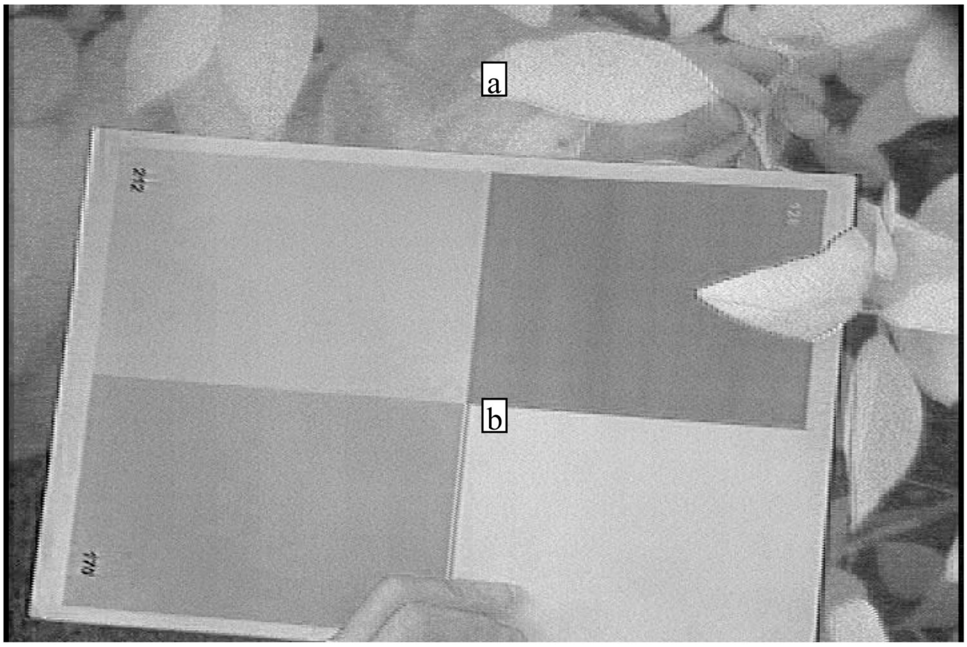


Figure 2. *Initial layout, November 2002.: leaf object to measure (a) and CP (b).*

Figure 2 shows initial layout, where most part of the flat field is covered by CP to record all 4 calibration plates, which ensures the absolute reflectance calibration in case of over- or under exposition. Due to this, image size, and pixel number of the leaf to measure is relatively small.

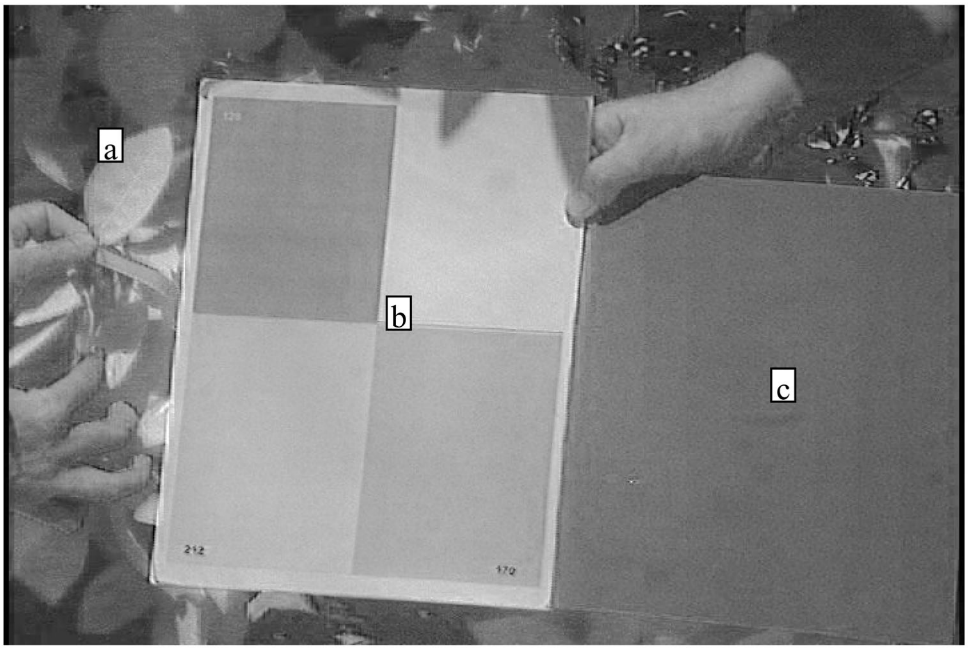


Figure 3. *August 2003.: leaf to measure (a), CP (b), GC (c).*

On *Figure 3* CP and GC are represented together to provide maximum possibility to a successful calibration, however leaf image size became even smaller.

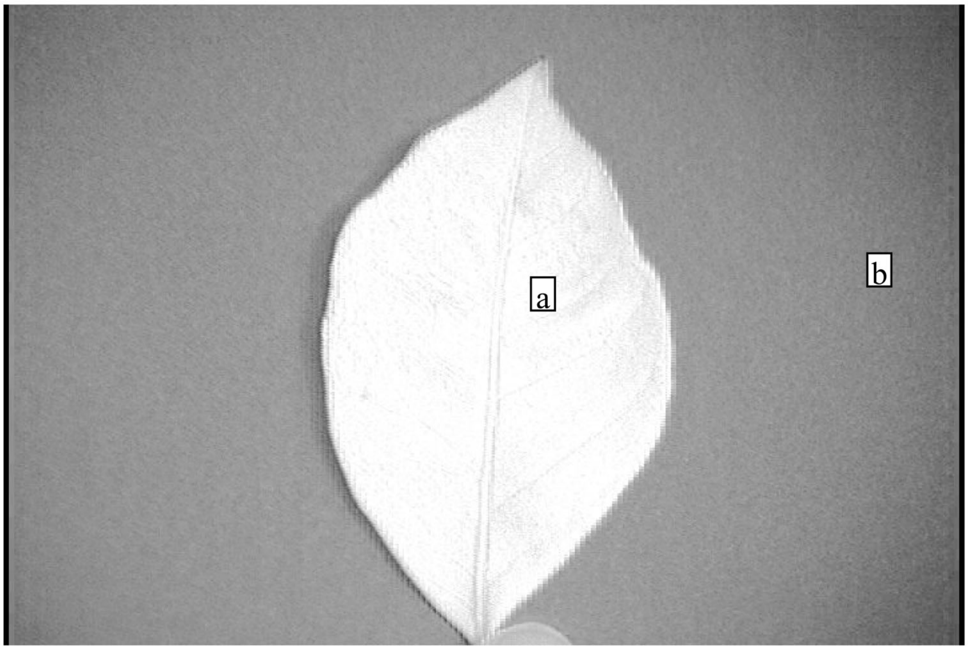


Figure 4. *December 2003.: Based on previous experiences this layout was found to be the most optimal with leaf to measure (a) and GC (b) also used as a high contrast background.*

Figure 4 depicts the most optimal image composition, that was developed by the previous experiences. Only two necessary objects are on the picture with maximal pixel numbers. This layout also gives the advantage that GC forms a high contrast background behind the leaf to determine exact leaf contour easily. In the image corners darkening, so-termed 'vignetting' effect appears.

During recording CP or GC must be exposed the same illumination conditions as the leaf to measure. Furthermore, objects must be oriented the way to avoid shadows on them, brightly colored objects reflecting light on them, and glaring (specular) reflections on objects themselves. *Figure 5* shows the optimal GC setup in daylight or artificial light.

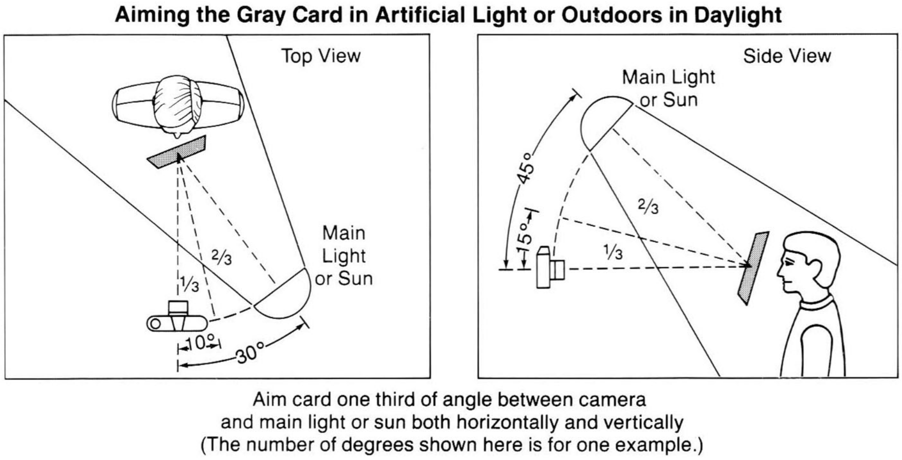


Figure 5. *GC optimal setup towards a main light source. [17]*

Image Analysis Scheme

1. Digital Video (DV) tape was played by Silvacam videocamera and DV data stream was transferred electronically via IEEE 1394 (firewire) port to the PC's hard disk. File specifications:

1.1. uncompressed Microsoft AVI file,
1.2. Field order A,
1.3. NTSC drop frame (29.97 fps),
1.4. 720 x 480 pixel resolution,
1.5. DV Video Encoder Type 1.

2. Type 1 AVI file was converted into Type 2 AVI file.

3. Frames were extracted from Type 2 AVI file into bitmap (BMP) file. File specifications:

3.1. compression: none,
3.2. color depth: 24 bits per pixel (RGB).

4. The RGB bitmap file was splitted into 3 grayscale channels representing NIR, Red, Green spectral channels.

5. All greyscale images were segmented and numerical information was extracted into frequency tables.

Segmentation workflow

Definition of the segmentation area's outline

In the course of the image analysis the foreground area – that contains valuable information – must be separated from the background area, that does not contain valuable information from the aspect of the research aim.

In present case leaf and reference object pixels were considered as foreground information. Universal segmentation method does not exist, the given task determines how to combine and customize known segmentation algorithms to get an optimal result. Aphelion image analysis software package contains several built-in threshold-based segmentation operators for this procedure.

Threshold-based segmentation operators

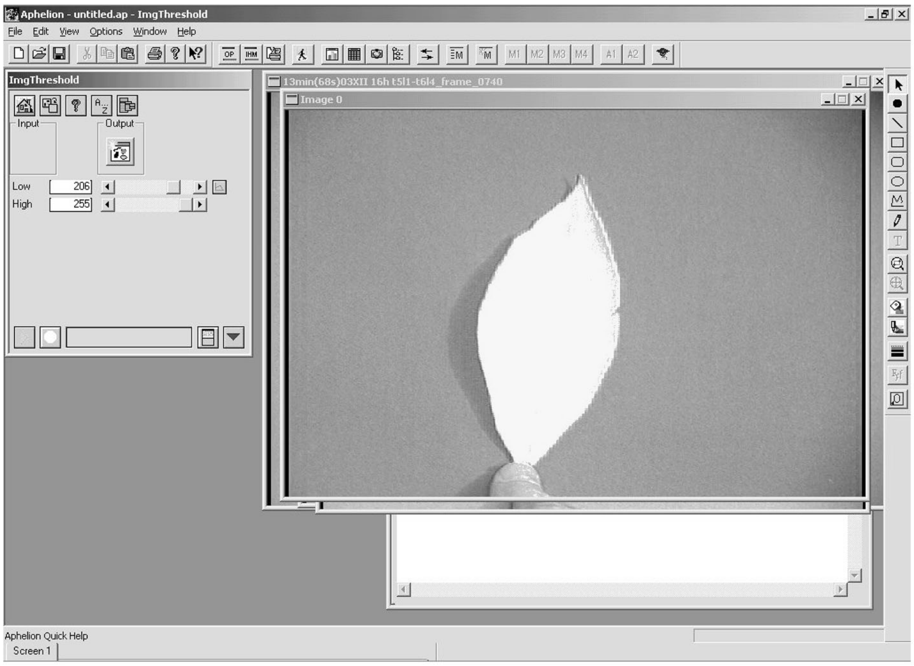
Threshold-based segmentation operators are algorithms with a greyscale image as an input image and a binary image as an output image.

`AphImgThreshold` operator demonstrates the operation method of these algorithms. This operator computes a binary image using the greyscale input image, low and high threshold values as parameters. It's syntax:

$$If\,(Inimg(i,j) \geq loThresh\,\&\,\&Inimg(i,j) \leq hiThresh,\ Then\ Outimg(i,j)=1\ else\ Outimg(i,j)=0$$

where:
Inimg : input image
Outimg : output image
loThresh : low threshold value
hiThresh : high threshold value

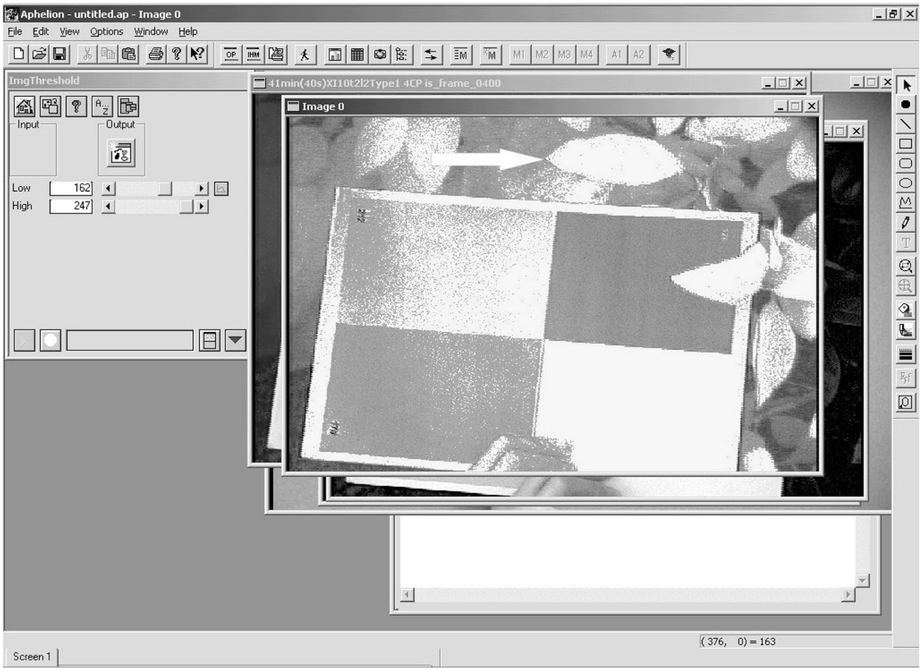
In case of a high contrast image (*Figure 6*), good segmentation efficiency can be resulted, however low contrast image shows less definite result (*Figure 7*).



