ROMANIAN JOURNAL OF BIOLOGY

ZOOLOGY

VOLUME 57, N^{o} 2 2012

CONTENTS

INTERPOPULATION DIFFERENCES OF FEMALE BODY SIZE AND CLUTCH SIZE IN *CHIROCEPHALUS SHADINI* (BRANCHIOPODA: ANOSTRACA)

DEMETER LÁSZLÓ[*], IOAN CĂRĂUŞ[**], ETELKA TÖRÖK[*]

Large between-population body size and up to an order of magnitude fertility difference was observed in an Anostraca species in the Eastern Carpathians. In this preliminary report we statistically compare several populations and discuss possible causes of this striking variability.

Key words: interpopulation differences, *Chirocephalus shadini*, *Drepanosurus hankoi*, fertility.

INTRODUCTION

Intraspecific life-cycle differences such as the variability of fertility have been observed in both vertebrates and invertebrates (Lack, 1967; Eldridge & Kapu, 1988; Drobney, 1991; Austin, 1998; Skow & Jakobs, 2003). Variations originate from environmental or genetic factors or both, fact which complicates their study (Austin, 1998). In Anostraca, it has been shown in a number of laboratory studies that food type affects life-history variables (*e.g.*: Maeda-Martinez *et al*., 1995; Ali *et al.*, 1999; Sarma *et al*., 2005). On the other hand, there are few studies on the intraspecific life history variations of wild populations in relation with environmental factors. The effect of habitat duration has been studied on growth rate, life span, sex ratio (Mura *et al*., 2003) and hatching phenology (Zarattini, 2004) in two *Chirocephalus diaphanus* populations. Belk *et al.* (1990) studied the relationship between egg size, body length and habitat type for *Streptocephalus seali*, but did not analyze clutch size variability. In a study of several Anostraca species, Mura (1992) found intraspecific differences that she attributed to biotope factors, and she observed differences in the egg production of laboratory and wild populations.

Chirocephalus shadini is a Western Palearctic cold-stenothermal fairy shrimp distributed from Austria to the Ural (Löffler, 1993; Vekhoff, 1993; Eder *et al.*, 1997). It was only recently found in Romania, in three mountain basins of the Eastern Carpathians (Demeter, 2004, 2005). While collecting the species from different habitats, we observed body size differences between the studied populations. The purpose of this study is to analyze (1) whether there is a true (statistical) difference between populations of *Chirocephalus shadini* regarding size (body length) and fertility (clutch size) and (2) whether the difference can be

explained by the difference of algal species composition and algal biomass between the habitats.

MATERIAL AND METHODS

We measured body length from the tip of the head to the end of *furca* to the nearest 0.01 mm with a hand calliper under a stereomicroscope. Some authors measure head to anus length excluding the furca due to possible variation in relation to environmental conditions (Mura, 1993), but there are studies where head to furca length was measured (*e.g*. Belk *et al.*, 1990). We counted egg number by opening the egg sac of fixed specimens, using 10-30X magnification. Samples were collected from 7 temporary ponds (totally 101 females, mean sample size 14.4 females/habitat) in the Lower Ciuc Basin (N 46^{o} 17', E 25^{o} 53'), between 2–5 May 2005 (Fig. 1). Habitats are situated on the floodplain (2 habitats: V1, V2 on Fig. 1, 640 m altitude) and on the second terrace (5 habitats: T1-T5 on Fig. 1, 650 m altitude) of the Olt River.

The seven habitats are considered to correspond to as many populations. The distance between the two temporary ponds on the floodplain site is approximately 400 m and on the terrace site it varies between 50 and 200 m (Fig. 1). Although the distance in the case of the ponds situated on the terrace is smaller, gene flow is probably lower than in the floodplain, where the Olt River floods in every 3–5 years.

Evaluating fertility in wild populations is difficult because the eggs may be released from the ovisac gradually and due to stress during collection and fixation (Daborn, 1976), and also egg production and maturation is continuous in species with a longer life span than 2 months (Anaya-Soto *et al.*, 2003; Sarma *et al*., 2005). The egg deposition procedure of *Chirocephalus shadini* is not known. Due to the fact that it is cold-stenothermal (Eder *et al*., 1997) with a short life cycle, we presume that egg maturation is simultaneous. We tried to avoid bias due to egg loss during collection by excluding females with an empty or near-empty ovisac from the analysis. We assumed that stress and consequent egg loss was equal in each sample. Another assumption in the analysis of egg number is that the females of different populations are of similar age and consequently in a similar phase of egg deposition (so that their egg numbers are comparable). All the eggs found in the examined specimens were mature. Mura (1992) considers that only similar size classes are comparable regarding fertility. Our approach is that mature individuals of different size classes are the result of environmental or genetic factors in a similar way as fertility, and consequently, they can be compared.

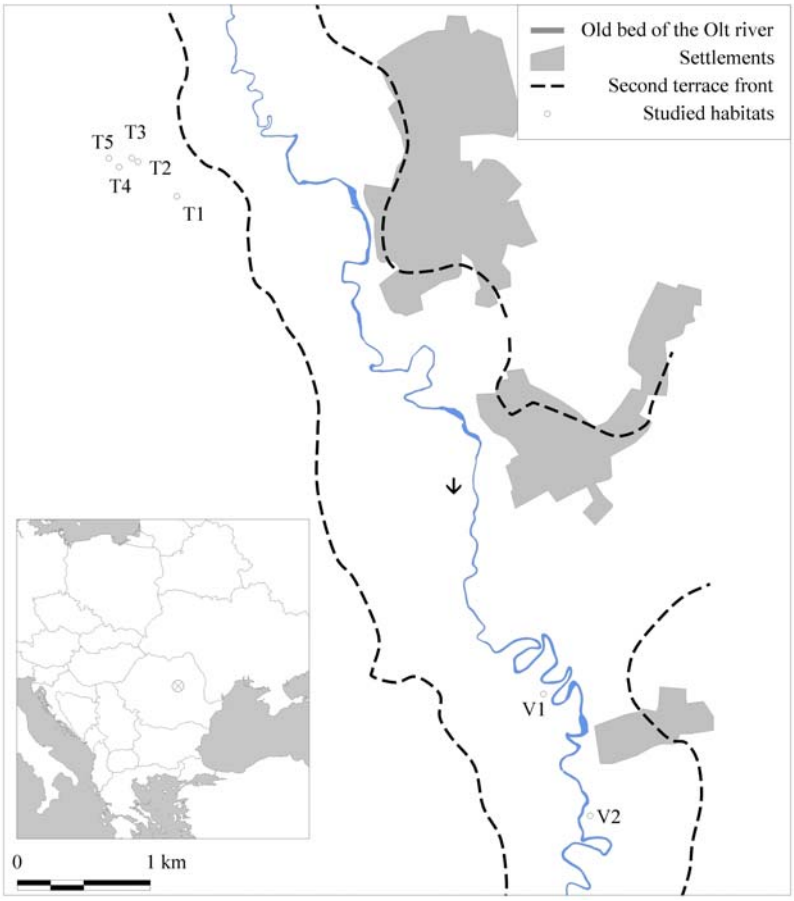


Fig. 1. Location of the studied habitats.

To quantify a possible environmental effect that causes differences between populations, we examined algal samples from 5 habitats, regarding species composition and the biomass of dominant species. Algal samples were collected by fixing one liter of pond water with Utermoehl solution, followed by sedimentation and decantation in the laboratory. Algae were identified by Cărăuş Ioan based on Starmach (1974), Nagy-Tóth & Barna (1998) and Godeanu (2002).

One-way ANOVA was run on means to show up between-habitat differences, while ANCOVA was used to eliminate the effect of body length in testing for differences in the number of eggs. Post-Hoc comparisons were made with Scheffe test. Log transformation was used. The collected data were used to estimate the needed sample size to achieve statistical power of 0.80 (Cohen, 1988). To check the accuracy of data the 95% confidence interval width was fixed at 25% of the expected value.

RESULTS AND DISCUSSION

Body length differed significantly among sampling sites ($F_{[6,\ 94]} = 40.59$, $P < 0.00001$) as well as the number of the eggs ($F_{[6,\ 94]} = 42.95$, $P < 0.00001$). Five populations had a mean body length of between 11.8 and 14.4 mm and two populations (T3 and V1) between 17.6 and 18.3 mm (Table 2). Mean egg number was between 19.1–50.6 mm for five populations and between 128.6–174.8 mm for two populations (Table 2). We found a significant positive correlation between average body length and egg number of the habitats (Spearman $r = .97$, $p < 0.001$, $n = 7$) and for all the data (Spearman $r = .85$, $p < .0001$, $n = 101$).

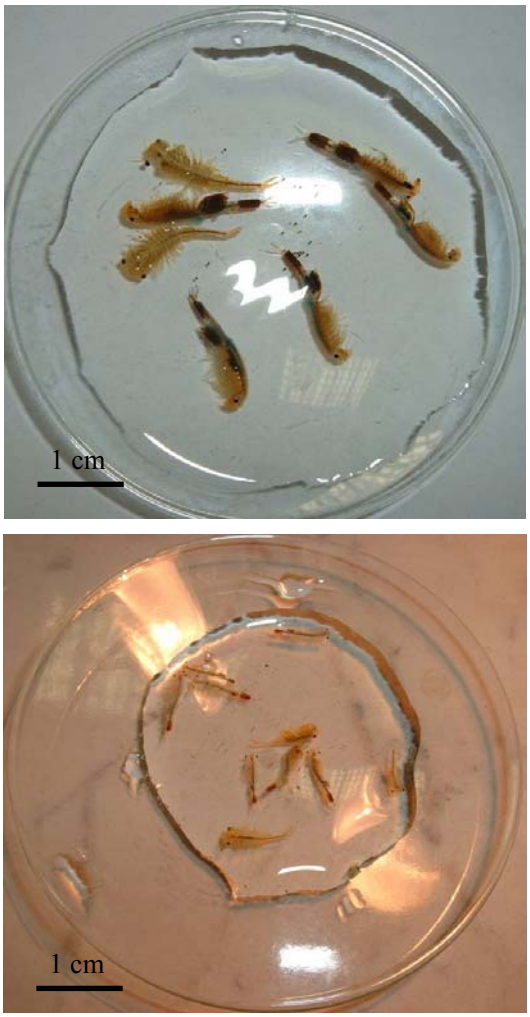


Fig. 2. Body size difference between two *Drepanosurus hankoi* populations.