Low 206
High 255

Figure 6. Segmentation with ImgThreshold operator on a high contrast image.

On these screenshots, fields labeled 'Low' (loThresh) and 'High' (hiThresh) indicates low and high threshold values. Segmented pixels are marked with pink color. In both cases threshold values must have been determined manually, and separately for each frames. This functional shortcoming disabled AphImgThreshold operator usage in an automatized segmentation script.



Low 162
High 247

(376, 0) = 163

Figure 7. Segmentation with ImgThreshold operator on a low contrast image. Arrow indicates the measured leaf object.

Similar to AphImgThreshold operator, Aphelion software contains several other threshold-based segmentation operators, that work specially (number of input parameters are shown in parenthesis):

- `ImgMomentThreshold (0),`
- `AphImgAdaptivePercentileThreshold (3),`
- `AphImgEntropyThreshold (0),`
- `AphImgHysteresisThreshold (4),`
- `AphImgMaximumContrastThreshold (1),`
- `AphImgMultiModalThreshold (6),`
- `AphImgExtremaThreshold (3)`

Operators generating Region Set

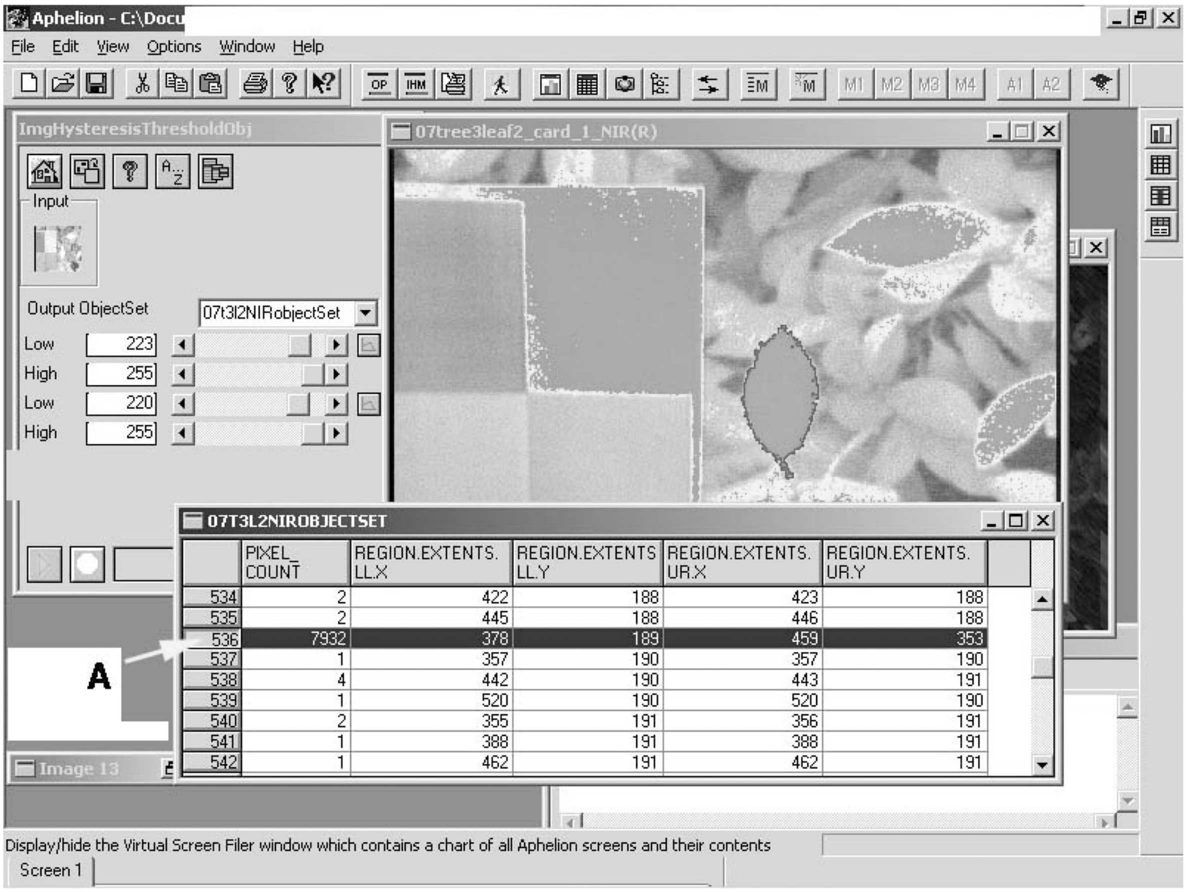
Each threshold-based operator has a pair with an additional function to generate Region Set. This means that in the first step the given operator returns a binary image, then in the second step, it groups the continuous pixels into objects to form a Region Set. These operators are named as threshold-based operators, following an …Obj ending.

To demonstrate Region Set generating operators function `AphImgHysteresisThresholdObj` operator is presented as an example. `AphImgHysteresisThreshold` operator applies two thresholds to the input image, outputting an image in which selected pixels are set to 1. A pixel is selected if its value is within the seed threshold range (first threshold), or if its value is within the hysteresis threshold range (second threshold), and there is a path of pixels connecting it to a seed valued pixel for which each pixel on the path has a value within the hysteresis threshold range. Requires the following parameters:

Inimg : input image,
Outimg : output image,

seedThresh : Seed threshold range (first threshold), constraint: SeedLoThresh value must be >= HysLoThresh value, and SeedHiThresh value must be <= HysHiThresh value,
    SeedLoThresh : seed low threshold value,
    SeedHiThresh : seed high threshold value,

hysThresh : Hysteresis threshold range (second threshold), constraint: HysLoThresh value must be <= SeedLoThresh value, and HysHiThresh value must be >= SeedHiThresh value,
    HysLoThresh : hysteresis low threshold value,
    HysHiThresh : hysteresis high threshold value.



Output ObjectSet 07t3l2NIRobjectSet

Low 223
High 255
Low 220
High 255

07T3L2NIROBJECTSET

	PIXEL_ COUNT	REGION.EXTENTS. LL.X	REGION.EXTENTS LL.Y	REGION.EXTENTS. UR.X	REGION.EXTENTS. UR.Y
534	2	422	188	423	188
535	2	445	188	446	188
536	7932	378	189	459	353
537	1	357	190	357	190
538	4	442	190	443	191
539	1	520	190	520	190
540	2	355	191	356	191
541	1	388	191	388	191
542	1	462	191	462	191

Figure 8. *A leaf object segmented by ImgHysteresisThresholdObj. 'A' indicates leaf object's properties.*

Figure 8 represents a screenshot result using AphImgHysteresisThresholdObj operator. Region Set objects' properties are described in the table shows the object's pixel number (PIXEL_COUNT), the bounding rectangle's lower left (REGION.EXTENTS.LL) and upper right (REGION.EXTENTS.UR) coordinates. Selected region's outline is red, unselected ones are marked yellow. Pink area indicates segmented pixels. Upper 'Low-High' values mean SeedLoThresh and SeedHiThresh values, HysLoThresh and HysHiThresh values locate under it as 'Low-High' values.

Region Set generating operators can extract segmented objects' outline, and convert into Region Of Interest (ROI), described below.

Region Of Interest (ROI)

It is practical to apply image processing methods not on the whole image, but select a part of the image and focus only that area. In Aphelion's terminology, this kind of area is called Region Of Interest (ROI), which can be defined either by Region Set or by upper left, and lower right coordinates of a rectangle-shaped ROI. Following a ROI definition, only the histogram of that area can be extracted, instead of the whole image's histogram. An adequately defined ROI can be used as a foreground after segmentation.

Automatic segmentation of an image sequence

The practical, automatized usage of threshold-based operators is obstructed by two factors:

- Due to natural illumination conditions, the upper and lower threshold values are not constant, but change dynamically frame by frame. Optimal threshold values can be found only after several manual 'fine tuning' steps.
- Operators themselves require large computation capacity, and the analysis of several thousand frames was expected to require too long time.

It is necessary to automatize as many image analysis steps as possible considering the several thousand frames.

A reductive method emerged to solve this problem. Instead of calculating the exact contour of a leaf object with a definitely uneven outline, the object's inscribable rectangle was determined as defined as ROI. This rectangle has the feature that all of its enclosed pixels are inside of the object i. e. those are foreground pixels. Coordinate pairs of upper left and lower right corner can define a rectangle, and these coordinate values can automatically be passed to an operator as input parameters. All along the whole image analysis procedure, we had to take into account that as many as 100-300 images per leaf can be analyzed, and the objects' position relative to the frame often changed slightly. This effect might cause the ROI rectangle to contain background pixels. To avoid this, the ROI coordinates must have been chosen carefully.

Results and discussions

ROI corner coordinate definition

Using a videocamera instead of a still image camera allowed us to record a large amount of images i. e. visual data in short time. Frames were extracted from Type 2 AVI file into bitmap format still images at image analysis scheme step no. 3. This meant ~ 3-10 seconds video length, that corresponds around 100-300 images per leaf (29.97 images per second). Beside of the advantage of collecting large amount of visual information, this method also had a drawback in the form of object-shifting. Images were recorded under natural conditions and objects could not be tightened completely. Through 3-10 seconds per leaf recording duration object-shifting effect occurred in several cases. ROI corner coordinates must be defined a way to avoid the influence of shifting therefore ROI does not contain any background pixel.

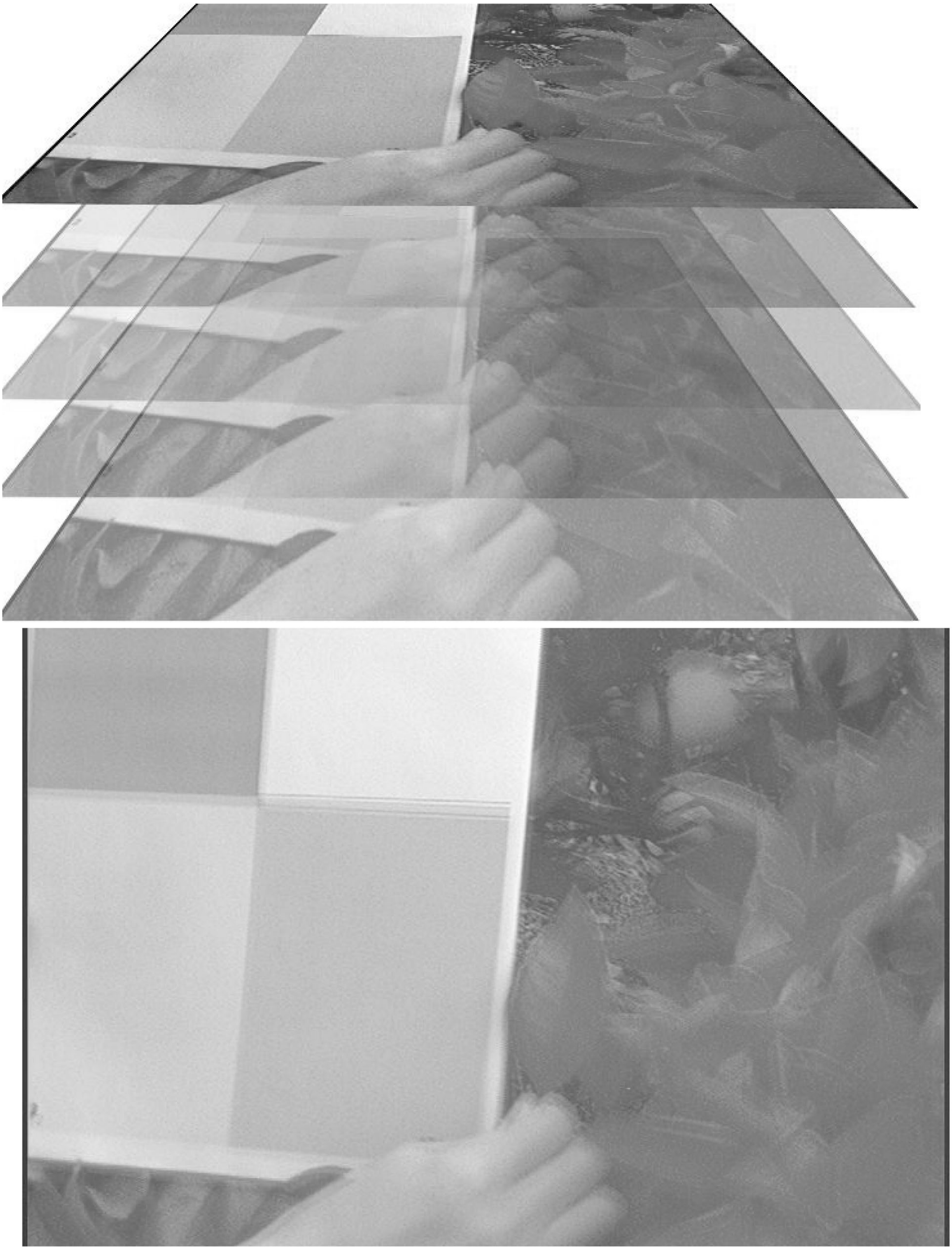


Figure 9. *Merged lower image is composed by the upper 50% transparency 5 image layers. Blurred object outlines indicate the shifting phenomenon.*

In the case of a given leaf object every twentieth-fiftieth frame of the series was opened in a graphical software with multilayer capabilities (*Figure 9*). After the manual definition of ROI coordinates, it was ascertained that ROI contained only foreground pixels on all layers. In case of ROI contained background pixels on any layer, then ROI coordinate modification become necessary. In case of ROI did not contain background pixels on any layer, ROI coordinates were accepted. This procedure must have been performed only once per each leaf, and was found as an effective segmentation method.