Table 1

Results of post hoc tests on body length and clutch size of *Chirocephalus shadini* samples

ANOVA for body length

Site	T1	T2	T3	T4	T5	V1	V2
T1		0.55	<0.001	0.04	0.51	<0.001	0.76
T2			<0.001	<0.001	<0.01	<0.001	0.02
T3				<0.001	<0.001	0.99	<0.001
T4					0.95	<0.001	0.69
T5						<0.001	0.999
V1							<0.001
V2							

ANOVA for clutch size

Site	T1	T2	T3	T4	T5	V1	V2
T1		<0.001	<0.001	0.9999	0.992	<0.001	0.54
T2			<0.001	<0.001	0.004	<0.001	0.058
T3				<0.001	<0.001	0.89	<0.001
T4					0.95	<0.001	0.36
T5						<0.001	0.96
V1							<0.001
V2							

ANCOVA for clutch size

Site	T1	T2	T3	T4	T5	V1	V2
T1		<0.001	<0.001	0.9999	0.99	<0.001	0.48
T2			<0.001	<0.001	0.002	<0.001	0.04
T3				<0.001	<0.001	0.87	<0.001
T4					0.94	<0.001	0.3
T5						<0.001	0.95
V1							<0.001
V2							

Dominant algal species were *Volvox globator,* with an exceptionally large biomass of 2333.6 mg/l in habitat T1, and *Cryptomonas reflexa*, *Cryptomonas erosa*, *Cryptomonas marssonii, Chroomonas nordstedtii* and *Chroomonas caudata* with biomass values between 0.43–2.99 mg/l in the other four habitats. The low sample size does not allow a reliable statistical analysis, but both body length and egg number seem to be positively correlated with algal biomass for 4 habitats where unicellular algae dominate. Body length and egg number is low in the habitat where *Volvox* dominated. Due to the large differences in the algal species composition between habitats, we were unable to identify any association between body length/egg numbers, respectively algal species composition.

Post-hoc tests show that there are significant differences between around 50% of habitat pairs considering body length and clutch size (Table 1). The number of eggs differs among sampling sites even after the removal of the effect of body length ($F_{[4, 64]} = 7.97$, $P = 0.00002$). Body length shows low variability while the variance is higher at the number of eggs (Table 2).

Table 2

Descriptive statistics for body length and egg number of *Chirocephalus shadini* and algal biomass values of the studied habitats

Site	N	Mean	SD	95%CI	95%CI	Mean	SD	95%CI	95%CI	Algal biomass (mg/l)
	body length					egg number				
T1	17	13.36	1.25	12.86	13.88	19.23	11.56	13.43	25.04	2333.6
T2	17	14.39	1.45	13.88	14.91	50.64	15.39	44.84	56.46	1.14
T3	8	18.26	0.72	17.52	19.02	174.75	19.51	166.28	183.22	2.99
T4	15	11.75	0.70	11.21	12.3	15.73	6.04	9.55	21.92	0.43
T5	13	12.29	0.57	11.71	12.89	19.07	4.49	12.43	25.72	1.12
V1	14	17.61	1.2	16.97	18.23	128.64	35.03	110.27	146.93	no data
V2	17	12.69	1.95	11.77	13.63	26.76	12.3	20.95	32.65	no data

Sample size calculations show that an ideal sample should consist of 50 individuals per habitat. We are not aware of previous studies that focus on interpopulation differences of clutch size in wild populations of large branchiopods. Our data show significant differences regarding body length and clutch size between *Chirocephalus shadini* populations in a small spatial scale. The difference in clutch size can be one order of magnitude. The small sample size and the heterogeneity of the algal species composition did not allow to identify a significant role of diet in causing these differences, but such a relationship is probable based on laboratory studies (Ali *et al*., 1999). The weakness of this study is that we do not have data on the egg deposition process of *Chirocephalus shadini*, and continuous egg loss may cause bias when comparing populations or individuals of different age. These should be clarified in further studies. However, the significant difference of body size alone suggests real differences between the populations, and the phenomenon has been observed in our study area in the case of another fairy shrimp species, *Drepanosurus hankoi* (Fig. 2). Further studies should attempt to clarify what habitat factors cause the differences, and whether the differences are constant in time, *e.g*. there are "better populations" or the variations change from year to year in a single habitat.

Acknowledgements. Thanks go to Nemes Szilárd for his help with the statistical analysis. The comments of Prof. Graziella Mura and Dr. Hartel Tibor were greatly appreciated.

REFERENCES

ANAYA-SOTO A., SARMA S.S.S., NANDINI S., 2003, *Longevity of the freshwater anostracan Streptocephalus mackini (Crustacea: Anostraca) in relation to food (Chlorella vulgaris) concentration*. Freshwater Biol., **48**: 432-439.

ALI A.J., SARMA S.S.S., DUMONT H.J., 1999, *Cyst production in the fairy shrimp, Streptocephalus proboscideus (Anostraca) in relation to algal and loricated rotifer diet.* Crustaceana, **72**: 517-530.

AUSTIN C.M., 1998, *Intraspecific variation in clutch and brood size and rate of development in the yabby, Cherax destructor (Decapoda: Parastacidae)*. Aquaculture, **167**: 147-159.

BELK D., ANDERSON G., SHEAU-YU H., 1990, *Additional observations on variations in egg size among populations of Streptocephalus seali (Anostraca)*. Journal of Crustacean Biology, **10** (1): 128-133.

COHEN J., 1988, *Statistical power analysis for the behavioral sciences*. 2nd Edition, New York, Lawrence Erlbaum Associates, 567 pp.

DEMETER L., 2004, *The spatial distribution of three species of Anostraca in the Ciuc–basin*. Studii şi Cercetări Ştiinţifice, Seria Biologie, Universitatea Bacău, **9**: 42-47.

DEMETER L., 2005, *Spatial distribution patterns and conservation status of large branchiopods in the Ciuc Basin, Romania.* Acta Biol. Debr. Oecol. Hung., **13**: 73-84.

DABORN G.R., 1976, *The life-cycle of Eubranchipus bundyi (Forbes) (Crustacea: Anostraca) in a temporary vernal pond of Alberta*. Can. J. Zool., **54:** 193-201.

DROBNEY R.D., 1991, *Nutrient limitation of clutch size in waterfowl: is there a universal hypothesis*? The Condor, **93**: 1026-1028.

EDER E., HÖDL W., GOTTWALD R., 1997, *Distribution and phenology of large branchiopods in Austria*. Hydrobiologia, **359**: 13-22.

ELDRIDGE J.L., KAPU G.L., 1988, *The influence of diet quality on clutch size and laying pattern in mallards*. The Auk, **105**: 102-110.

GODEANU S.P. (ed.), 2002, *Diversitatea Lumii Vii – Determinatorul ilustrat al florei şi faunei României*, Vol. II – Apele continentale (partea 1, 2), Edit. Bucura Mond, Bucureşti, 489 pp.

LACK D., 1967, *The significance of clutch size in waterfowl*. Wildfowl, **18**: 125-128.

LÖFFLER H., 1993, *Anostraca, Notostraca, Laevicaudata and Spinicaudata of the Pannonian Region and in its Austrian area*. Hydrobiologia, **264**: 169-174.

MAEDA-MARTINEZ A.M., OBREGÓN-BARBOSA H., DUMONT H., 1995, *Laboratory culture of fairy shrimps using baker's yeast as basic food in a flow-through system*. Hydrobiologia, **298**: 141-157.

MURA G., 1992, *Preliminary testing of Anostraca from Italy for use in freshwater fish culture*. Hydrobiologia, **241**: 185-194.

MURA G., 1993, *Habitat and life history of Chirocephalus marchesonii Ruffo & Vesentini 1957, an endemic fairy shrimp from Monti Sibillini, Central Italy.* Riv. Idrobiol., **32**: 73-104.

MURA G., FANCELLO G., DI GIUSEPPE S., 2003, *Adaptive strategies in populations of Chirocephalus diaphanus (Crustacea, Anostraca) from temporary waters in the Reatine Apennines (Central Italy)*. J. Limnol., **62** (1): 35-40.

NAGY-TÓTH F., BARNA A., 1998, *Alge verzi unicelulare (Chlorococcales).* Determinator. Presa Universitară Clujeană, Cluj, 200 pp.

SARMA S.S.S., BELADJAL L., NANDINI S., CERÓN-MARTÍNEZ G., TAVERA-BRISEÑO K., 2005, *Effect of salinity stress on the life history variables of Branchipus schaefferi Fisher, 1834 (Crustacea: Anostraca)*. Saline Systems, Jul. 4; 1: 4.

SKOW C.D., JAKOB E.M., 2003, *Effects of maternal body size on clutch size and egg weight in a pholcid spider (Holocnemus pluchei)*. The Journal of Arachnology, **31**: 305-308.

STARMACH K., 1974, *Cryptophyceae-Kryptofity. Dinophyceae-Dinofity. Raphidophyceae-Rafidofity.* In: *Flora slodkowodna Polski.* Tom **4**, Starmach K. & Sieminska J. (Eds), Warszawa, Kraków: Panstwowe Wydawnictwo Naukowe, 520 pp.

VEKHOFF N.V., 1993, *The fauna and zoogeography of fairy and tadpole shrimps of Russia and adjacent lands (Crustacea: Anostraca, Notostraca)*. Arthropoda Selecta, **2** (3): 11-42.

ZARATTINI P., 2004, *Intraspecific differences in hatching phenology of the fairy shrimp Chirocephalus diaphanus Prévost, 1803 (Crustacea: Anostraca) in relation to habitat duration*. J. Limnol., **63** (Suppl. 1): 85-89.