Determine empirical density function by frequency table

Applying the AphImgBandHistogram, ROI pixels' intensity was extracted into a frequency table. SPSS software package was used to process data stored in frequency table. Further statistical analysis seemed to be difficult, because the type of objects' empirical density function was not uniform. Largely, objects' empirical density function showed normal (Gaussian) distribution (*Figure 10*).

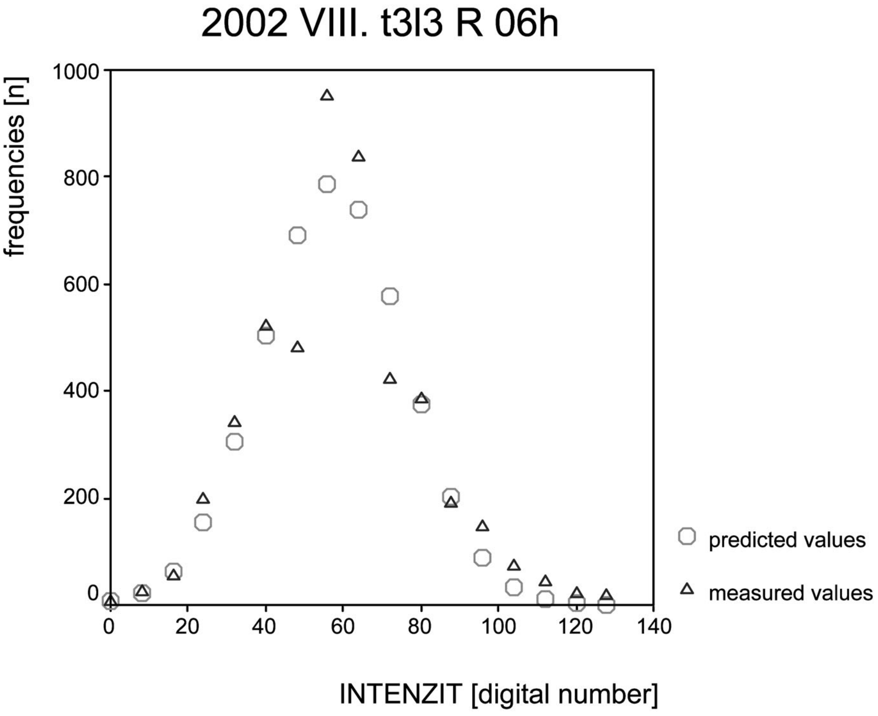


Figure 10. *Measured and predicted values of an object, whose reflectance shows normal distribution.* $\mu_{pred} = 57$; $\sigma_{pred} = 18$; $R^2 = 0{,}91$

Although normal distribution was found as a typical object density function, distinct type of functions also occurred. Both over- and under exposition caused 'shifted out' Gaussian curves from the perceptional range. Under exposition (*Figure 11*) evidently effected R and G bands where typically dark images were resulted due to the intensive light absorption. Over exposition (*Figure 12, 13*) effected the bright images of NIR band where light reflectance was intensive. Bimodal distribution also occurred. The reason of that phenomenon can be due to the non-flat surface of a leaf, and self-shading can occur: one part of the leaf seems shaded, another part seems sunlit (*Figure 14*).

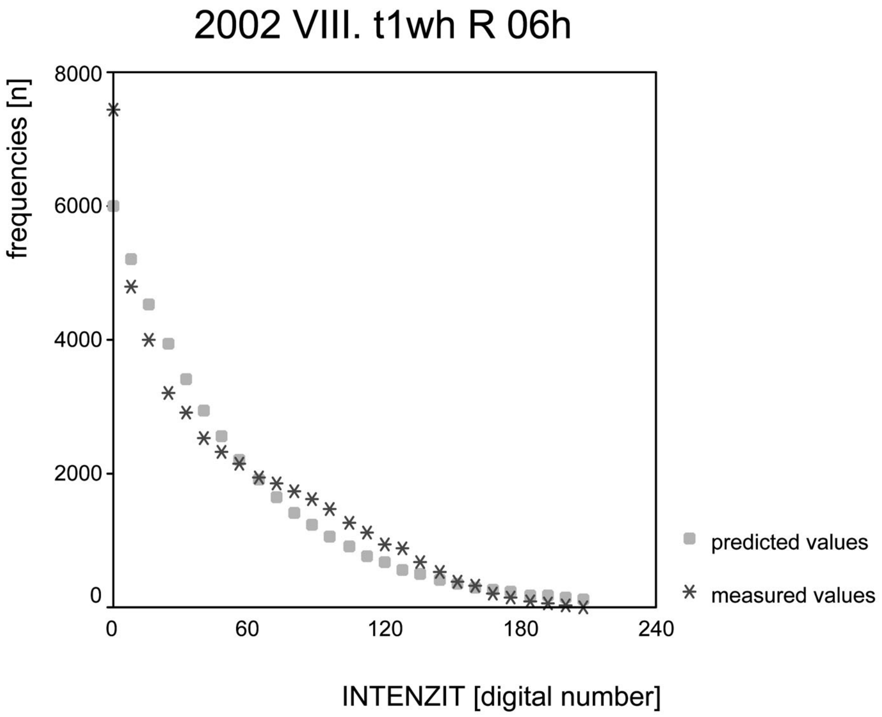


Figure 11. *Negatively shifted and truncated normal density function of an underexposed object's image.* $\mu_{pred} = -1255$ *(not interpretable);* $\sigma_{pred} = 268$ *(not interpretable);* $R^2 = 0,94$

Figure 12. *Image of an overexposed leaf object. White and gray areas indicate pixel intensity between 230 and 254, black areas indicate pixel intensity 255 or over.*

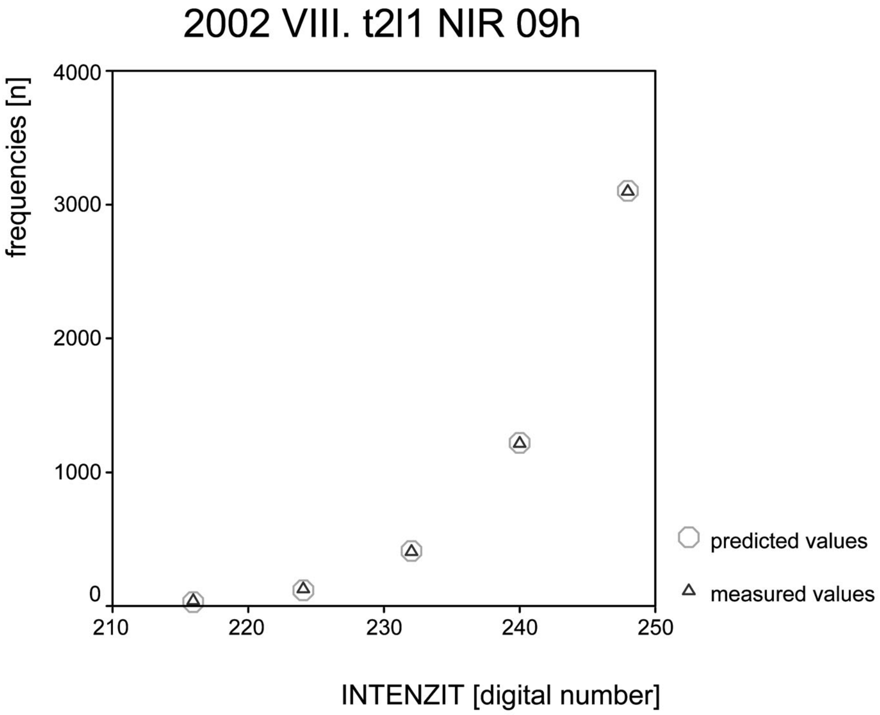


Figure 13. *Positively shifted and truncated normal density function of an overexposed object's image.* $\mu_{pred} = 298$ *(not interpretable);* $\sigma_{pred} = 21$; $R^2 = 0{,}99$

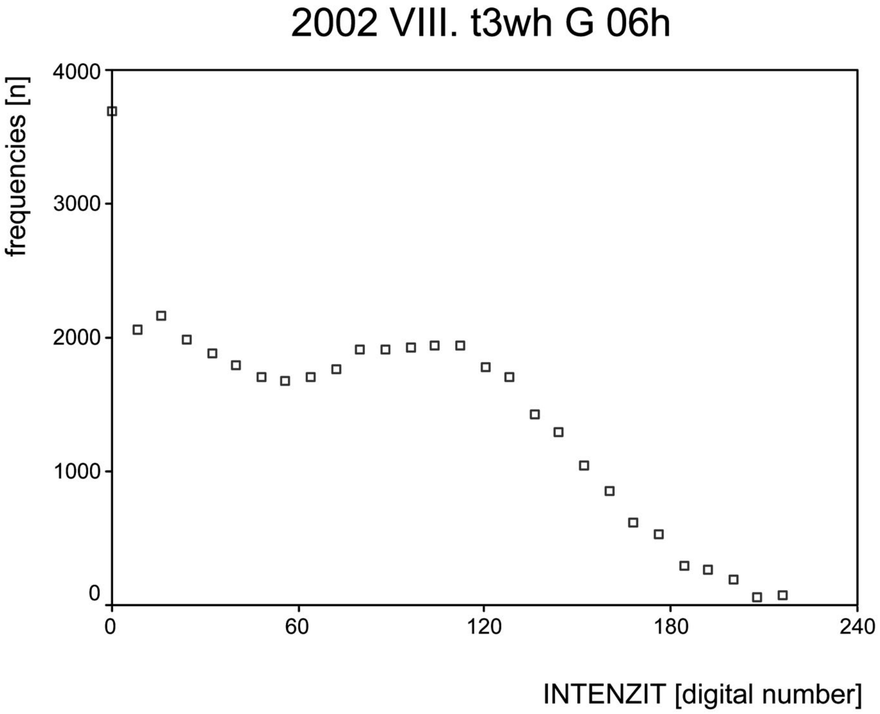


Figure 14. *Bimodal density function of a self-shaded leaf.*

In case of data showing normal distribution the application of nonlinear regression (Eq. 1) was found to be practical, which resulted high R^2 value showing strong correlation.

This method was inadequate at cases showed non-normal distribution. Predicted mean value result became smaller than zero in the case of underexposed images, and greater than 255 in the case of overexposed images. These results were not interpretable. Parameterization caused an other problem, because it could not be automatized. The estimated μ and σ values must have been provided manually one by one, separately for each objects (leaf or calibration plate), and this extremely slowed down the data analysis. The development of a uniform, algorithmizable statistical method was required to be able to analyze several thousand images, that showed several different distribution types. Weighted averaging (Eq. 2) was found to be a suitable method to solve the problems caused by self-shading, under- and over exposition. It ensures the μ and σ value results falling into the measurement range between 0 and 255, and did not require prerequisite, manual parameter estimation.

$$y = \frac{e^{-\frac{(x-\mu)^2}{2\sigma^2}}}{\sqrt{2\pi\sigma}} \qquad (1)$$

$$\bar{x}_a = \frac{\sum_{i=1}^{k} f_i x_i}{\sum_{i=1}^{k} f_i} \qquad (2)$$

As f_i values were discrete numerical values, so they are commutative and associative. This means that averaging (Eq. 3) can be used equivalently.

$$\bar{x}_a = \frac{\sum_{i=1}^{N} x_i}{N} \qquad (3)$$

Summary

In the frame of supporting precision agriculture fruit production system, the correlation between remotely sensed visual information and fruit tree physiological parameters were investigated. Significant amount of data was analyzed.

After successfully converting visual information into digital values, the objective became the characterization of a given object’s (leaf, reference plate) reflectance. In the practice during statistical analysis of the visual information, technical and natural phenomena prevented the algorithmized, automatized application of normal curve fitting by nonlinear regression.

Experiments showed, that data-averaging method gives solution for practical statistical data interpretation problems caused by under-, over exposition, and self-shading due to a non-flat leaf surface.

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GENETIC CONSERVATION OF LIVESTOCK AND FACTOR ANALYSIS

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Genetic Conservation of Livestock

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Abstract. It is necessary to keep diversity in livestock as well as in wild life. Decreased variety in wild life leads to extinction of species whereas in the aspect of livestock it may lead to hunger. This can be because of inbreeding depression or lost genes that are still needed. Different lines of livestock should be kept to prepare for a future that requires attributes not needed today. The different lines can be determined using factor analysis. Separate lines then can be merged by random mating; making way to a herd that has the variance of the original herd. Alternatively, biological material can be stored cryogenically. Live animals can adapt to environmental changes in time and may prove more useful though keeping live animals may cost more than cryogenic storage of biological material.

Keywords. cryogenic storage, livestock, biotecnology, biodiversity, sperm, oocites

Introduction

Humans like old things and try to keep old structures and memories, such as old buildings and photographs. People bring children into the World and want them to follow their footsteps. They try to reach out in time backwards by keeping old things and forward by having children. Humans like to see old things from their early life to forget the fact that their time is passing.

The same reason "trying to stop the time" might be true for the people who try to keep rare breeds or wild species that are about to vanish. There is certainly an emotional drive in keeping those animals in the World. Wild animals are widely regarded as part of the World's heritage and it is the duty of present generation to keep them for the future ones. We did not inherit those from the past generation; we borrowed them from the next generation (an old-Native-American-saying).

Another chief reason to keep those animals may be their possible benefits to the industry in the future. It is a well known fact that much genetic improvement in plants has been obtained by introducing genes from wild, "unproductive" species into cultivated crop plants today. A gene preventing flounder from freezing is introduced to a domestic tomato breed [1]. Another example of a useful character would be the isolated population of feral pigs found on Ossabaw Island, a coastal island off Georgia, USA [2, 3]. Those animals have a unique lipid physiology that makes them useful in medical and nutritional research [4]. The animals can either be kept alive or biological material can be collected to be recovered when needed.

Methods

Live Animals

The animal preservation used to be a hobby and some zoos existed keeping a few animals. Lately, some governments have started paying owners for keeping and breeding an endangered breed, such as the prolific Taihu sheep in China. In 1950's, the US Congress passed a law to ensure the conservation of the Texas Longhorn cattle breed as part of the country's living heritage. Today there is a revival of interest among farmers and the breed is no longer in danger [5]. The advantage of keeping live animals is that a breed can respond to the changes in external circumstances progressively and a performance evaluation is possible. However, because of high costs, only small populations can be kept; therefore genetic variance declines [5].

It was clearly shown by Sewall Wright in the 1930s that inbreeding depression causes fitness problems and extinction in small, isolated populations [6]. In addition, danger of losing a unique herd due to disease is high, especially in poultry. Smith [7] estimated minimum size of a breeding unit and number of animals that should be replaced to keep inbreeding levels to about 2 % a year (*Table 1*). The main strategy used to achieve a maximum genetic variation in most conservation programs is to set up a breeding system that maximizes the effective population size [8]. These kinds of programs have been shown by Bodo [9] using Hungarian Grey Cattle and by Alderson [10] using Portland and British Milksheep, to be effective in maintaining genetic variation at least when the populations were in a growth phase. According to Alderson a rapid population growth can maintain genetic variability even in the presence of intensive selection. However, when breeds with less than 100 females and a few males are taken into consideration, keeping the variance at a high level can be a serious problem. Lacy [11] showed by computer analysis that a random change by genetic drift may override all changes arising for other reasons in such populations. Borlase [12] reported that keeping the family variance at a low level (equalizing the family size) is recommended in captive breeding programs as it increases the effective population size, reduces inbreeding and slows down the loss of genetic variation. Berger [13] concluded by analyzing 122 populations of mountain sheep that populations of fewer than 50 animals were subject to rapid extinction within 50 years. Krausman [14] reported six populations – ranging in size from 8-46 – persisted for 34 or more years using data from Arizona.

Table 1. *Minimum number of animals required for conservation by management (Smith, 1984a).*

	Cattle		**Sheep**		**Pigs**		**Poultry**	
	Male	Female	Male	Female	Male	Female	Male	Female
Size of breeding unit	10	26	22	60	44	44	72	72
No. of breeding animals entering/ year	10	5	22	12	44	18	72	72

Maintaining Genetic Diversity and Factor Analysis

A breed, which is in danger of extinction, may possess characteristics that could be useful in the future. Chiefly disease resistance and adaptation to stressful environments such as drought and temperature stress are more likely to be some of those [2], as well as anything preparing future animals to eat novel types of feed and crop or industrial by-products [5]. However, it is very hard to determine exactly what those attributes might be. Therefore, one might have to keep lines with different attributes with the ones that are not economically valuable today. The various lines of live animals can adapt to environmental changes in time and may prove to be more useful than storage of biological material cryogenically. Keeping live animals may prove beneficial because they can be used immediately without complicated laboratory techniques of biotechnology.

The lines to be kept can be determined using factor analysis. By defining covariance relationships among many variables and by describing those in terms of some underlying but unobservable random quantities called factors [15], one could separate all the animals into well defined-groups. This enables the owner of the herd to keep different lines of animals for the future. Animals that are resistant to specific diseases or perform better in various environments can be obtained using these lines. The idea in using factor analysis is that one can make groups of animals according to an underlying factor. This can be e.g. a disease resistance gene that gives way to a group of animals that are resistant to hunger, or bad nutrition. This can be an enzyme or protein or some other biological factor that makes the animals, for example, behave calmly and also induces higher milk production. Whatever the underlying factor is, grouping animals using factor analysis may lead to a better separation, increasing the overall variance. Keeping different lines of animals will cause some genes to be fixed and some genes to be lost due to genetic drift. The subpopulations will have smaller variances, but the original variance will be restored when all subpopulations are merged back together, assuming that all circumstances are optimal and there is no selection, migration or mutation [16]. This may be a good way to keep the original variance intact since keeping the whole herd together is costly and results in mating of relatives, which increases inbreeding.

Spearman [17] invented the common factor analysis. Kim and Mueller [18, 19] presented a basic discussion of the common factor model. Mulaik [20] is a good general reference on factor analysis. Usually, the term factor is a source of confusion in factor analysis. It refers to a hypothetical, unobservable variable, as in the phrase common factor. In this sense, factor analysis must be distinguished from component analysis since a component is an observable linear combination. A common factor is an unobservable, hypothetical variable that contributes to the variance of at least two of the observed variables. The unqualified term “factor” often refers to a common factor. A unique factor is an unobservable, hypothetical variable that contributes to the variance of only one of the observed variables. The model for common factor analysis posits one unique factor for each observed variable [21].

Simon [22] reported that animals kept for conservation can be used in the future to overcome possible selection limits within the present breeding populations and within the prevailing environment. Simon wrote that based on Robertson’s [23] theory on selection limits and on experimental results with laboratory animals, animal breeders have to face the hazard that the response to selection “will cease sooner or later, after a continuous decline in magnitude” [24].

Cryogenic Storage

By using cryogenic methods now it is possible to store a variety of cells for a long period. Living material is stored at –196 Celsius in a liquid nitrogen tank and the length of storage time seems to be indefinite. Most disadvantages that apply to live animal preservation can be avoided by storage of frozen cells at lower costs. Brem [25] compared the costs of the methods (*Table 2*). Techniques of cryogenic storage can be summarized as deep-freezing of sperm and oocytes, deep-freezing of embryos and storage of genes as DNA.

Table 2. *Comparison of the different methods of genetic conservation for cattle (m= male, f= female; DM= Deutsche Mark)(Brem, 1984).*

Method	No. of animals needed	Costs (DM)	
		To establish	Per annum
Small population	5m 25f	50000	15000
Frozen semen (500 doses)	25m	2500	500
Frozen embryos (100)	25f	40000	500
And semen	25m	2500	500

Sperm and Oocytes

Deep-freezing semen is possible in all domestic animals, including poultry. The techniques are well documented. One difficulty is that a relatively complex breeding system is needed to regenerate a purebred population from semen alone [5]. Brem [25] wrote that at least 5 generations of backcrossing are required to achieve 97 % genes of the rare breed. Brem added that inbreeding and genetic drift has to be avoided which can be done by using a rotational breeding system. Smith [26] estimated that 25 sires per breed are needed to prevent inbreeding when males are used rotationally on each other’s daughters. Loskutoff [27] reported that in vitro fertilization (IVF) is already proving to be a powerful tool for rescuing gametes (sperm and oocytes) directly from gonads after death or gonadectomy. Coulter [28] reported that manipulation of spermatozoa provides opportunities for the predetermination of sex of resulting offspring, the introduction of foreign DNA into oocytes and formation of transgenic individuals. The cryopreservation of oocytes of most animal species remains a challenge due to their complex structure [29]. Using these techniques and new ones, one can control the structure of a newborn herd in the future and direct it to the needs of that future time.

Embryos

Cryopreservation of mammalian embryos has been successfully used in cows, sheep, goats and horses [5]. The entire genetic information is stored in a single diploid embryo and no complicated backcrossing programs are necessary. Once the embryos are obtained -which can be done non-surgically now- storage costs become very low. On one hand, Sciewe [30] reported that transportation of embryos can reduce/eliminate the need for shipping live animals. On the other hand, the same authors also stated that efficient worldwide movement of germ plasm requires established cryobanks. In addition, Loskutoff [27] wrote that traditional approaches of superovulation and non-surgical embryo recovery have been hampered in non-domestic ungulate species by inconsistent responses to commercially available gonadotropin preparations; requiring IVF which is apparently a more expensive procedure than the non-surgical method.

Storage of DNA

If a breed becomes extinct, one can bring the stored DNA into the active gene pool of the species by insertion of DNA into the embryos of another breed of the same species. DNA is a chemical and not viewed as biological material by animal health and quarantine authorities, so there should not be any problems with international transportation. The costs of DNA collection is lower than collecting semen, embryos etc. since this is possible simply by taking some blood samples [5].

Conservation of rare breeds or vanishing species is necessary. It is clear that one day humans will need those animals. In a group of bacteria, some individuals carry disease resistance genes in their plasmids. Those individuals are heavier and slower than the others and they require more energy. However, the other bacteria in the group do not leave behind those who carry the resistance genes. In case of a new disease, the bacterium carrying the resistance gene for that specific disease passes along the gene to the others and thus, the whole group is saved. In conclusion, it is necessary to keep the animals, which may not be useful today, but can be beneficial in the future.

Conclusion

Among all of the conservation methods, the easiest and cheapest one is taking and storing DNA/cell samples. Although wild animals are meant to be kept alive continuously, rare livestock breeds can be kept as DNA/cell and regenerated when needed. In the near future, it can be possible to generate a herd from one cell using the cloning technique (first one animal from one cell and then a herd from that animal's cells). Keeping different lines of animals requires extension of resources, not only for the costs of these animals, but also on management and monitoring the attributes. Various kinds of animals should be kept to make sure that the gene pool is available when needed, and keeping different lines can be accomplished by using factor analysis to separate the animals into different lines. Live animals can adapt to environmental changes in time and may prove to be more useful though keeping live animals may cost more than cryogenic storage of biological material.

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MIGRATION OF FISHES IN ROMANIAN DANUBE RIVER (Nº 1)

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Abstract. Present review paper tries to show the main aspects of migration of fishes in Romanian sector of Danube River. The Danube River has a large hydrographical basin, being the second largest river in Europe. The main channel flows trough seven European countries. There are many species of fish that annually or seasonally migrate either for reproduction or feeding in different areas of Danube and its main tributaries.

Keywords: migration, fish, capture dynamic, Danube, fulton factor, protection

Introduction

Some recent studies [8] have increasingly demonstrated the widespread existence of spatial and temporal variations in the abundance and distribution of the populations of freshwater fishes, many of them previously assumed not to move between habitats. These movements are based on seasonal or ontogenetic causes, for spawning, feeding and refuge, and in many cases are crucial for the successful completion of the fish life-cycles [15]. This text tries to cover the incidence and types of migration exhibited by the freshwater and anadromous fishes in the Romanian Danube River and their different capacity and stimulus related to the migration.

The Danube River has a large hydrographical basin, wide of about 817000 km^2, being the second largest river in Europe. The main channel flow trough seven European countries. A large part of its lower sector flows either nearby or into Romanian territory where it forms a large delta (Danube Delta) before reaching Black Sea. The ecological importance of Danube is underlined by the new studies or monitoring programs driven by international organizations or associations such as International Commission for the Protection of Danube River (ICPDR) and International Association for Danube Research (IAD).

Into the Danube River basin, fish fauna is still quite well represented by more than 100 fish species among 69 species in the main channel of Lower Danube. A percentage of about 75% fish species is still the main object of activities for the local fisheries.

The fish migration in Danube River is a common biological phenomenon. There are many species of fish that annually or seasonally migrate either for reproduction or feeding in different areas of Danube and its main tributaries. Usually, the fishes migrate from delta and main channel to the recently flooded plains of Lower Danube, especially for spawning. There is also a migration from delta to some particular places in the Black Sea

- the area of Danube River Mouths, where the water salinity is diluted by the fresh water supply. The fishes migrate in these marine areas probably for taking advantage of the characteristic richness of these waters due to the large amount of nutrients that the Danube is caring out. In this off shore zones it is a common fact to find many species of the native fresh water fishes, which migrate in for feeding.

The migration of the fresh water fish species is usually a seasonal event. The fishes migrate in the spring for spawning in the most naturally protected areas. In these places the offspring find out the best conditions to grow up at least in their first months and even in their first year of life. After the spawning time, the fishes usually swim back into the feeding areas. These places are the riparian zones of Danube River that include, beside some permanent lakes and small swamps, also some similar spots in the main tributaries such as River Prut, River Siret, River Arges and River Olt).

The main fresh water species that perform that type of migration belong to the following families: Cyprinidae and Percidae (*Aspius aspius* L., *Leuciscus idus* L., *Cyprinus carpio* L., *Scardinius erythrophthalmus* L., *Rutilus rutilus carpathorossicus* Vlad., *Stizostedion lucioperca* L., *Perca fluviatilis* L. and *Silurus glanis* L.)

Migration of the fishes in the flooding areas

The seasonal migration of fresh water fish is very close related, beside the water temperature, to the hydrological regime of Danube River that determinates the characteristics of the flooding areas. It has been estimated in the middle of the last century a huge wetland area of about 800000 ha, including Danube Delta (about 400000 ha), which were potentially flooded or benefited of the water supply provided yearly by Danube River.

The connection between main channel of the river and the flooding plains was generally assured trough many small creeks and the natural morphology of the relief (e.g. ground depressions). Similar formations are also usual into the Danube Delta where they provide the water supply. The flood is regularly producing every spring and its intensity depends on the amount of the melted snow and spring precipitations, mainly from the hydrographical basin of the Lower Danube River.

The ecological richness of these areas could be explained by the large amount of nutrients Danube River is carrying out and deposing on the flooding plains and its delta. The annual average of the transported alluvia in Danube River is 57 - 83 million tons per year with an average debit up to 2200 kg/sec [5]. The other general characteristics of flooding area such as the geo-morphological aspects that provides heterogeneous habitats with an average water level of 0.5 m to 2 m, many shelters and plenty of food, make these zones a real paradise for the spawners of many migratory fishes and their offspring.

Figure 1. Romanian Danube River from Iron Gates dams to Black Sea. Source: The Research Institute for Danube Delta.

At this time, the status of the Romanian Danube River flooding zone (*Figure1*) is dramatically changed. First, because the building of Iron Gates I dam and, situated 80 km downstream, Iron Gates II dam. Second, do to the former Romanian management policy regarding the agricultural activities in flooding planes. Many specific constructions dammed the most of Romanian Danube river's banks preventing natural flooding and reducing drastically former wetland areas. Whether in 1921 to1 km of river corresponded 612 ha floodplain, in the middle of 70ies the corresponding area for 1 km of river was only about 120 ha [2].