Received August 28, 2012

[*]*Sapientia University*
Department of Technical and Nature Sciences
530104 Miercurea Ciuc, P-ţa Libertăţii, nr. 1, Romania
e-mail: domedve@gmail.com
[**]*University of Bacău, Department of Biology, 600115, Bacău, Calea Mărăşeşti, nr. 157, Romania*
e-mail: carausi@yahoo.com

VERTICAL DISTRIBUTION OF ANOSTRACA RESTING EGGS IN RELATION TO OTHER DORMANT INVERTEBRATE FORMS AND PLANT SEEDS IN THE SEDIMENT OF A TEMPORARY POND (CIUC BASIN, EASTERN CARPATHIANS, ROMANIA)

DEMETER LÁSZLÓ[*], JAKAB GUSZTÁV[**], GABRIELLA PÉTER[*], ALAIN THIÉRY[***], PÁSZTOHY ZOLTÁN[****]

We studied the vertical distribution of an Anostraca egg bank in the sediment of a temporary pond. Simultaneously, we extracted other invertebrate dormant forms (Cladocera ephippia, Bryozoa statoblasts) and plant seeds (*Lemna* sp. and *Potentilla supina*). The studied pond bed was formed through landslides on permafrost in the Early Holocene. Increased infilling of the pond happened in the last 1000 years probably related to massive deforestation in the watershed. Anostraca (*Chirocephalus shadini*) eggs were the deepest found remains in the studied cores, and it seems that they were present in this habitat throughout the pond's history, in spite of a longer hydroperiod in the earlier phase. The egg bank documented here is the deepest known for Anostraca.

Key words: Anostraca, Bryozoa, Cladocera, egg bank, seed bank, temporary pond.

INTRODUCTION

Egg banks and seed banks play an important role in the ecology of plant and invertebrate communities adapted to temporary ponds, and they constitute an archive of the community history of these habitats (Brendonck & DeMeester, 2003). Eggs and seeds are analogous structures and they display similar adaptations that ensure survival in unpredictable habitats like prolonged dormancy (Simovich & Hathaway, 1997; Brendonck & DeMeester, 2003). Both structures are widely used in paleoecology (Frey, 1964; Grosse-Brauckmann, 1972; Birks, 1980; Birks & Birks, 1980; Wasylikowa, 1996; Beaudoin, 2007; Mauquoy & Van Geel, 2007; Muller *et al*., 2008). Egg banks have been used in assessing species richness of zooplankton as an alternative to active community sampling or hatching ephippia (Vandekerkhove *et al*., 2004) and for completing information about the community (Beladjal & Mertens, 2003).

The eggs of Anostraca are often morphologically distinct (Alonso & Alcaraz, 1984; Thiéry & Gasc, 1991), although in some cases their identification is difficult because of large cyst variability (Mura, 1991, 1992; Beladjal & Mertens, 2003). In studies of Anostraca, usually only the top 3 cm is considered to be the active egg bank (Brown & Carpelan, 1971; Eriksen *et al.*, 1986; Thiéry, 1997) and Mura

(2004) considers that through disturbance, the eggs found in the upper 10 cm have the chance to get to the surface and hatch. However, data on the lower limit of the active egg bank and the transition between the active egg bank and subfossil egg remains are not available.

The horizontal distribution of Anostraca egg banks was studied by several researchers (Thiéry, 1997; Brendonck & Riddoch, 2001; Maffei *et al*., 2002; Mura, 2004), but very few studies are available on the vertical distribution of Anostraca resting eggs (Mura, 2004; Hulsmans *et al*., 2006). Quaternary paleoecological studies rarely use Anostraca remains (Lundquist, 1936), because mainly Tertiary and older remains of this group are known (Frey, 1964).

The goal of this study is to find out the depth and age of Anostraca egg bank in relation to other macro-remains in the sediment of a temporary pond in the Ciuc Basin, Eastern Carpathians, Romania.

MATERIAL AND METHODS

STUDY AREA AND HABITAT

The studied temporary pond (Fig. 1) is situated in the Ciuc Basin, which is one of the large tectonic mountain basins of the Eastern Carpathians, Romania. The Ciuc Basin has a bottom altitude between 640 m and 750 m, it is surrounded by 1000-1800 m high mountains. It represents the upper catchment area of the Olt river. The central part of the basin (up to approximately 800 m altitude) is occupied by agricultural land represented by a mixture of arable fields, hay fields and pastures. The surrounding mountains are to a large extent covered by coniferous and mixed forests.

The studied pond is situated at 671 m above sea level, near the town Ciceu (46.4^{o}N, 25.79^{o}E). It has a maximum surface area of 800 m^2 and a hydroperiod of approximately 2 months per year beginning at the end of February. Its current vegetation contains *Carex vesicaria*, *C. acuta*, *Lysimachia nummularia, L. vulgaris, Potentilla anserina, Scirpus silvaticus*. It is surrounded by small scale crop fields and it is usually mown. Its sediment is black peat-mud between 0–110 cm, grey silty lacustrine sediment between 110–250 cm and gravel under 250 cm. The black mud has a larger fraction of very fine sediment (<0.02 mm) and a smaller fraction of coarse particles (0.2–2 mm) (Fig. 2). The black peat-mud with varying organic content is rich in plant and animal remains, while no remains were found in the grey silty lacustrine sediment using a stereomicroscope.

Currently, the pond is inhabited by the Anostracans *Chirocephalus shadini*, *Drepanosurus hankoi*, the Notostracan *Lepidurus apus*, calanoid copepod *Hemidiaptomus amblyodon*. Other zooplankton groups were not studied. Bryozoans and the plant *Lemna trisulca* were not detected in the habitat, perhaps because the present hydroperiod is too short for these organisms.

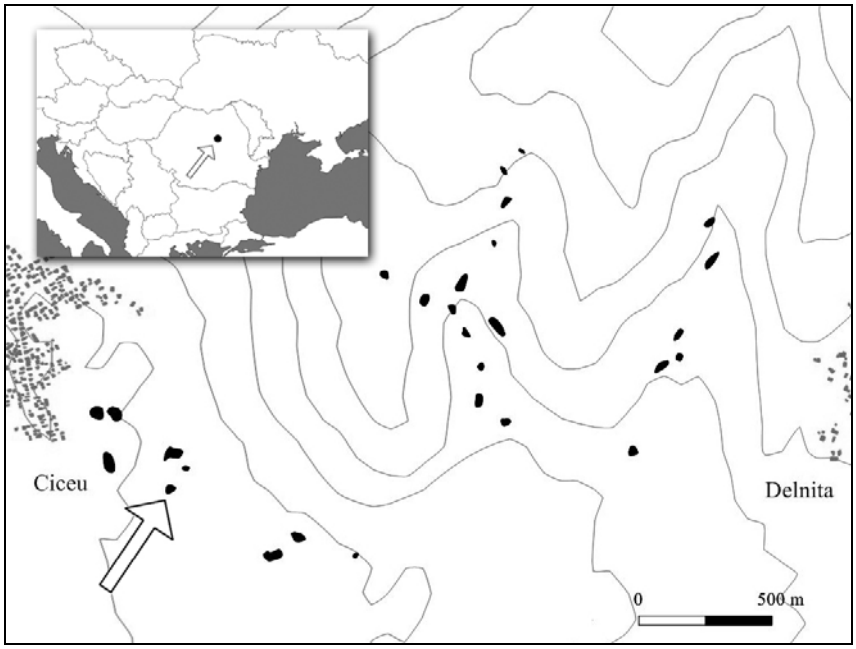


Fig. 1. Location of the studied pond in Romania (small picture), and within a pond site in the Ciuc Basin. Black areas represent ponds, gray areas are buildings, lines are hypsographic curves (between 670 m on bottom left and 740 m on top right). The arrow in the left bottom shows the studied habitat.

FIELD AND LABORATORY METHODS

Two undisturbed sedimentary sequences were taken from the temporary pond using a 5 cm diameter Russian type corer (Jowsey, 1966) in 2008. Coring was carried out in the central part of the bog, now occupied by a tall sedge fen. The first core was 150 cm deep with 110 cm black mud, and the second core was 83 cm deep black mud. The cores were sliced into 5 cm thick segments. Three subsamples from each core segment of 7 cm^3 or 10 g were soaked in tap water for 24 hours. Then they were filtered through a 1 mm mesh size filter to retain large particles. The fraction that passed the filter was diluted to 1000 cm^3 tap water. After a few seconds of sedimentation the floating fraction was filtered through plankton net with 80 μ mesh size, the filtrate was transferred to an eyeglass and observed through a stereomicroscope at 15–60 × magnification. The remaining sediment was thoroughly inspected under the stereomicroscope.

The Psimpoll (Bennett, 1992) software was used for plotting data. Plant seeds were identified using Bojňanský & Fagarašová (2007).

Five types of remains were distinguished: Anostraca eggs (*Chirocephalus shadini*, *Drepanosurus hankoi*), Cladocera ephippia, Bryozoa statoblasts, seeds of *Lemna sp.* and *Potentilla supina*. Anostracan eggs were identified by comparing the size and surface pattern of eggs extracted from the sediment with eggs collected

from adult females, and using Nagorskaja *et al.* (1998). There is a clear size and surface pattern difference between *C. shadini* and *D. hankoi* at the magnification range used by us. Cladocera ephippia were determined to the genus level (*Simocephalus*, *Ceridodaphnia*) using Vandekherkhove *et al.* (2004) or were classified according to their size and morphology. The extracted material was preserved in 70% ethyl-alcohol. The amount of remains is given for 10 cm^3 on the diagrams.