Actually, with some small exceptions (i.e. Little Island of Braila and "The Little Delta" of Somova-Parches area), most of Romanian sector of the Danube River is dammed along its banks. Excepting the Danube Delta, only few and small flooding plains have remained. One significant example is the sector of about 23.8 km, situated on the right bank and roughly located between Iasccea and Tulcea Town. In this area, the seasonal flood facilitates the persistence of some complex aquatic ecosystems included into a characteristic wetland formed by marshes, small lakes and creeks, known as Somova-Parches Complex.

Along the Romanian sector of the Danube, there are some differences concerning the structure of fresh water fish populations. In the upper sector of the river, the fish fauna consists mainly in small cyprinids, breams and perches. Some species as *Perca fluviatilis, Stizostedion lucioperca* and *Silurus glanis* are quite well represented.

The lower sector of the river, which includes the main stream, branches, no dammed pools, lakes and Danube Delta has, beside a similar fish fauna, bigger cyprinids such as the common (European) carp (*Cyprinus carpio*) and the Asian carps. The other species that populate those habitats are *Silurus glanis, Esox lucius, Tinca tinca, Carassius auratus gibelio, Stizostedion lucioperca.* The capture studies show in these locations the permanent presence of large individuals, medium size and younger individuals along. These sites are considered main areas for adult fishes feeding.

In the lowest part of Danube Delta (*Figure 2*), some fresh water tolerant marine species mainly belonging to the Clupeidae, Gasterosteidae, Atherinidae and Gobiidae families, seasonally enter into the delta form Black Sea. Some scientists consider that at least Gobiidae family play actually a very important role in the energetic balance of the local ecosystems, as a very important input of food resource for many indigenous carnivore species [3].

The spatial distribution and the migration of fishes in the flooding plains of Romanian Danube River were not rigorously studied. However, fish distribution and density in various food plain habitats depend on the local water level, flooded vegetation and the major biological needs of fishes as feeding and reproducing. The amount of fish that remains for some reasons in the floodplain pools over the year and the seasonal intensity of migration of fish coming in here from the main channel should be also considered.

The earliest stage of the spring migration for reproduction starts in February or March, depending on the water temperature. Usually, the fresh water fish species such as *Silurus glanis, Leuciscus idus, Stizostedion lucioperca* and *Pelecus cultratus* leave their winter shelters from the main channel of Danube for entering into the spawning areas when water temperature is about 4 - 6°C.

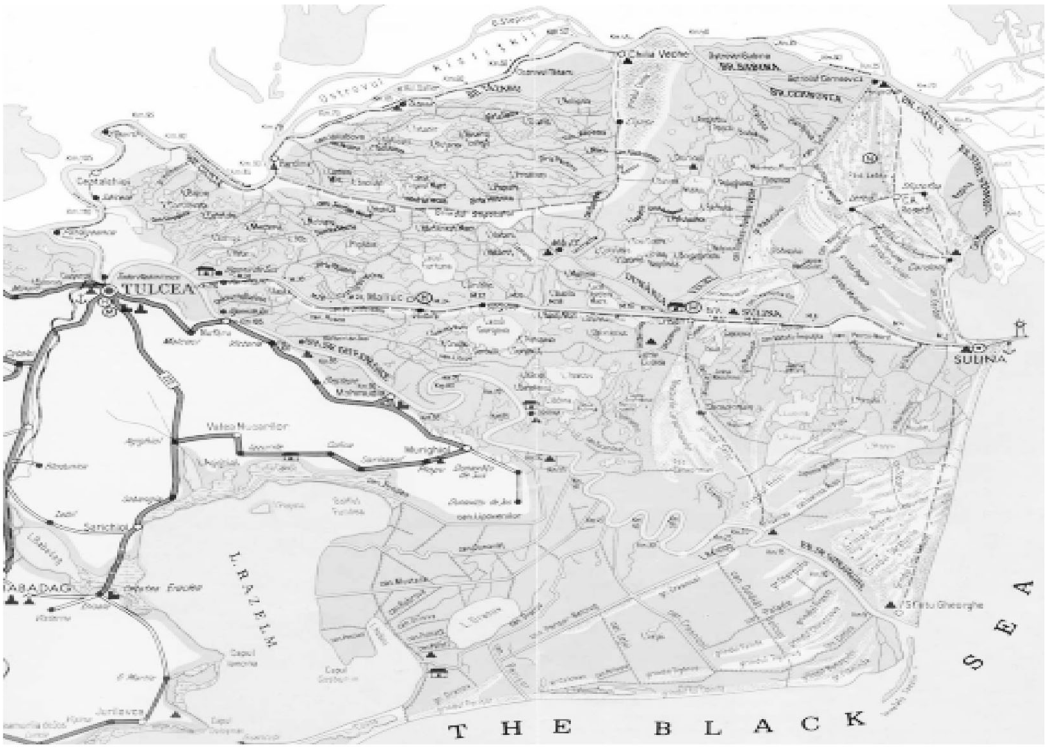


Figure 2. The network of channels, creeks, pools and lakes in Danube Delta. Source: The Research Institute for Danube Delta.

In the spring, when water temperature is about 10°C – 14°C, the other group of fishes becomes to head to the flooding areas for spawning. They are the main species of Cyprinids (common carp, breams and goldfish) which crowd at this time the fresh flooded areas of the banks, entering also into the similar places on the lower sectors of the main tributaries of Danube such as Prut River, Siret River, Arges River and Olt River.

After the reproduction, in the same time with the water retreat, the spawners migrate to the deeper pools or lakes. In the mean time the offspring goes to the not so deep areas using the network of small creeks and channels of flooded plain.

The offspring stay in these places, for feeding and growing, until the next flood occurs. Many times, the communication ways between those places and the main channel disappear because the decreasing of the water level in the main channel of Danube

River. Some times, mainly in the droughty summers, a large amount of juveniles dies because the lack of the water and solved oxygen. This way, many of the remaining flooding areas become a deadly trap for the offspring.

Migration of the common carp

The common carp (*Cyprinus carpio* L.) is quantitatively one of the most important species of the fresh water fishes in lower section of Danube River. Its migration could be representative for many other related species. The density and the dispersal of the carp populations in Danube are certainly depending on the way that the migration and the reproduction is occurring. There is a supposition that a migration of carp from Danube Delta areas to the recent flooded plains upstream located is a yearly event.

Even there are not clearly scientific proved, some local observations over a large period of time bring out the idea that this particular migration of carps is a real fact, its intensity being related to the water level evolution. When the water level in Danube Delta are not too high and, as a result, the local reproduction places become less available and not appropriate for reproduction, the carps intensely migrate to the Danube flooding plains. In the years with more convenient water level, when a large area of delta is flooded and many places become more acceptable for reproduction, the upstream migration of common carp is not as intense as usual.

Either less or more intense, the carp migration from delta to main channel and nearby flooding areas is a permanent process, probably due also to the lack of the convenient conditions for the offspring survival and development in the Danube Delta.

How long is the way of common carp migration from the delta to up stream place of reproduction is still a sensitive issue to be investigated, because there is not relevant experimental data. The best way to perform this research is of course the tagging methods that suppose catching, tagging and recapture of tagged fish. Probably because the high costs in time and money supposed by the use of these more precise methods, there are not recent rigorous studies concerning any migratory fish species in Romanian Danube River. The older studies on 130000 individuals of adult carps in Danube Delta and some upstream locations [10], that included tagging methods, did not offer any conclusive result concerning the real intensity of the carps migration.

However, the increased abundance of the common carp into the main channel in spring mounts versus other seasons can be a good indicator that the carp migration for spawning either from delta or similar places is a real event. The carp migration usually starts in April when the water temperature is about 12°C and it has the highest intensity in Mai when the water temperature is around 16°C. The water temperature of 18°C to 20°C is the optimum for spawning.

The biology of common carp is quite well known mainly because the importance of this species in some Central and Eastern European countries that have developed an intensive fishculture based on the genetically selected races of cyprinids. There are not recent studies on similar aspects regarding bio-ecology of the wild carp in the Danube River. Therefore, some results of our research in middle 90ies concerning the common carp in the area before the Danube Delta could bring out some interesting issues.

The common carp becomes sexually mature in the Romanian Danube River at the age of 3 years for males and 4 years for females. There is information that some times, in special situation (in the seasons warmer than usual) the males became sexually developed even at the age of 2 years old [4]. Our studies on the carp population in the Lower Danube did not confirm that supposition that could be explained by some accidental errors involved by the age determination methods.

The males-females ratio (M/F) calculated in 1996 in different locations on more than 200 adult individuals was of 0.42 to 0.65, a value of about 0.5 being considered as a normal ratio for the common carp. The average fecundity of the females was estimated at about 85000 follicles but the fecundity could be very variable depending on the age and the size of each adult female.

The yearly growth rate of common carps in Lower Danube River could be an indicator of the environmental conditions for their natural development. Comparing the carp size at different ages from different ecosystems we observe many similarities (*Table 1*).

For a better evidence of the natural life conditions of the common carp in Danube River, we have calculated also the values of Fulton isometric conditional factor (a) for different age classes (*Table 2*). As shown, the values for the different ages are relatively close and quite high, indicating a really good condition of the carp development and growth.

Table 1. *Size of the common carp at different ages in some ecosystems.*

Ecosystems	**Average values of total body length (cm) at the age of year:**						
	1	2	3	4	5	6	7
Central Danube River [1]	13.6	22.2	30.1	36.2	41.5	47.2	54.3
Central Volga River [7]	8.9	19.8	28.9	37.2	43.6	48.1	52.9
Azovean Sea [5]	13.0	24.5	34.9	41.0	45.5	48.0	-
Lower Danube River [6]	13.5	23.8	30.5	37.5	44.6	48.0	53.3

Table 2. *Fulton isometric conditional factor calculated for different ages of the common carp in Romanian Danube River (W: body weight, L: body length).*

Elements of calculus and Fulton factor (a)	**Classes of age (years)**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
W (g)	61	290	580	1200	2250	2450	3350	4750	6200
L (cm)	13.5	23.8	30.5	37.5	44.6	48.0	53.3	58.0	63.6
Fulton factor (**a** = W/L^3)	0.0247	0.021	0.02	0.0227	0.0253	0.022	0.02	0.0243	0.024

The stock of common carp in Danube River has significant decreased in the last time. The main reason is the reducing area of the flooding plains the restraint of the best places for carps spawning. The overfishing and a very high level of the poaching, especially in the migration time, contribute also to the decline of the carp populations. As a result a lot of large adults are caught in the small lakes and pools using traps, nets and other gears either in the time of spawning or on their return way to the main channel after the spawning period of time.

Even the last rules and regulations concerning the fishing activities in the Romanian Danube River have improved, the management of these fisheries is still inadequate and the common carp stocks protection need more attention from the official institutions that coordinate the Romanian fisheries.

The existing programs related to the establishing of few more protected areas, not only in the Danube Delta but also upstream, in the areas of the flooding planes must be implemented in a daring manner. It should be also considered the opportunity of some fish repopulation plans in diverse appropriate areas of the river.

Migration of other fish species

There is a similar situation also for other fish species. The environmental changes related to the damming of the major part of the Danube River in the last time had the most important contribution to the decreasing number of fish in many other fish populations. That situation can be observed studying the evolution of the yearly amount and the structure of the captures in *Table 3.*

We can see that some species not very sensitive at the environment degradation as *Carassius auratus gibelio* Bloch, 1783 or other species which are comfortable in main channel, as Asian carps, became more present in the captures than others species as *Tinca tinca* (tench).

Table 3. *Yearly capture of the fresh water fish species in theRomanian Danube River from 1991 to 1999.*

Fish species	**Annual capture (tons)**								
	1991	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**
Acipenser ruthenus	-	0.2	0.03	0.03	0.3	0.3	-	0.32	0.50
Cyprinus carpio	6.5	5.3	21.8	29.8	26.0	14.2	26.0	20.4	83.18
Abramis brama	21.5	51.4	59.8	64.9	127.7	72.7	140.8	33.52	22.17
Blicca bjoerkna	16.2	13.1	9.3	6.85	9.01	1.88	12.58	7.52	16.72
Stiyostedion lucioperca	0.27	3.04	3.08	3.54	9.93	17.05	9.81	29.97	33.65
Silurus glanis	16.1	5.58	5.81	7.27	4.35	6.4	5.90	16.09	43.47
Asian carps	16.30	49.38	75.11	89.29	102.3	101.4	72.99	20.75	91.30
Esox lucius	-	-	1.13	0.68	1.63	1.39	1.29	4.86	9.4
Rutilus rutilus carpatho-rossiucus	7.71	13.95	37.06	22.98	10.20	14.36	35.8	31.90	8.0
Carassius auratus	33.08	66.97	107.6	197.0	262.6	177.7	113.9	102.8	142.0
Perca fluviatilis	2.30	0.12	5.70	46	1.69	0.22	0.625	0.46	0.8
Tinca tinca	-	0.05	-	-	-	-	-	0.32	0.42
Barbus barbus	5.15	8.09	6.20	15.95	21.72	21.70	6.05	0.14	1.26
Aspius aspius	0.07	0.90	6.88	0.65	2.01	6.22	2.03	1.27	4.04
Alburnus alburnus	9.10	8.89	10.24	26.13	13.88	14.45	4.41	0.78	10.76
Pelecus cultratus	0.01	0.10	-	0.32	1.32	0.048	3.65	2.07	2.83
Alias sp.	8.10	44.70	5.18	19.2	18.81	20.70	9.00	6.51	28.13

The evolution of the annual amount of capture is better shown in *Table 4.* Covering a lager interval of time, and presenting separate data from the Danube Delta area and the upstream sectors of the Danube River (main channel), the table show obviously the decreasing level of the captures in ones of the last twenty years.

If considering an almost constant fishing effort during the investigated period of time, which is not quite real because we do have information that the fishing effort has significantly increased in 1990ies, we observe that in 1982 was officially registered the largest capture ever, of almost 12000 tons. This amount of the capture is more than twice higher than the average capture over twenty years (about 4994 tons) and more than 22 times higher than the smallest ever level of capture (approximately 531 tons) in 1999.

Even the official statistic data cannot be truly considered as the most accurate information, for some years (form 1990 to 1999) there is the evidence of the dramatically decreasing of the capture, indicating a proportional fish stock decrease.

Table 4. *Fish capture evolution in different sectors of the Danube River from 1980 to 1999.*

Year	**Danube Delta**		**Main channel of Danube River**		**Total Romanian Danube River**	
	Tons	**%**	**Tons**	**%**	**Tons**	**%**
1980	6683.0	76.7	2035.0	23.3	8718.0	100
1981	8834.4	79.8	2035.0	20.2	10069.4	100
1982	9494.8	80.0	2330.3	20.2	11825.1	100
1983	7603.0	84.6	1388.3	15.4	8991.3	100
1984	6578.9	67.8	3124.7	32.2	9703.6	100
1985	7461.8	80.6	1772.8	19.2	9234.6	100
1986	7565.6	89.2	913.5	10.8	8479.1	100
1987	5870.6	82.2	1268.9	17.8	7139.5	100
1988	6822.6	88.2	908.9	11.8	7731.5	100
1989	4481.6	89.8	505.9	10.2	4987.5	100
1990	4281.6	98.2	76.2	1.8	4357.7	100
1991	388.4	68.61	176.214	31.39	564.7	100
1992	936.0	84.89	166.635	15.11	1102.7	100
1993	633.3	81.41	144.681	18.59	778.0	100
1994	1179.5	79.78	298.888	20.22	1478.4	100
1995	930.8	72.0	361.9	28.0	1292.7	100
1996	655.4	66.57	329.1	33.43	981.5	100
1997	970.3	81.04	237.2	18.96	1207.5	100
1998	521.5	69.84	225.3	30.16	746.8	100
1999	436.58	82.22	94.46	17.78	531.04	100

The Asian carps

One of the most interesting issues of the ecology of fishes in the Danube River and particularly in the Romanian Danube River is the presence of some relatively new acclimated fish species, so called “Asian carps” which include three species: the grass carp (*Ctenopharyngodon idella* Vallenciennes, 1844), (*Figure 3*), the silver carp (*Hypophthalmichthys molitrix* Vallenciennes, 1844) (*Figure 4*) and the big head (*Aristichthys nobilis* Richardson, 1845) (*Figure 5*).

The story of the acclimatization of the Asian carps in Romania starts in 1957 when 100 young individuals of grass carp have been imported form former USSR. That action continued in the early 1960ies by the introducing of the young individuals of the other species brought in from the People Republic of China. These species were introduced first in some fish culture research facilities and the fish farms located in Western and Central Romania, as well as few fish farms from Danube Delta [9].

The acclimatization of the Asian carps has been considered successful and positive for the fish farming production, even in the local climate, these species still require a hormonal stimulation, the artificial fecundation and incubation for obtaining the offspring.

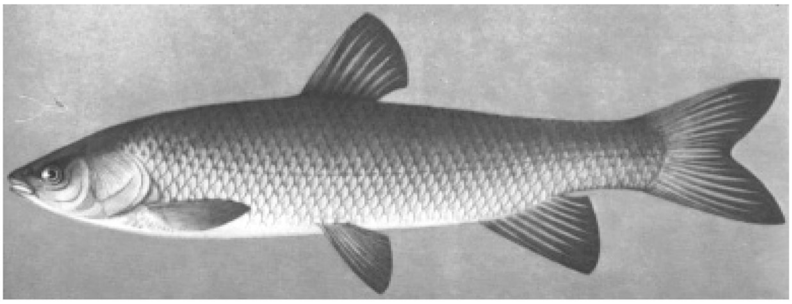


Figure 3. *Grass carp (Ctenopharyngodon idella Vallenciennes, 1844). Source: Manea, 1985.*

The juvenile Asian carps probably enter the natural environment accidentally, by escaping from some fish farms, either directly in Danube River or in its main tributaries. Nobody knows certainly the dates of the event or how many individuals have escaped in the wild. Some of these accidents could be related to the large floods occurred in the middle 70ies. However, at the end of 1970ies, the Asian carps start to be an increasing part of the capture in Danube Delta and the upper sectors of the Romanian Danube River. Their weigh in the structure of the total yearly capture arrives at more than 100 tons in the middle of 1990ies (*Table 5*).

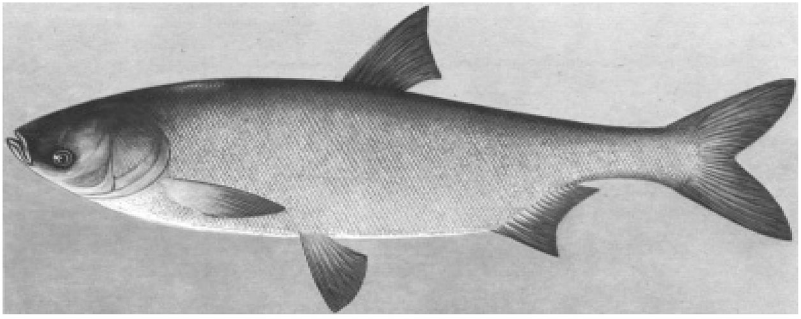


Figure 4. *Silver carp (Hypophthalmichthys molitrix Vallenciennes, 1844). Source: Manea, 1985.*

One of the main bio-ecological issues concerning the Asian carps is still the possibility of the natural reproduction of those alien species in the Danube River. There is an opinion that does not recognize the eventuality for the Asian carps naturally reproduce in the new habitats. The meaning of that is the unsuccessful acclimatization of these species. So, it is supposing that the presence of the Asian carps in the Danube is only related to the escape events of the small individuals from the fish farms facilities.

On the other side, the idea of an existing process of reproduction of the Asian carps in Danube River is confirmed by the information related to the capture of the individuals in their first stages of development. Unfortunately, we do not have yet any scientific prove concerning the presence of the roes or the embryos of Asian carps in Danube River, even there are some unpublished information about.

However, the increasing number of the adult individuals caught in the last decade and also some biological and ecological characteristics of these species could confirm their real acclimatization in Danube River.

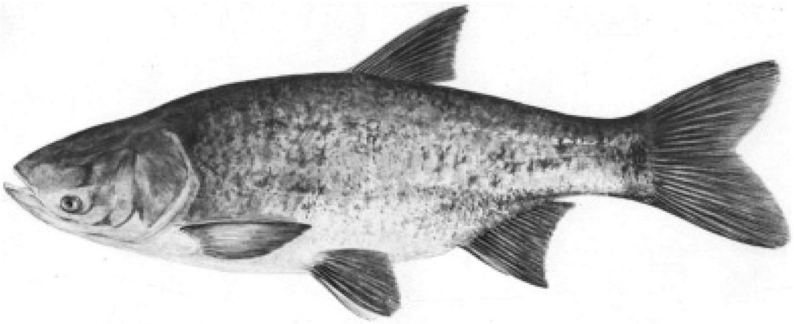


Figure 5. *Big head (Aristichthys nobilis Richardson, 1845). Source: Manea, 1985.*

First, the evolution of the captures in the last time shows, with few exceptions, the annual amount of the annual capture is of about 100 tons. It is hard to believe that the increased number of fish in natural populations could be sustained only by the accidentally escapes from fish farms.

Table 5. *Dynamics of the Asian carps capture in the Romanian Danube River from 1991 to 1999.*

Year	**Danube Delta (tons)**	**Danube River (tons)**	**Total (tons)**
1991	0.221	34.123	34.304
1992	25.368	24.020	49.388
1993	56.785	18.432	75.217
1994	60.079	29.211	89.290
1995	64.929	37.680	102.609
1996	33.560	67.857	101.407
1997	22.960	70.696	72.992
1998	5.891	14.866	20.757
1999	3.313	87.980	91.293

Second, even the biology of the Asian carps in the fish farms, closely related to their artificial reproduction, is well known, there is a lack of information regarding their behaviour in Danube River.

In their endemic habitats in the Asian rivers these species of fish need some particular environmental conditions to be accomplished for having a normal reproduction. The most important of these conditions are concerning the following aspects:

- The water temperature stabilized for a relatively large period of time in the interval of 18°C to at least 22°C.
- The existence and the persistence of the increasing water level.
- The water flow up to 3m per second.
- A certain degree of water turbidity.
- The appropriate places for the offspring development and growing up, similar to the Danube River native cyprinids.

All these conditions must be accomplished for a successful spawning, which includes the gonads maturation, the stimulation of the spawning and a good offspring survival.

In Danube River these requirements are basically accomplished in some specific locations. Even the opinion supposing the action of a limitative factor, such as the concentration of the solid water suspensions of about 1.2 kg/m^3 [10], necessary for the big head and silver carp spawning stimulation is true, that condition is also accomplished. As a result, there is not significant motivation, regarding the environmental conditions, for the Asian carps not to spawn in Danube River.

A study of the capture performed over ten years (*Table 5*) shows a relative equilibrium between the capture amount of the Asian carps in Danube Delta and the total capture in the main channel of the river. This is an indicator either for a quite uniform distribution of the fish population in this environment or, the most probably, for the manifestation of a real migration for reproduction of the adult Asian carps from lower sector of the river to the upstream areas, where they are finding appropriate places for spawning. The migration of the Asian carps occurs usually in June. This fact is obvious even in some years such as 1998, when the adult flocks had a reduced number of individuals (*Table 6*). The result of an experimental fishing campaign in some places situated upstream Danube Delta, which registered only the individuals bigger than 4 kg (potential spawners) put in evidence the massive presence of the Asian carps in the migration time.

Table 6. *The amount of the adult Asian carps captures in 1998.*

Area	**Monthly amount of capture (kg)**						
	Mars	**April**	**May**	**June**	**July**	**August**	**September**
Isaccea	12	56	212	945	230	124	56
Galati	43	94	198	1008	357	60	20
Braila	45	101	202	1122	157	45	12

Analysing the monthly capture for a larger period of time we observe a specific seasonal behaviour of these species, which consist in the following aspects:

- In March and April, there is a timid presence of the spawners in the different sectors of the lower Danube River.
- In May, there is an increasing amount of the adults, probably as a result of the start of the crowding process that usually precedes the upstream migration.
- In June, the capture was the largest one; it signalises the peak of the migration. The water temperature is of 19°C to 24°C.
- In July, there is a decreasing presence of the adults; there is possibly to find the evidence of their offspring presence.
- Most of the caught individuals weighted 6 kg-12 kg; this size is a characteristic of the sexually mature individuals.

In some other ecosystems the natural reproduction of the Asian carps is certified. In Amu-Daria River and Kuban River in the former USSR, Asian carps are certainly acclimatized in similar environment [10].

In fact, there is information that in Danube River the Asian carps could reach their sexual maturity even one or two years earlier than the similar individuals from fish farms [7]. A comparative study of the average growing rates of the grass carp in captivity versus natural ecosystems of the Danube River (*Figure 6*) sustain the validity of that information.

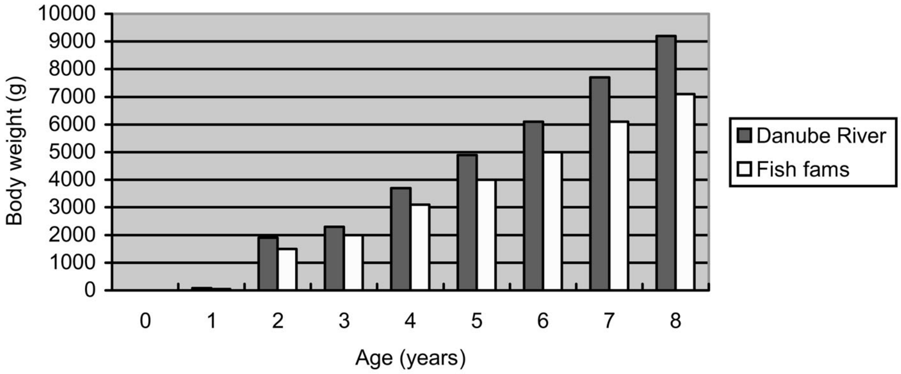


Figure 6. The grass carp dynamic of the growth in the fish farms and Danube River.

The other biological information regarding some large spawners of about 12 kg, which have completely developed gonads and a quite high gonad-mass ratio of 6.41 to 9.2, confirm again the possibility for the grass carp to reproduce in Danube River.

Some scattered observations related to the specific behaviour of the Asian carps, regarding the reproduction process, such as the crowding, indicated few particularly spots in Danube River which are considered the preferred places for spawning. There are few differences concerning the specific biology of each species of Asian carps, depending of the environmental conditions, such as the water temperature and the water deep. The grass carp spawns earlier, at a lower water temperature than silver carp and big head. Also, the silver carp and the grass carp seem to prefer the superficial waters as well as the big had stay in the deeper horizons of the water.

The main characteristic of the fresh water fish migration in Romanian Danube River is supposing to be the relative short migration way trough the ecosystems with similar environmental conditions. The few differences regard some parameters such as the water temperature and the amount of the solved oxygen, but only in some particular situations.

The fishes migrations depend not only of the needs related to reproduction but also of the need to assure the nutritional basis for the offspring and the availability of food for them self after a sensitive and high consumption of energy demanded by their movement and spawning A different type of migration, not yet studied in Danube River, could happen in the late fall and in the early winter when some species such as *Cyprinus carpio*, *Silurus glanis* and few other species crowd, for wintering, in some particular habitats with deep waters and reduced water flow.

The fish wintering is a very interesting adaptation of certain species that allow them to spend the cold season by reducing their metabolic rate and living mainly on their fat reserves cumulated in the warm seasons. That is an interesting modality to avoid the lack of food in winter and preserve the energy, common to many poikilothermous (cold-blooded) species.

The commercial fishermen, who know well the fish behaviours use some specially designed gears for fishing into the particular spots where the fish is crowded in the winter (which is not a normal fishing season in Danube River).

Typical migration of fishes

Most of biologists consider the migration of fresh water fish is in fact a semi-migration, mainly because the relatively short migration way and the little differences between the ecosystems they use to leave or to enter. As a result, these species of fish are often called the semi-migratory fishes.