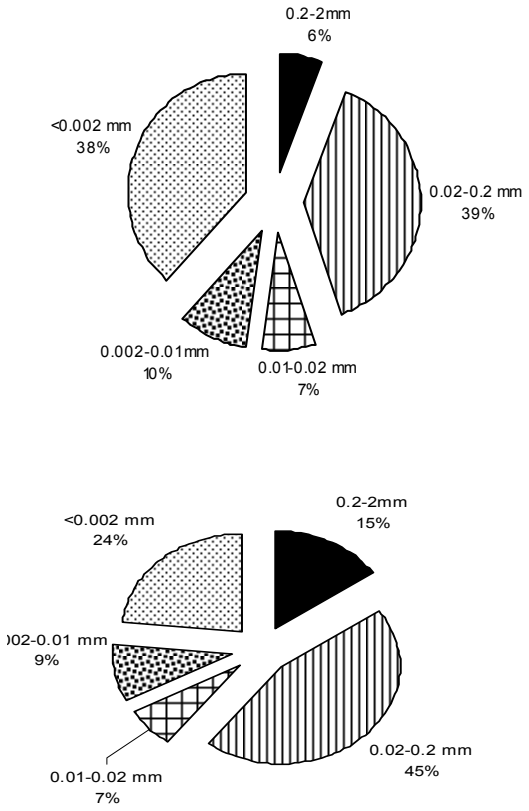


Fig. 2. Particle composition of the cores of pond sediment at two depths (80–100 cm on the left and 140–160 cm on the right).

Sediment samples from three depths of the first core (4–5 cm, 62–63 cm and 94–95 cm) were AMS (Acceleration Mass Spectroscopy) radiocarbon dated by the Poznań Radiocarbon Laboratory. At our request, the measurement of the 62–63 cm

sample was repeated. The measurement accounted for carbonate contamination thus decreasing the reservoir effect (Libby, 1962).

RESULTS

The first step of our extraction method (filtration) yielded between 71–85 % of Anostraca eggs, 72–73 % of *Lemna* seeds, 100 % of Bryozoa statoblasts, 94–100 % of Cladocera ephippia and 76–85 % of *Potentilla supina* seeds (Table 1).

Table 1

The compared efficiency of the two steps of the extraction in the two cores (marked for example as *Lemna* 1 and *Lemna* 2).
Step 1: flotation and filtration of the suspension;
Step 2: direct search of the sediment (see Methods)

	Step 1 (%)	**Step 2 (%)**	**N**
Anostraca 1	84.4	15.6	58
Anostraca 2	71.8	28.2	117
Lemna 1	72.2	27.8	36
Lemna 2	72.8	27.2	202
Bryozoa 1	100	0	34
Bryozoa 2	100	0	33
Cladocera 1	94.4	5.6	36
Cladocera 2	100	0	48
Potentilla 1	76.9	23.1	26
Potentilla 2	84.2	15.8	19

The maximum depths in which remains (eggs or seeds) of the selected organisms were identified vary between 65 and 105 cm in the first core and between 45 and 85 cm in the second core. The maximum amount of remains varies between 5 and 14 per 10 g in the first core and 5 and 37 per 10 g in the second core (Figs. 3, 4).

In the first core the two organisms found at greatest depth (100–105 cm) are Anostraca eggs and *Lemna* seeds. The Anostraca eggs show a more continuous distribution. In the second core Anostraca eggs were found at the greatest depth (80–83 cm). The distribution of Anostraca, Bryozoa and *Lemna* peaks was between 35–50 cm in the first core and 40–50 cm in the second core.

Cladocera have a fluctuating vertical distribution in both cores, with a peak on the surface in the second core. *Potentilla* has a high peak on the surface in the first core and a more gentle decrease in the second core.

Lemna seeds were missing from 0–15 cm in the first core and they were very rare between 0–20 cm in the second core (Figs. 3, 4).

The results of the radiocarbon measurement analysis of the sequence described in this study are shown in Figs. 3, 4. The age-depth relationship of core 1 is presented on Fig 3. It is apparent from the age-depth curve that the rate of sediment accumulation above 60 cm is much higher compared to the rest of the sequence. The age of upper sediment level (300 ± 25 yr BP) suggests some sediment hiatus.

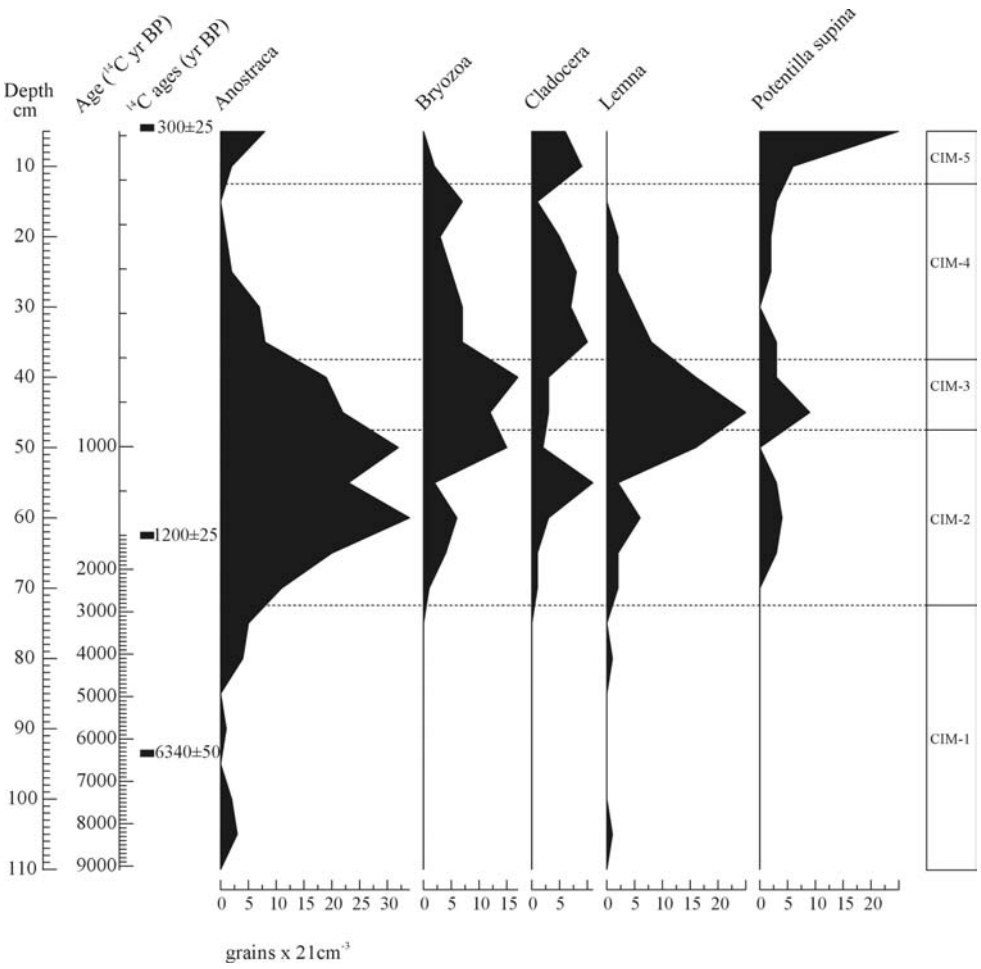


Fig. 3. The vertical distribution of the identified remains in core nr. 1.

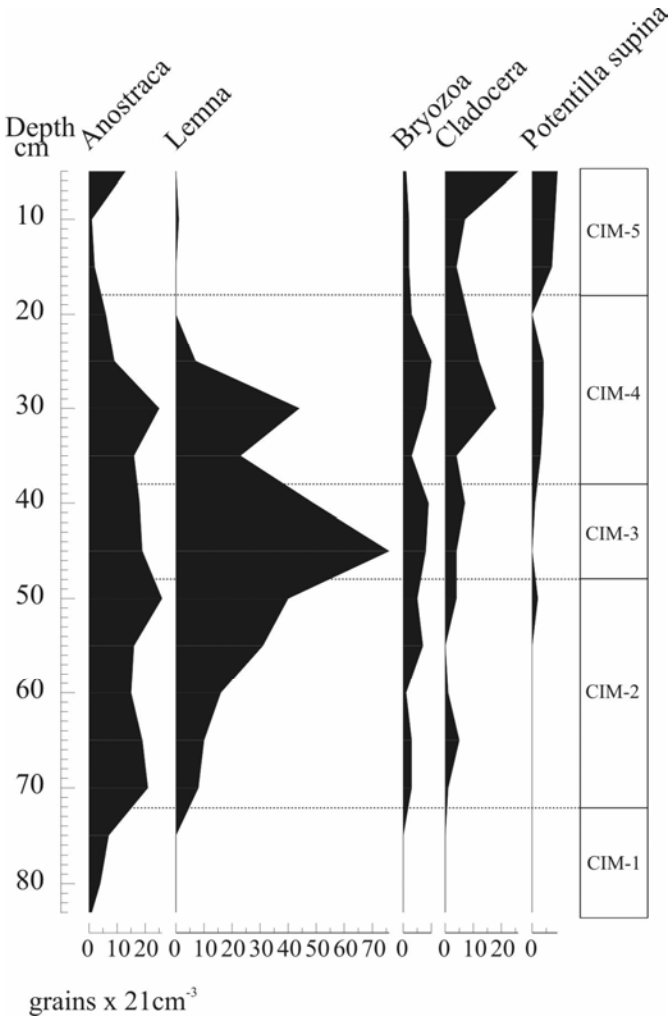


Fig. 4. The vertical distribution of the identified remains in core nr. 2.

DISCUSSION

To our knowledge, the *Chirocephalus shadini* egg bank documented here is the deepest known in Anostraca, although we do not know precisely the boundary between the viable and fossil egg bank. It is often considered that fairy shrimp eggs do not accumulate beyond the first 5–10 mm of sediment (Brown & Carpelan, 1971; Eriksen *et al.*, 1986; Thiéry, 1997). Mura (2004) found eggs of *Chirocephalus ruffoi* down to 15 cm, while Hulsmans *et al.* (2006) found eggs of *Phallocryptus ornata* to a depth of 13 cm and *Branchinella spinosa* to a depth of

11 cm in Botswana temporary ponds in a 1 m deep core. In our study pool, eggs of *Chirocephalus shadini* were found at a depth around 80 cm in two cores, reaching maximum at 45–50 cm. Mura (2004) mentions bioturbation as the cause of a deeper than usual vertical distribution of an Anostraca egg bank (15 cm).

The method presented here is a fast and low-cost alternative to the ones most frequently used in the literature for the study of dormant egg banks (*e.g.* Onbé, 1978; Hulsmans *et al*., 2006). Flotation and filtration alone were highly efficient in the case of Cladocera ephippia (94–100%) and Bryozoa statoblasts (100%), and moderately efficient in the case of Anostraca (71–80%) and seeds. Broken eggs and seed fill up with sediment and sink fast, therefore a direct examination of the sediment under the microscope was necessary.

WETLAND DEVELOPMENT

According to the radiocarbon measurements the studied pond basin was probably formed at the Pleistocene/Holocene transition by landslides on permafrost, and it is a typical landscape element of the tectonic mountain basins of the Eastern Carpathians. As a result of the landslides, a shallow oligotrophic pond formed in the lake basin with sparse vegetation (period CIM-1 in Figs. 3, 4). The sedimentation in the basin was very slow.

The radiocarbon data suggest a Late Holocene transition from oligotrophic to eutrophic pond, started at *ca*. 4000 yr BP, and accelerated after 3000 yr BP (period CIM-2 on Figs. 3, 4). The abundance of invertebrate macroremains and *Lemna* seeds grows, indicating an oligotrophic/eutrophic water quality transition. The pond seasonally dried out and *Potentilla supina* appeared on the mud surfaces.

According to the radiocarbon data the sedimentation accelerated after ca. 1000 yr BP. The infilling of this eutrophic pond was probably triggered by the massive deforestation of the region in the 11–13th century by the growing human population, shown by palynological investigations at the near Lake St Ana (Magyari *et al*., 2006, 2009). The amount of *Lemna* and *Potentilla supina* seeds increased, indicating a highly fluctuating water level (period CIM-3 on Figs. 3, 4). The increasing amount of Bryozoa and *Lemna* seeds below 40 cm could indicate a longer hydroperiod. One would expect a smaller frequency of Anostraca under longer hydroperiods, however Anostraca are also most abundant at this depth. This indicates that although hydroperiod was longer, the pond probably dried out regularly.

With the infilling of the lakebed the vegetation/cover increased, and the amount of *Lemna* and *Potentilla supina* seeds decreased (period CIM-4 on Figs. 3, 4). Probably the present-day tall sedge fen occupied the lakebed. The organic content of the sediment increased to a great extent.

The low density of Anostraca, Bryozoa and *Lemna* remains in the top 40 cm could be explained by the disturbance of the sediment combined with some filling

that "diluted" the egg and seed bank. According to land owners, no filling has happened, however ploughing could have moved in soil from the surroundings of the pond.

CONCLUSIONS

This is the first study on the sediment of early Holocene temporary ponds in the mountain basins of the Eastern Carpathians. Our results show that there is much scope to further explore the paleoecology of these ponds. Among others, they could bring information on the evolution of the vegetation of these areas during the Holocene.

The main outcomes of this study can be summarized as follows.

1. The studied lakebed was formed through landslides on permafrost in the Early Holocene.
2. After 3000 yr BP the oligotrophic lake became eutrophic and water levels fluctuated seasonally.
3. The sedimentation accelerated after ca. 1000 yr BP, probably triggered by the massive deforestation of the region in the 11–13th century.
4. Subfossil Anostraca eggs were recorded since the Early Holocene layers, but most abundant in the Late Holocene, between 3000 and 800 yr BP.
5. The vertical distribution of the Anostraca egg bank in this case is much deeper than those documented in the literature.

Acknowledgements. This study was part of a research and conservation project on temporary ponds funded by Dr Barbara Knowles. In 2008 we benefited from a grant of the Sapientia Institute for Research Programmes. Thanks go to Richard Schoenbohm for his help in the field work and to Kapás Árpád for fixing the broken corer. The comments of Dragana Milicic-Cvetkovic and Federico Marrone are gratefully acknowledged.

REFERENCES

ALONSO M., ALCARAZ M., 1984, *Huevosr esistentesd e crustóceos eufilópodos no cladóceros de la península lbérica: Observación de la morfología externa mediante técnicas de microscopía electrónica de barrid*. Oecologia Aquatica, **7**: 73–78.

BEAUDOIN A.B., 2007, *On the laboratory procedure for processing unconsolidated sediment samples to concentrate subfossil seed and other plant macroremains*. Journal of Paleolimnology, **37**: 301–308.

BELADJAL L., MERTENS J., 2003, *Interstitial remains for fauna reconstruction of desert pools using fairy shrimps as an example (Anostraca)*. Journal of Crustacean Biology, **23** (1): 60–68.

BENNETT K.D., 1992, *PSIMPOLL – A quickBasic program that generates PostScript page description of pollen diagrams.* INQUA Commission for the study of the Holocene: working group on data handling methods, Newsletter, **8**: 11–12.

BIRKS H.H., 1980, *Plant macrofossils in Quaternary lake sediments*. Arch. Hydrobiol. Beith. Ergebn. Limnol., **15**: 1–60.

BIRKS H.J.B., BIRKS H.H., 1980, *Quaternary palaeoecology*. University Park Press, Baltimore, 289 pp.

BOJŇANSKÝ V., FAGARAŠOVÁ A., 2007, *Atlas of seeds and fruits of Central and East-European flora. The Carpathian Mountains Region*. Springer, Dordrecht, 1046 pp.

BRENDONCK L., DE MEESTER L., 2003, *Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment*. Hydrobiologia, **491**: 65–84.

BRENDONCK L., RIDDOCH B.J., 2001, *Hatching characteristics of the fairy shrimp Branchiopodopsis wolfi in relation to the stochastic nature of its habitats, desert rock pools*. Verh. Internat. Verein. Limnol., **27**: 3931–3935.

BROWN L.R., CARPELAN L.H., 1971, *Egg hatching and life history of a fairy shrimp Branchinecta mackini Dexter (Crustacea: Anostraca) in a Mohave Desert playa (Rabbit Dry Lake)*. Ecology, **52**: 41–54.

ERIKSEN C.H., PRETTYMAN G.E., MOEUR J.E., 1986, *The effects of soil disturbance by off road vehicles on the eggs and habitat of playa lake crustaceans*. ln: R.G. ZAHARY (Ed.) Desert ecology: a research symposium, pp. 50–65. Southern California Academy of Sciences and Southern California Desert Studies Consortium. Los Angeles, California.

FREY D.G., 1964, *Remains of animals in Quaternary lake and bog sediments and their interpretation*. Arhiv f. Hydrobiol. Beih. Ergebn. Limnol., **2**: 1–114.

GROSSE-BRAUCKMANN G., 1972, *Über pflanzliche Makrofossilien mitteleuropäischer Torfe. I: Gewebereste krautiger Pfanzen und ihre Merkmale*. Telma, **2**: 19–55.

HULSMANS A., BRACKE S., MOREAU K., RIDDOCH B.J., DE MEESTER L., BRENDONCK L., 2006, *Dormant egg bank characteristics and hatching pattern of the Phallocryptus spinosa (Anostraca) population in the Makgadikgadi Pans (Botswana)*. Hydrobiologia, **571**: 123–132.

JOWSEY P. C., 1966, *An improved peat sampler*. New Phytologist, **65**: 245–248.

LIBBY W.F., 1962, *Radiocarbon; an atomic clock*. Annual Science and Humanity Journal, pp. 190–200.

LUNDQUIST G., 1936, *Hochasiatische Binnenseesedimente*. Mem. Conn. Acad. Arts Sci., **10**: 193–204.

MAFFEI C., MURA G., ZARATTINI P., 2002, *Assessing anostracan (Crustacea: Branchiopoda) cyst bank size: An attempt at a standardized method*. Hydrobiologia, **486**: 255–261.

MAGYARI E., BUCZKÓ K., JAKAB G., BRAUN M., SZÁNTÓ ZS., MOLNÁR M., PÁL Z., KARÁTSON D., 2006, *Holocene palaeohydrology and environmental history in the South Harghita Mountains, Romania*. Földtani Közlöny, **136** (2): 249–284.

MAGYARI E.K., BUCZKÓ K., JAKAB G., BRAUN M., PÁL Z., KARÁTSON D., 2009, *Palaeolimnology of the last Eastern Carpathian crater lake - a multiproxy study of Holocene hydrological changes*. Hydrobiologia, **631** (1): 29–63.

MAUQUOY D., VAN GEEL B., 2007, *Mire and peat macros*. In: ELIAS S.A. (Ed.): *Encyclopedia of Quaternary Science*, Vol. **3**. Elsevier, pp. 2315–2336.

MULLER S.D., BRUNETON H., SOULIÉ-MÄRSCHE I., REY T., THIÉRY A., WATERKEYN A., BRENDONCK L., SCHEVIN P., YAVERCOVSKI N., GRILLAS P., 2008, *Long-term dynamics of a Mediterranean alkaline vernal pool (Rhone Delta, Southern France)*. Wetlands, **28** (4): 951–966.

MURA G., 1991, *Additional remarks on cyst morphometrics in Anostracans and its significance. Part I: Egg size.* Crustaceana, **61**: 241–252.

MURA G., 1992, *Additional remarks on cyst morphometrics in Anostracans and its significance. Part II: Egg morphology*. Crustaceana, **63**: 225–246.

MURA G., 2004, *Structure and functioning of the "egg bank" of a fairy shrimp in a temporary pool: Chirocephalus ruffoi from Pollino National Park (Southern Italy) as a case study*. Internat. Rev. Hydrobiol., **89** (1): 35-50.

NAGORSKAJA L., BRTEK J., MURA G., 1998, *The Anostraca of the Republic of Belarus*. Hydrobiologia, **367**: 21-30.

ONBÉ T., 1978, *Sugar flotation method for sorting the resting eggs of marine Cladocerans and Copepods from sea-bottom sediment*. Bulletin of the Japanese Society of Scientific Fisheries, **44**: 1411.