The real or the typical migratory fishes are considered those species of fish that perform a long migration into the marine ecosystems or from marine to fresh water environments, either for spawning or feeding. These species are usually named the typical migratory fishes.

There are two categories of fish that perform long migrations to or from large rivers. The first category includes the catadromous fishes that spawn in the marine environment and spend most of their life for feeding and growing up in fresh water ecosystems at least until they reach the sexual maturity age. The second includes the anadromous migratory fishes, which spend most of their lifetime in seas or oceans and migrate to spawn in the fresh water environment.

In Danube River both catadromous and anadromous migratory fish species are present in certain period of time. The only one catadromous fish in Danube River is the eel (*Anguilla anguilla* L.).

At this time the number of the eels caught in Romanian Danube River is so less, as well as one individual every two or three years. There is the idea supposing the eel does not enter any more the river from the Atlantic Ocean in natural way but is sill present in a few places as a result of the stocking with elvers.

Much better represented in Danube River is the group of the anadromous migratory fishes which include mainly two fish families: Acipenseridae (sturgeons) and Clupeidae (shads).

The Sturgeons

The most important marine migratory sturgeons in Danube River belong to two genders: Huso, which is represented by beluga (*H. huso* Linnaeus, 1758) and Acipenser having two, quantitatively, more representative species: Russian sturgeon (*A. güldenstaedti* Brandt, 1833) and stellate sturgeon (*A. stellatus* Pallas, 1771). These species are common to the ecosystems of the Black Sea, Caspian Sea and Azovean Sea, being considered a relict species remained after the old Sarmatian Sea that formed before a huge marine basin, disappear in the Pliocene.

Beluga (Huso huso L.)

Beluga (*Figure 7*) is the largest migratory sturgeon in Danube River. Its huge size became legendary. The oldest data recorded in 1827 show one beluga of 1500 kg caught in Caspian Sea. The other data shows also few individuals weighting up to 1200 kg. In 1922 a female individual caught in the lower sector of the Volga River weighted 1220 kg. In 1924 another female caught in almost same place weighted almost 1228 kg and had 246 kg of roes (about 7.5 millions follicles) [16]. An individual caught in the Azovean Sea had an estimate age of 58 years old and weighted 640 g [4].

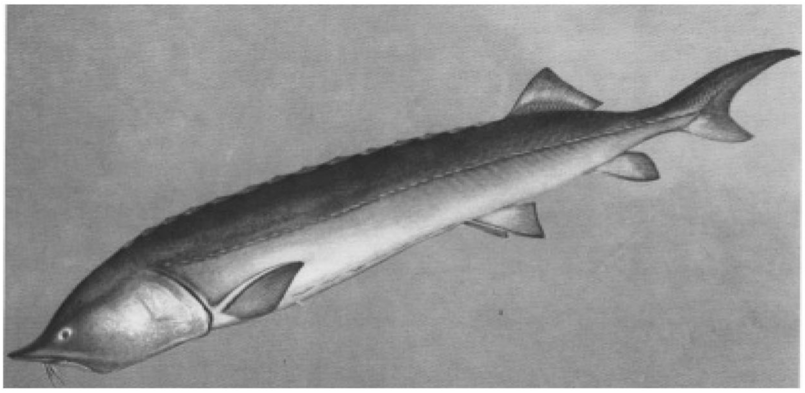


Figure 7. Beluga (Huso huso Linnaeus, 1758). Source: Manea, 1980.

There is also recorded information on very large individuals of beluga caught in Danube River. In 1890 has been caught in the Mouth of Saint George Branch of Danube one individual of 882 kg and later another one of 560 kg. The last one is preserved in Bucharest at the “Grigore Antipa” Natural History Museum. Some biologists consider credible captures of beluga individuals weighted up to 2000 kg and more than 6 m long [11]. The absolute longevity of beluga is also supposed to be up to 100 years. Beluga needs to live almost 75 years for reaching 1000 kg [12].

The adult individuals of beluga in Danube River usually size from 1.5 m to 3 m and weight from 45 kg to more than 100 kg. In the Black Sea belugas live in waters of 50 m to 100 m deep, some times deeper (up to 160 m).

The presence into the beluga stomach of certain amount of shells, mainly *Modiola phaseolina* that forms large populations on the bottom of sea at 60-70 m, could be a confirmation for the presence of beluga in these places. The belugas could be found also in certain places corresponding to some Romanian onshore localities at the Black Sea (Constanta Town, Agigea Town and Caliacra Cape) and even (less frequently) in some southern locations of the Bulgarian coasts. It is supposing that beluga is also wintering in similar places [5].

Beluga’s nutritional spectrum in the marine environment is different depending on the individuals’ age. In their very firs age of life, the belugas feed mainly on the inferior crustaceans. At the age of 2 years old beluga starts to eat also some mollusks for after that to become mostly fish eater. More than 80% of their food may consist in diverse species of fish. The large adults feed on fishes (mainly clupeids and also cyprinids) to the exclusion of different food and occasionally they are capable of hunting bigger pray as young Caspian seals [16].

Frequently, the belugas follow the flocks of the pelagic fishes for feeding on them. Some accidental captures of beluga in marine fish traps, specially designed for small pelagic fish, confirm that behaviour.

When became sexually mature, at a certain stage of the maturation of gonads, in spring months (March-April) or even much earlier [13] and also in the fall (September-October), beluga starts to crowd in the front of the Mouths of Danube River and swims upstream a little bit later.

The migration of the beluga in Danube River may start earlier than usual if the water temperature is higher than normal, in the early spring or in warm winters. In these situations the beluga swims upstream in January. The beginning of the first period of migration (spring migration) in Danube River generally happen at the end of February when water temperature is about 4-5°C [14]. Beluga is the first migratory sturgeon, which starts to migrate in Danube River, in front of Russian sturgeon and stellate sturgeon.

The intensity of the migration slowly decreases until early July, when only very few individuals can be found in Danube, for rapidly disappearing in the middle of July or the first days of August.

The second migration of beluga has not the same intensity as the first one. It starts in late summer and has a maximum of the intensity in October and November. The fall migration is more specific to the younger spawners and to the other individuals, which not fully accomplished the gonads maturation process. These immature sturgeons remain in the river and spend the winter in deep location. They will spawn in the next spring season.

Even there are some suppositions concerning a different behaviour, characteristic to some other biological forms of beluga, which are capable to spend more than one year in Danube River, there is not any documented data to prove it. Also the idea of two existing biological forms of beluga, the first one migrating in the autumn and the second one in the spring, has not scientifically proved arguments.

The differences regarding the age and the degree of the maturation of gonads between the individuals that compose the flocks in the spring, respectively in fall, can be rather do to the different precocity. The belugas spawn in Danube River on the rocky or sandy bottoms placed in the deep locations of 8 to 20 m, in the late spring when the water temperature is 13°C or more. These favourable places for the beluga reproduction are usually located into the upper sector of the Romanian Danube River and even in the upper Danube.

After 8 or 9 days, depending on the water temperature the young hatches and after another 8 days they start to actively feed. The beluga offspring slowly returns to Black Sea in late summer and in the fall. They usually remain in the front of the Mouths of the Danube until the late fall when start to swim to the deeper waters of the Black Sea, along the continental platform, for wintering.

Russian sturgeon (Acipenser güldenstaedti Brandt, 1833)

The second large species of sturgeon that migrates for spawning in Danube River is the Russian sturgeon (*Figure 8*). It has up to 2.4 m and weights 80-100 kg. Usually, the adult individuals size 101.5 m and weight 8-25 kg. Similar to beluga, the Russian sturgeon needs long time to become sexually mature: 8-12 years for males and 12-15 years for females.

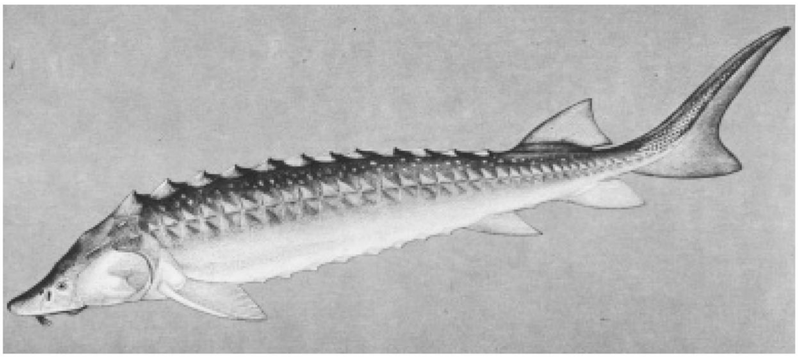


Figure 8 *. Russian sturgeon (Acipenser güldenstaedti Brandt, 1833). Source: Manea, 1980.*

Beside Black Sea, the Russian sturgeon can be found also in the Caspian Sea and Azovean Sea. Some biologists consider the existence of three subspecies and many biological forms [9]:

- *Acipenser güldenstaedti güldenstaedti* is considered the nominate form of this species, being common to the Northern Caspian Sea. It migrates for spawning in the Volga and Ural rivers.
- *Acipenser güldenstaedti persicus* lives the South of the Caspian Sea and migrates mainly into the Iranian rivers and Kura River.
- *Acipenser güldenstaedti colchinus* is the most frequent in Black Sea. It is supposing to have the following bio-morphological forms (natio): *A. güldenstaedti colchinus* natio colchica which migrates in the Caucasian rivers, *A. güldenstaedti natio tannaica* - the biological form of the Azovean Sea and *A. güldenstaedti colchinus natio danubicus* which migrates in Danube River.

The Russian sturgeon has also two periods of migration in Danube River, relatively balanced from the point of view of their intensity. In the spring, a maximum of the migration intensity is in April. In fall, the migration starts earlier and is the most intense in September.

The Russian sturgeons spawn in the late April or May and prefer for spawning the deep, rocky and sandy bottom places, similar to those of the beluga. There is some old information regarding the possibility for the Russian sturgeon to swim also into the main tributaries of the Danube, mainly in the Prut and Siret rivers [1].

After the reproduction period of time, the spawners head back to the Black Sea. The offspring feed on some aquatic worms and small crustaceans until they go back to the sea, in late summer. The nutritional spectrum of the adult individuals consist generally in molluscs up to 50%, crustaceans about 30% and fishes 20%.

Stellate sturgeon (Acipenser stellatus Pallas, 1771)

The stellate sturgeon (*Figure 9*) is the smaller marine sturgeon that migrates in Danube River. It is also the most frequent sturgeon in Danube and Black Sea. It can reach up to 1.1 m long and weights up to 30 kg. The stellate sturgeon may weight 60-70 kg.

There is the idea that stellate sturgeon has four subspecies:

- *Acipenser stellatus stellatus*, which can be found in the Northern areas of the Caspian Sea.
- *Acipenser stellatus natio* cyrensis located mainly in the Southern zones of the Caspian Sea.
- *Acipenser stellatus donensis*, which lives in the Azovean Sea and migrates for reproduction in the Don and Kuban rivers.
- *Acipenser stellatus ponticus* is a Black Sea endemic species, which include two populations with some distinct morphologic characters; the one migrates for spawning in the Dnepr River and the other one in the Danube River.

Into the Black Sea, the stellate sturgeon spends winter in not too deep locations, generally situated relatively close to the shore. It starts to migrate to Danube River in March or April when the water temperature is about 8°C. The maximum of the migration intensity is recorded generally in May. There is also a fall migration that has almost the same characteristics with the fall migration of the Russian sturgeon.

Figure 9. *Stellate sturgeon (Acipenser stellatus Pallas, 1771). Source: Rang et all., 2003.*

The stellate sturgeon spawns in almost the places similar to the other sturgeons: deep waters with the hard bottom and speedy water flow. Normally, the incubation of the roes takes 2-4 days, depending on the water temperature. The offspring remains in Danube River for feeding until the fall season when migrates to the Black Sea for wintering and growing up. Exceptionally, some young individuals can be found in the lower locations of the Romanian Danube River even in the winter. They may spend the winter in the river instead of the sea [5].

The sturgeons have complex life cycles that have been sporadically studied in the past 50 years. Their life cycle is reviewed in six stages: non-spawning adults, female spawners, male spawners, egg and larval, early juvenile, and late juvenile.

All species are long-lived, mature at advanced age. They have rapid and similar growth during the first few years of life and are feeding on similar taxa. They use deep river habitats for juvenile life stage deep marine locations for higher ages and have similar migratory patterns with seasonal concentration areas either in the sea before the moment of migration or in the river at the spawning time. These species of sturgeons differ however in ages and sizes at the maturity, in maximum size, time of spawning and migratory behaviours.

Their behaviour in marine habitats is sill not very well known but it is probably to make some coastal migrations restricted to the Black Sea area. The juveniles reside in the riverine habitats along the coastal areas, in front of the Danube Delta and in the southern areas.

The Danube shad

Another interesting anadromous migratory fish in Danube River is the Danube shad (*Alosa pontica* Eichwald, 1838). Its migration is different than the sturgeons' migration, being more related to the environmental factors as the water temperature and levels. Also the way of the Danube shad migration up the river is shorter.

Generally, the shads are herring-like fishes with a few differences regarding their external aspect. Belonging to the Clupeidae family, they are old forms of fish, attested from the Tertiary Period. They have populated also the former Sarmatian Sea, that can explain the presence of some species and biological forms that migrate for reproduction in rivers (even if most of clupeids, herrings for example, remain all the time into the marine environment).

Among the relict clupeids in that old area there are those belonging to the Black Sea, Sea of Azov and Caspian Sea. However, only few species enter in Danube River for spawning: *Alosa pontica, Alosa maeotica, Alosa nordmanni* and *Clupeonella delicatula.*

The Danube shad (*Figure 10*) lives in Black Sea. It is a pelagic species and prefers the Southern areas of the Black Sea. The adult individuals size about 40 cm and rarely weight more 1 kg.