SIMOVICH M.A., HATHAWAY S., 1997, *Diversified bet-hedging as a reproductive strategy of some ephemeral pool anostracans (Branchiopoda)*. Journal of Crustacean Biology, **17** (1): 38-44.

THIÉRY A., 1997, *Horizontal distribution and abundance of cysts of several large branchiopods in temporary pool and ditch sediments*. Hydrobiologia, **359**: 177-189.

THIÉRY A., GASC C., 1991, *Resting eggs of Anostraca, Notostraca, Spinicaudata, Laevicaudata (Crustacea, Branchiopoda) occurring in France: identification and taxonomical value*. Hydrobiologia, **212**: 245-259.

VANDEKERKHOVE J., DECLERCK S., VANHOVE M., BRENDONCK L., JEPPESEN E., CONDE PORCUNA J.M., DE MEESTER L., 2004, *Use of ephippial morphology to assess richness of anomopods: potentials and pitfalls*. Journal of Limnology, **63**: 75-84.

WASYLIKOWA K., 1996, *Analysis of fossil fruits and seeds*. In: BERGLUND B.E. (Ed.) *Handbook of Holocene Palaeoecology and Palaeohydrology*. John Wiley and Sons Ltd., pp. 571-590.

[*]*Sapientia University*
Department of Technical and Nature Sciences
530104 Miercurea Ciuc, P-ţa Libertăţii, nr. 1
Romania
e-mail: domedve@gmail.com; ptrgabriella@gmail.com

[**]*Szent István University*
Institute of Environmental Sciences
Szabadság Str. 1–3
5540 Szarvas
Hungary
e-mail: cembra@freemail.hu

Received July 30, 2012

[***]*ER Biodiversité & Environnement*
Laboratoire de Biologie Animale, Case 18
Université de Provence
3 Place Victor Hugo, 13331 Marseille Cedex 3
France
e-mail: alain.thiery@up.univ-mrs.fr

[****]*Oficiul de Studii Pedologice şi Agrochimie*
530104 Miercurea Ciuc, str. Progresului, nr. 16
Romania
e-mail: pasztohyz@yahoo.com

EYE LENS DIAMETER AND WEIGHT AS AN AGE INDICATOR IN *AUXIS THAZARD* (LACEPEDE, 1800) FROM THE SEA OF OMAN

LAITH JAWAD[*], JUMA AL-MAMRY[*], NADIR AL-ABRI[*],
LUQMAN AL-HASSANI[**]

Eye lens diameter and weight were analyzed in *Auxis thazard*, collected from the coasts of Muscat City on the Sea of Oman, in order to determine the possibility of using these data for age determination. The results showed that the diameter of the eye lens can be used for identifying I and I^+ animals from those of II^+ while the eye lens weight proved unreliable for age determination. Due to the inability of the eye lens to detect changes in growth rate between year classes the accurate age determination using this parameter is ultimately invalidated. The method is especially useful for age determination when otolith or scale rings are not visible or when false rings may give erroneous readings.

Key words: *Auxis thazard*, eye lens diameter, eye lens weight, the Sea of Oman.

INTRODUCTION

Both eye lens diameter and eye lens weight have been used for the age determination of a variety of animals. Eye lens weight was also used for studying the effect of nutrition on the process of age determination in vertebrates (Teska & Pinder, 1986).

In teleost fishes, studies using these eye lens parameters as age indicators include: Carlton & Jackson (1968); Burkett & Jackson (1971); Crivilli (1980); Douglas (1987); Saleem *et al*. (1990); Al-Hassan *et al*. (1991, 1992); Al-Hassan & Al-Sayab (1994); Conides & Al-Hassan (2000); Jawad *et al*. (2001); Jawad (2001, 2003, 2004). The aim of this study is to determine the validity of the eye lens diameter and weight as age indicators in the Oman Sea fish *Auxis thazard*.

MATERIAL AND METHODS

190 specimens of *Auxis thazard* were obtained from the coasts of Muscat City on the Oman Sea. The eye lens diameter and weight were measured to the nearest millimeter and gram (Jawad, 2003). The large bone such as operculum and preoperculum were used to determine the age following Al-Hassan & Al-Sayab (1994). The bones on both left and right sides were twice read independently, using an ordinary dissecting microscope for verification. One way analysis of variance

followed by Duncan's multiple range test (Harraway, 1997) were applied to test the differences between the total length of the fish and its age.

RESULTS AND DISCUSSION

Based on the bone, the age of *Auxis thazard* ranged from one year to II^+ years. There was a clear overlap in the body length of different age classes, with no significant difference ($p > 0.05$) (Fig. 1). There was a slight increase in the average lens diameter with age within different age groups. The results showed that the eye lens diameter of the fish individuals in their first year and I^+ of life did not overlap with the ranges of the eye lens diameter of II^+ year class (Fig. 2). The ages of fishes in year classes I, I^+ and II could not be determined using the present technique (Fig. 3).

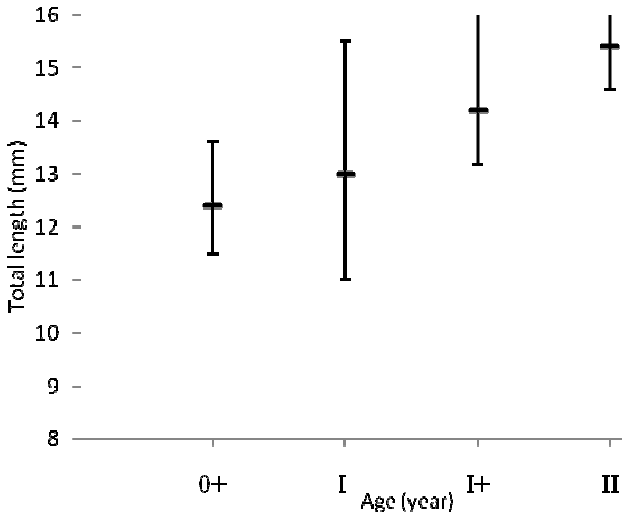


Fig. 1. Total length *vs* age (determined from opercular bone) of *Auxis thazard*
(Vertical bars represent range of fish total length and horizontal lines represent mean fish length).

Similar results were obtained for the Brown Trout, *Salmo trutta* (Douglas, 1987) and *Lithognathus mormyrus* and *Diplodus vulgaris* (Conides & Al-Hassan, 2000) and *Saurida undosequamis* and *Sillago sihama* (Jawad, 2003). Carlton & Jackson (1968) and Burkett & Jackson (1971) also failed to find such a relationship during their study of different fish species.

Different environmental factors can alter the growth rate in different fish species living in different habitats (Wootton, 1990). Temperature is probably the most important environmental parameter for tropical fish species as regards their growth rate. Several authors reported on the relationship between temperature and the growth rate of fish that live in such tropical habitats (Ahmed, 1982; Khalaf

et al., 1986). In such habitats, almost uniform temperature regimes dominate. *Auxis thazard* experiences a narrow range of variation in water temperature all year round. There is thus a great possibility that changes in growth rate in this species from different year classes cannot be detected in its eye lens diameter, and that an accurate age determination using this parameter is ultimately invalidated. This situation is similar to fish ageing in tropical regions, where well-defined annual rings failed to appear on their scales (Wootton, 1990).

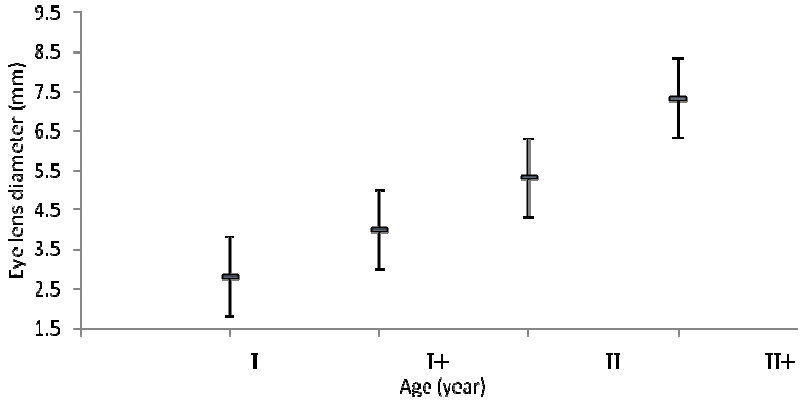


Fig. 2. Lens diameter *vs* age (determined from opercular bone) of *Auxis thazard*.
(Vertical bars represent total range of lens diameter and horizontal lines represent mean diameter).

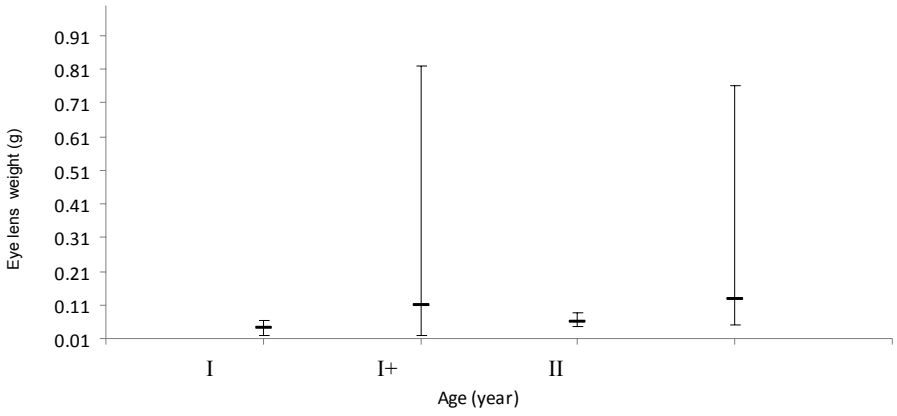


Fig. 3. Lens weight *vs* age (determined from opercular bone) of *Auxis thazard*.
(Vertical bars represent range of lens weight and horizontal lines represent mean lens weight).