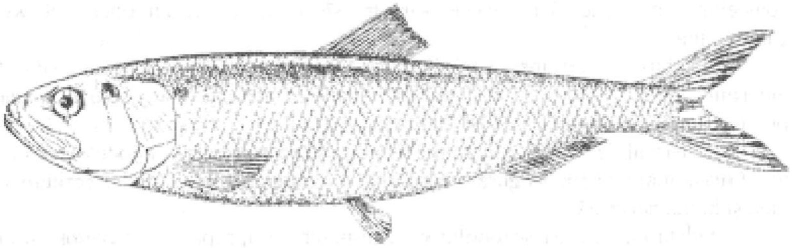


Figure 10. *Danube shad (Alosa pontica Eichwald, 1838). Source: Rang et all., 2003.*

Its behaviour in the marine environment is not well studied but it is known that in the winter, the Danube shad can be found along the South-Eastern Coast of the Black Sea in the relative deep locations (more than 40 meters) [1].

As the water become warmer, the Danube shads crowd closer to the Romanian coasts of Black Sea in the less deep areas. They start to swim into the Danube River when the water temperature becomes relative stable in the interval of 5 to 6°C. This happens usually in the last decade of March and the beginning of April. The maximum intensity of the Danube shad migration is recorded in April-May when the water temperature is about 9-13°C [7].

In addition to the water temperature the other important ecological factors for the migration intensity are water turbidity and the amplitude of the water level oscillations. A high degree of the water turbidity, quite usual in spring, in Danube River, can delay the beginning of the Danube shad migration. Also the unusual low water level has a negative impact on the migration start and the migration intensity.

The lastingness of the Danube shad migration is between 100 days and 150 days, depending on the temperature evolution, frequently 120-130 days. The migration is almost finished in June when the water temperature is about 19-20°C. Being a pelagic species, the Danube shad lays the spawn in the mass of the water. Depending to the water temperature, the incubation of roes takes from 43 to 72 hours.

The offspring enters the flooded areas for feeding and developing and leaves those areas as soon as the water level starts to decrease. An important amount of the offspring can be found in the Mouths of the Danube River areas almost in the same time in which the spawners return to the Black Sea, after the reproduction.

The spawning areas into the Danube River are located mainly up to Calarasi Town (*Figure 1*). Even there are recordings on Danube shad captures upstream this particular location (until the Mouth of Timok River), these captures are less important.

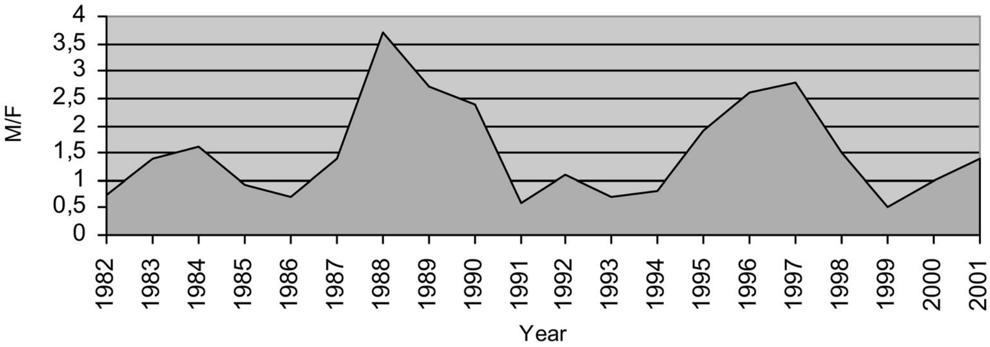
The structure of the Danube shad flocks shows spawners with different ages. From this point of view, the individuals of 3 and 4 years old represent the core of flocks. Together, they mean up to 90% of all adults that migrate in Danube for reproduction. There are also few older individuals of 5, 6 and exceptionally 7 years old. The younger individuals of 2 years old can be found in certain years in a larger number than usual, as a result of their precocity in the sexual development (probably do to some better environmental conditions in the Black Sea).

Based on these evaluations, most of the Danube shads are very possible to participate to the spawning process only once or twice. That could explain the reduced percentage of the older individuals in the migratory flocks. Generally, an important number of older spawners may to die after the reproduction, exhausted by the long way of the migration, some times more than 1200 km. Because the Danube shads do not feed them self during the migration period of about two months, the old individuals finish their fat reserves and are not strong enough to return into the sea. However, even exhausted, the younger spawners come back to the Black Sea and they can be found in June, in the coastal areas of the Romanian Black Sea offshore.

The studies on the dynamic of the captures show some particular aspects related to the migration intensity. It decreases or increases following a quite precise cycle of about 10 to 12 years. Nobody knows what precisely ecological factors are responsible for. They could be related to some cyclic changes in the meteorological factors, which periodically affect either the Danube shad populations in Black Sea or the success of the reproduction and the offspring survival in Danube River.

The variability of the Danube shad amount that migrates yearly can be also a result of some biological factors such as the value of the males-females ratio (M/F). The normal value of the M/F ratio is around the unit. In fact, it is a very variable value in the interval of 0.38 to 3.72, most frequently of 0.9 to 2.77 (*Figure 11*). It means a large difference between the number of the Danube shad females which participate to the reproduction year by year and, as a result, a different amount of offspring that will size the number of potential spawners in the next 3 or 4 years.

Figure 11. *Evolution of the Danube shad flocks M/F ratio from 1982 to 2001.*



The Danube shad fishery is one of most important fisheries in the Romanian Danube River and the actual tendency of the decreasing number of shads that migrate for reproduction, respectively, the smaller captures recorded in the last time are good enough motivations for interested people to be concerned about, and to find out the real causes of this decline.

There are a couple of smaller clupeids that migrate also from Black Sea for spawning in Danube River. Alosa caspia nordmanni is a species of shad similar but smaller than the medium sized Danube shad. It has a maximum length of 22 cm and rarely weights more than 600 g. It prefers warmer waters and enters the Danube River about two weeks later than the Danube shad, generally in April when water temperature is 9-12°C. It could be found up to Iron Gates II Dam and uses for spawning (in May and first decade of June) many different location, even the pools and lakes of the Danube Delta. The offspring return to the marine environment in the late summer and crowds the Mouths of the Danube area.

The Caspian sprat (*Clupeonella delicatula*) is the smallest species of clupeids that migrate in Danube River. It is also common to the Caspian Sea where reach its maximum size of 17 cm. In Black Sea, Caspian sprat sizes no more than 12 cm, usually 6-8 cm. It spawns starting from its very first year of life in May and June, using the pools and lakes of the Danube Delta and the flooded areas up to Iron Gates dams.

Some times, the Caspian sprat remains in the fresh water environment for wintering in the appropriate spots. It is a quite important commercial fish, it being caught with the seine nets, mainly in the shallow water of the Black Sea. The Caspian sprat is in addition the main food source for the large shads in the Black Sea.

The last environmental changes occurred over all Lower Danube sectors [14] and particularly in the Romanian Danube River, especially as a result of dam constructions either for energetic (Iron Gates dams) or agricultural (flooding plains damming) purpose, have seriously shortened and damaged normal migratory pathways. Following their natural instinct, the migratory fishes still swim upstream but they find less appropriate opportunities for spawning, feeding and developing. As a result, the fish stocks in Danube River are decreasing year by year. Adding to this general picture of fish fauna status other human interventions such as the over fishing, the poaching and the accidental or permanent pollution of certain areas of the river, it should be easy to understanding the decline of the migratory fishes and the other species of fish in the Romanian Danube River.

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J. Balogh & P. Balogh

IDENTIFICATION KEYS TO THE ORIBATID MITES OF THE EXTRA-HOLARCTIC REGIONS. VOLS. I–II.

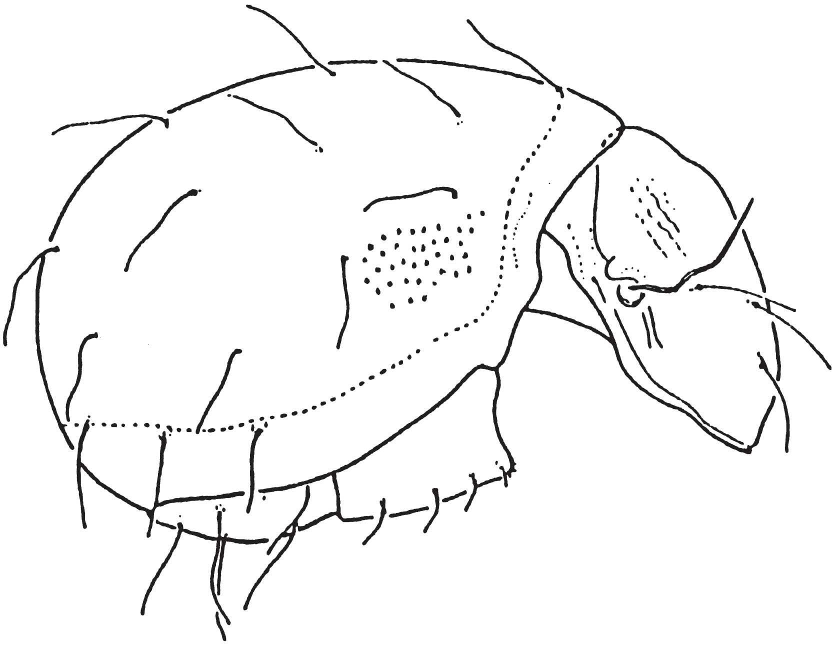
Well-PRess Publishing Limited, Miskolc, 2002; vol. I. 453 pp, vol. II. 504 pp.
ISBN 963862518x. Paperback, Available from Dr. P. Balogh, Eötvös Loránd University,
Dept. of Systematic Zoology and Ecology, H-1117 Budapest, Pázmány P. sétány 1/c, Hungary.
e-mail: baloghom@axelero.hu Price per copy EUR 80 (postage included).

The oribatid mites are one of the most speciose and most abundant group of arthropodes living at the soil-, ground-, moss- etc. level. In spite of the extensive research of outstanding specialists in the last few decades, obviously, only a small fraction of extra-Holarctic species has hitherto been described, and the actual number of species can hardly be estimated. The identification of the species is rendered more difficult due to the great number of the species and also to the small number of synthetizing works. Therefore, the oribatid mites can be identified almost exclusively by very specialized zoologists, mostly taxonomists; it is in close connection with the fact that our data on the ecology of this group are very limited.

Prof. János Balogh and his son Dr. Péter Balogh, with their extensive experience on Oribatida, now present the first practical guide for identification of all hitherto described extra-Holarctic oribatid mites. The background of the authors on oribatids is indicated – among others – by the fact that they are the describers (as author or co-author) of about 970 species included in the book – nearly 25% of all hitherto known extra-Holarctic species. Habent sua fata libelli – it is particularly true for this book. Tragically, during the compilation of the book the senior author died. The keys processed by him were not ready for press. However, the junior author corrected and completed his manuscript.

The primary objective of this book is to present keys to all known species and thereby to facilitate their identification and future research. Two bulky paperback volumes, 378 pages of identification keys for 3929 oribatid species and subspecies in volume I, and more than 6000 figures combined into 499 tables in volume II – these are the main data of this grandiose work. The work aims at completeness and is strikingly up-to-date; unfortunately, it is not mentioned, up to which year has the literature been processed, but some species described in the year of 2000 are also included.

Volume I contains (after a very short introduction) the identification keys. The keys are brief and are based mostly on only a few distinguishing characters. For each species, the type localities are given. The authors – with few exceptions – practically do not use higher taxa above generic level, the species are grouped into „groups". These groups are based mostly on few easy-visible characters, therefore most of them are totally practical and without taxonomical value (species belonging to the same genus are often divided into two or more groups). This method often makes the use of the keys easy; however, sometimes it is difficult to follow the hierarchy of the diverse „groups", which are divided into other “groups" and so on. Furthermore, some subordinate groups are not included in the key of their superior group. Nevertheless, the book contains almost any information on the higher systematics of Oribatida. Therefore, it would be very important and helpful to present a systematic list of the species treated in this book. The volume is supplemented with an index to scientific names. Unfortunately, not the generic and specific



names but their combinations are listed, which sometimes makes the finding of a name extremely difficult.

Volume II consists of an enormous compilation of figures, which increase the practicability of the keys considerably. Altogether 3754 species are figured. The figures are taken mostly from the original descriptions of the species; mainly habitus illustrations, and also a great number of diagnostic characters. Unfortunately, some figures are of poor quality.

The value of the book is raised by 128 species inquirendae and presumable synonyms listed, as well as numerous taxonomical and nomenclatural remarks and changes incorporated. A species is downgraded to subspecies, 2 specific and 1 family names are placed in synonymy, new status is established for 4 taxa, 17 preoccupied names are recognized and replaced with new names. (Unfortunately, it is not pointed out which name is replaced with „Paulinacarus sarkari nom. nov.".) The book also contains 7 „sp. n.". It is to be regretted that no descriptions and diagnoses are presented for these species; they are only incorporated in the identificaton keys.

The book does not treats the basic external morphology of the Oribatida. The reason for this is easy to guess: this information is readily accessible in numerous other works. However, a brief survey – at least a glossary of the setation of the notogaster, which plays an especially important role in the taxonomy of Oribatida – would be helpful mainly for students in developing countries that are without library resources. The compilation of the list of references, the most important ones at least, would be also helpful for similar reasons.

It is a pity that the book contains several mistakes (even in scientific names!). Some keys can not or only with great difficulties due to their erroneous compilation (e.g. on pages 34, 111, 112, 179–180). Furthermore, some keys contain missing items (e.g. on pages 304, 315, 317) or run out to missing specific names (e.g. on pages 106, 108, 110). The great number of mistakes as well as the numerous «???» insertions in the text suggest that the final proofing of the manuscript was not thorough enough.

In spite of all its faults, this work is highly recommended not only to all students and workers concerned with Oribatida but also to the ones interested in soil ecology. The advantages of the keys, completed with the extensively collected figures, are self-evident: having this information compiled together makes the identification of the species of this important group far easier, especially for non-specialists like ecologists – and the correct identification of species is the underpinning for all other zoological investigations. We are sure that this work could efficiently facilitate not only the taxonomical but the ecological investigations on this group of arthropodes in the future. Moreover, it would highly contribute to increasing our knowledge on the ecology of the soil-living animal communities.

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