The data on *Auxis thazard* show complete overlap in eye lens weight among the age groups (Fig. 3), precluding accurate age determination. The presence of this overlap may correlate with the development of sexual maturity (Crivilli, 1980). During the reproductive period, energy is transformed from somatic to gonadal

growth. Since the increase in lens weight is closely correlated with somatic growth, the variation in individual reproduction development (hence variation in somatic growth) could result in an increased variation in lens weight within an annual group.

CONCLUSIONS

The diameter of the eye lens of *Auxis thazard* can be used for identifying I and I^+ animals from those of II^+ while the eye lens weight proved unreliable for age determination. The method is especially useful for age determination when otolith or scale rings are not visible or when false rings may give erroneous readings.

Acknowledgements. We would like to thank the Ministry of Agriculture and Fisheries Wealth, the Agriculture and Fisheries Development Fund and the Marine Science and Fisheries Centre for giving us the opportunity to work on the fish samples within the qualitative and quantitative distribution of marine organisms in the Sultanate of Oman and to provide the appropriate financial support.

REFERENCES

AHMED H.A., 1982, *Growth of the cyprinid fish, Barbus luteus (Heckel) in Tharthar Reservoir, Iraq*. Bulletin of Basrah Natural History Museum, **5**: 3-15.

AL-HASSAN L.A.J., AL-DAHAM N.K., HASSAN S.S., 1991, *Eye lens as an age indicator in Mystus pelusius (Bagridae)*. Cybium, **15**: 171-172.

AL-HASSAN L.A.J., AL-DUBAIKEL A.Y., WAHAB N.K., 1992, *Ocular lens diameter as an age indicator in two teleost fishes*. Acta Hydrobiologica, **34**: 275-279.

AL-HASSAN L.A.J., AL-SAYAB A.A., 1994, *Eye lens diameter as an age indicator in the catfish, Silurus triostegus.* Pakistan Journal of Zoology, **26**: 81-82.

BURKETT D.D., JACKSON W.B., 1971, *The eye lens as an age indicator in freshwater drum*. American Midland Naturalist, **85**: 222-225.

CARLTON W.G., JACKSON W.B., 1968, *The eye lens as an age indicator in carp*. Copeia, **1**: 633-636.

CONIDES A.J., AL-HASSAN L.A.J., 2000, *Using eye lens diameter as age indicator of young Lithognathus mormyrus and Diplodus vulgaris*. Naga, the ICLARM Quarterly, **23**: 21-22.

CRIVILLI A., 1980, *The eye lens weight and age in the common carp Cyprinus carpio* L. Journal of Fish Biology, **16**: 469-473.

DOUGLAS R.H., 1987, *Ocular lens diameter as an indicator of age in brown trout, Salmo trutta.* Journal of Fish Biology, **31**: 835-836.

HARRAWAY J., 1997, *Introductory statistical methods for biological, health and social sciences.* Otago University Press, Dunedin, New Zealand, 342 pp.

JAWAD L.A., 2001, *Eye lens diameter and age determination in the tilapia fish, Tilapia zilli.* Biologia, Bratislavia, **56**: 573-575.

JAWAD L.A., 2003, *Ocular lens diameter and weight as age indicators in two teleost fishes collected from the Red Sea of Yemen*. Zoology in the Middle East, **29**: 59-62.

JAWAD L.A., 2004, *Preliminary study on the use of eye lens diameter and weight as an age indicator in two cyprinid fishes collected from Basrah, Iraq*. Bolletino del Museo Regionale di Scienze Naturali, Torino, **21**: 151-158.

JAWAD L.A., TAHER M.M.A, NAJDI H.M.H., 2001, *Age and asymmetry studies on the Indian mackerel, Rastrelliger kanagurta (Osteichthyes: Scombridae) collected from the Red Sea coast of Yemen.* Indian Journal of Marine Science, **30**: 180-182.

KHALAF A.N., AL-YAMOUR K.Y., ALLOUSE S.B., AL-JAFERY A.R., SADEK S.E., 1986, *Age, growth, length-weight relationship and distribution of Khishni Liza abu (Heckel) (Mugilidae) in a polluted habitat*. Journal of Biological Science Research, **17**: 63-81.

SALEEM S.D., AL-HASSAN L.A.J., MELKONIAN M.K., 1990, *The eye lens weight and age in some fish species collected from Basrah waters, Iraq*. Proceedings of the 15th International Conference for Statistics, Computer Science, Social and Demographic Research, Cairo, pp. 17-22.

TESKA W.R., PINDER T.E., 1986, *Effect of nutrition on age determination using eye lens weight*. Growth, **50**: 362-370.

WOOTTON R.J., 1990, *Ecology of teleost fishes*. London; New York: Chapman and Hall, 450 pp.

Received 1 March, 2012

[*] *Marine Science and Fisheries Centre, Ministry of Agriculture and Fisheries Wealth,*
Sultanate of Oman, P.O. Box 427, Postal Code 100 Muscat,
e-mail: laith_jawad@hotmail.com
[**] *High Technical College, Muscat, Sultanate of Oman*

OPTIMIZING THE GROWTH AND SURVIVAL OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) THROUGH PHOTOPERIODIC MANIPULATIONS

MOSHOOD MUSTAPHA, TAOFEEK OLADOKUN, MUBARAK SALMAN, PRAISE AFOLAYAN

One hundred and eighty juveniles of Nile tilapia (*Oreochromis niloticus*) were reared in three photoperiods of 24L: 0D; 0L: 24D and 12L: 12D for thirteen weeks in triplicates to determine the best photoperiod for the growth and survival of the species and thus optimize this photoperiod for their culture. Fish in OL: 24D were placed in a well ventilated dark room, 24L: OD was illuminated with a 40W fluorescent lamp with light intensity of 400 lx at the water surface, while 12L: 12D were placed in normal daylight and darkness, all fed with coppens at 5% of body weight. 24L: OD produced the highest significant ($P < 0.05$) final mean weight gained, specific growth rate, food conversion efficiency and lowest food conversion ratio, while 0L: 24D showed the lowest final mean weight gained, specific growth rate, food conversion efficiency and highest food conversion ratio. Survival rate was significantly higher ($P < 0.05$) in 12L: 12D than the other photoperiods. The highest growth recorded in 24L: OD was due to diurnal feeding activity which enabled the fish to utilize feed in continuous light as well as its low activities (lack of aggression). Stress coupled with low dissolved oxygen and pH in the 0L: 24D tank caused the low growth rate and poor survival. To optimize the growth of the species in culture, 24L: OD should be employed with the light intensity > 400 lx and 40 watts with four weeks of acclimatization before culture of the species.

Key words: *Oreochromis niloticus*, photoperiod, growth, survival, food conversion ratio, food conversion efficiency, stress.

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is one of the most cultured fish in the world. This is due to its hardy nature, high protein content, ease of culture, ability to eat natural and artificial foods, disease resistance, and consumers' preference among other reasons. The high cost of feed, coupled with the time it takes to rear table size and other challenges in the culture and management of the fish species has led to the evolution of simple, cheaper, low-cost technique to rear the fish to table size in less time.

One of such techniques is the use of photoperiodic (light duration) manipulations, which has been studied in many fish species including the Nile tilapia *Oreochromis niloticus* (Brummet, 1995; El-Nady *et al*., 1999; El-Naggar *et al.*, 2000; Campos-Mendosa *et al*., 2004; Biswas *et al*., 2005; Onumah *et al*.,

2010). Different stages of the fish species respond differently to different photoperiods. For instance, El-Naggar *et al.* (2000) showed that natural photoperiod enhanced the rate of growth and reproduction of Nile tilapia brood stock, while complete darkness lowered the survival rate of fry. El-Sayed & Kawanna (2004) and Rad *et al*. (2006) reported that fry and not fingerlings were significantly affected by longer day light producing higher growth in the fish. Campos-Mendoza *et al*. (2004) reported that *O. niloticus* showed higher growth and better spawning with long photo periods of 18L: 6D, while Alvarez-Rosario *et al*. (2009) observed that the growth and survival of red tilapia fry were not significantly affected by different photoperiods. Even in the wild, Gwinner (1986) reported that photoperiod is an important environmental signal in the control of variety of seasonally changing processed, including growth rate.

The enhanced growth performance in fish on the account of photoperiod manipulation has been traced to change in plasma growth hormone and pituitary somatotrop activity (Bjornson *et al.*, 1989), immunology (Valenzuela *et al*., 2008) and endogenous rhythms (Simensen *et al*., 2000), modification of fish appetite, food conversion and growth energy requirements (Donaldson *et al*., 1979), regulation of feeding activity (Boujard & Leatherland, 1992), reproductive habits (Lowe-Mc Connell, 1999), and behavior (Britz & Piennar, 1992; Piaia *et al*., 1999). In spite of the positive effect of enhanced growth in fish, photoperiod has also been reported to compromise fish welfare (FSBI 2002; Huntingford *et al*., 2006; Ashley, 2007) and this could affect their survival especially the fry and fingerlings often leading to mortality.

The objective of this study is to test for the best photoperiod for the growth of and survival of the juveniles of the Nile tilapia without compromising the welfare and thus optimize this photoperiod for the culture of the species.

MATERIAL AND METHODS

One hundred and eighty juveniles of *O. niloticus* were obtained from a private fish farm (Nefraday Nigeria Ltd) in Ilorin, Nigeria with average mean weight of 7.05 ± 0.05g. The fishes were transported to the fish laboratory of the University of Ilorin, Ilorin, Nigeria where they were acclimatized for one week in laboratory conditions set for the experiment in three 200L plastic tanks filled with borehole water before the start of the experiment.

After the period of acclimatization, the fish were distributed randomly into nine 200L plastic tanks (1×1×0.5m) corresponding to three replicates for each photoperiod of 24 hours continuous light, (24L: 0D) 24 hours continuous darkness (0L: 24D) and 12 hours light and 12 hours darkness (12L: 12D). Each tank was stocked with 20 fish species and the inside of the tanks had black colouration. Fish

in OL: 24D were placed in a well ventilated dark room, while fish in 24L: OD was illuminated with 40W fluorescent lamp with light intensity of 400 lx at the water surface. Fish in 12L: 12D were placed in normal daylight and darkness. The fish were fed with commercial feed of coppens twice daily (8.00am and 6.00pm) at 5% of body weight. The feed composition was 45% protein, 12% fat, 9.55% oil, 1.5% crude fiber and 1–2% total phosphorus. The experiment was conducted for 13 weeks (91 days).

Fish sampling was carried out at the beginning of the study and weekly thereafter by removing 10 juveniles randomly from each treatment at each time. The growth rate and survival rate were measured by weighing the fish to the nearest 0.01g and counting their numbers at each sampling time. The specific growth rate (SGR) and survival rate, food conversion ratio (FCR) and food conversion efficiency (FCE) were calculated according to Ghomi *et al*. (2011).

Physico-chemical parameters of the tanks water such as dissolved oxygen, carbon dioxide, temperature and pH were measured weekly with the aid of Lamotte Aquaculture Lab Model SCL-08. The water in the tanks was changed weekly after fish sampling.

Data analysis

Data are expressed as means ± SE. One way Anova and Duncan's multiple range tests were used to test significant differences between each photoperiod groups at $P < 0.05$.

RESULTS

The weekly variations in the mean weight of the juveniles of *O. niloticus* cultured under the three photoperiods of 24L: OD; 0L: 24D and 12L: 12D are presented in Fig. 1. The highest significant ($P < 0.05$) final mean weight gained, specific growth rate (SGR), food conversion efficiency and lowest food conversion ratio was in the 24L: OD photoperiod. This was followed by 12L: 12D photoperiod, while the lowest final mean weight gained, specific growth rate, food conversion efficiency and highest food conversion ratio was observed in the 0L: 24D photoperiod (Table 1). Survival rate was the highest in 12L: 12D which was significantly higher ($P < 0.05$) than the other photoperiods. 0L: 24D photoperiod recorded the lowest survival rate and the highest mortality, while the survival rate and mortality in 24L: 0D were moderate (Table 1). Dissolved oxygen and carbon dioxide contents of the tanks ranged between 4.0–8.5mg/l and 0.01–0.05mg/l respectively, while temperature was in the range of 26.0–28.0ºC and pH varied between 6.5 and 7.5.

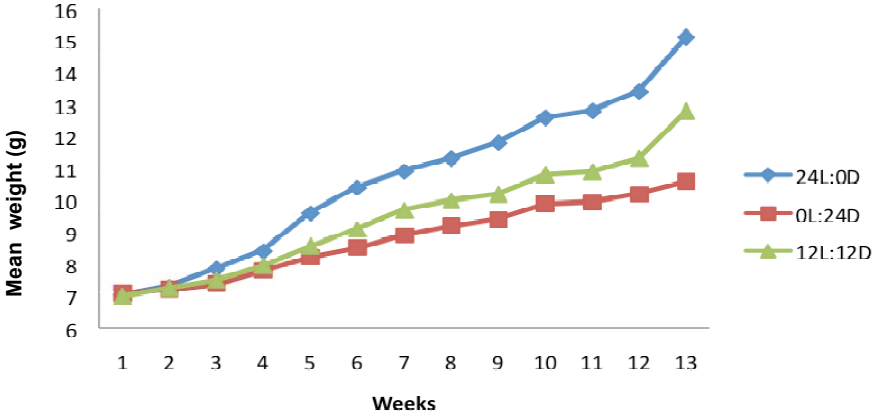


Fig. 1. Growth rate of *Oreochromis niloticus* cultured at three different photoperiods.

Table 1

Growth parameters and survival rate in juveniles of *O. niloticus* subjected to three different photoperiods

Photoperiod treatments	Initial weight	Final weight	Mean weight gain	% weight gain	SGR %	FCR	FCE	Initial number stocked	Number of survival	% survival	Number of mortality	% mortality
24L:0D	7.05	15.10	8.05	114.2	0.84	1.48	67.59	60	36	60	24	40
0D:24L	7.10	10.60	3.50	49.3	0.44	2.64	37.88	60	14	23.3	46	76.7
12L:12D	7.00	12.80	5.80	82.9	0.66	1.82	54.92	60	48	80	12	20

DISCUSSION

Various studies have shown that growth responses of fish species to different photoperiods depend on the developmental stages of the fish and the fish itself (Barlow *et al*., 1995). This work showed that juveniles of *O. niloticus* exposed to 24L: 0D had significant higher SGR, mean weight gain and FCE with lower FCR than the other photoperiods. This occurred on the account of the diurnal feeding activity of the juveniles which enabled the fish to utilize feed in continuous light as well as its low activities (lack of aggression) at that photoperiod. Thus, continuous light improves the feeding of the juvenile of the fish resulting in the high FCE and low FCR. The complete utilization of the feed in continuous light was due to higher motor activity of the fish (Alkahem & Ahmad, 1987). Another possible explanation could be that continuous light increased the growth hormone levels in the species as observed in Salmon Smolts (Handeland *et al.*, 2003).

McCormick *et al*. (1995) also reported that increased photoperiod increases plasma growth hormone levels in fish species. Positive correlation between growth and continuous photoperiod of 24L: 0D has been reported in several fish species

juveniles such as Nile tilapia (El-Sayed & Kawanna, 2004; Rad *et al*., 2006; Bezerra *et al*., 2008), Mirror Carp (Danişman-Yağci & Yiğit, 2009), Mahseer (Sawhney & Gandotra, 2010), rainbow trout (Sonmez *et al*., 2009), Atlantic Salmon (Berg *et al*., 1992).

Absence of light slowed down the rate of food consumption resulting in the lowest mean weight gain, SGR and FCE in the 0L: 24D. Darkness in the tank may have caused poor visibility of the feed, since *O. niloticus* is a visual feeder. Darkness or dark colour in tanks has been reported to impede fish growth (Strand *et al.*, 2007; Elnwishy *et al*., 2012). Ruchin (2007) also observed that darkness inhibited food consumption in the juvenile of Siberian sturgeon (*Acipenser baerii*), while Meske (1982) reported decreased food coefficient in the dark in European eel fry. It could therefore be deduced that motor activities that control feeding and growth hormone production are at the lowest in the dark in this species. Low weight gain and SGR and FCE in 0L: 24D could also be attributed to stress the fish undergoes in the tank coupled with low dissolved oxygen and pH cumulatively resulting in poor feeding. Dissolved oxygen less than 4mg/l and pH < 6 has been reported to decrease growth FCE, SGR in *O. niloticus* (El-Sherif & El-Feky, 2009). Another possible reason for the low in weight in 0L: 24D could be due to high energy expenditure by the fish to survive in the dark and for synchronization of the Endogenous rhythm of the fish to the external environment as observed by Biswas & Takeuchi (2003). Similarly, inability to physiologically adapt to conditions in continuous darkness (0L: 24D) could be responsible for the low weight recorded in the photoperiod. Ruchin (2007) noted that physiological deterioration in continuous darkness often resulted in poor growth of fish.

Result of the mortality and survival in this study has shown that juveniles of *O. niloticus* should not be cultured under continuous darkness (0L: 24D). This is apart from the low growth, SGR, FCE and high FCR recorded in the photoperiod. High mortality and low survival rate in this photoperiod resulted from stress and low dissolved oxygen and pH (4mg/l and 6.5) contents of the water in this photoperiod. High mortalities were recorded in the first 5 weeks of the experiment in all the photoperiods with the 0L: 24D particularly more severe and occurring throughout the experimental weeks. Stress, low dissolved oxygen, acidic pH, and inability to adapt and express its social behaviors in continuous darkness may be responsible for the high mortality. *O. niloticus* is a territorial species that exhibits high social hierarchy and which requires long daily light cycle and space to express its behavior. Tank colour and light have been shown to cause stress in fish (Rotllant *et al*., 2003; Papoutsoglou *et al*., 2005).

The low mortality in 12L: 12D and moderate mortality in 24L: OD was similar to that obtained in other species such as carp (Danişman-Yağci & Yiğit, 2009), Red tilapia (Rosario *et al*., 2009), Nile tilapia (Ridha & Cruz, 2000), Giant cat fish (Giri *et al*., 2002), Arctic Char (Burke *et al*., 2005).

The result in this work has shown that photoperiod has a relationship with growth and survival with increased growth in 24L: OD resulting in increased survival of the juveniles of *O. niloticus*. Similar trend was observed in African cat fish *Clarias gariepinus* juveniles where increased growth and survival were found in OL: 24D (Britz & Pienaar, 1992; Hossain *et al*., 1998; Appelbaum & Kamler, 2000; Adewolu *et al*., 2008, Mustapha *et al*., 2012).

CONCLUSION

Improved growth and survival in 24L: OD in the juveniles of *O. niloticus* is related to its feeding and behavioural expressions. In order to optimize the growth of the species in culture, 24L: OD should be employed with the light intensity of above 400 lux and 40 watts. This will provide increased photoperiod (lighting) and temperature of the water since *O. niloticus* performed better in increased temperature. The light source should also be very close to the water surface. Enough time for acclimatization to the 24L: 0D photoperiod, probably about four weeks, should be allowed before culture of the species is started. This will reduce mortality and increase survival since *O. niloticus* is photophilic. Mortality in the 24L: OD could be reduced by pumping additional oxygen into the system, i.e. oxygen supplementation during culture.

There is a relationship between photoperiod, growth, survival and mortality with optimal photoperiod for growth of a species that will also increase the survival of the species and vice-versa.

REFERENCES

ADEWOLU M.A., ADENIJI C.A., ADEJOBI A.B., 2008, *Feed utilization, growth and survival of Clarias gariepinus (Burchell 1822) fingerlings cultured under different photoperiods*. Aquaculture, **283**: 64-67.

ALKAHEM F., AHMAD Z., 1987, *Studies on oxygen consumption of Oreochromis niloticus and Cyprinus carpio*. Zeitschrin fur Angewandte Zoologie, **74** (4): 471-477.

ALVAREZ-ROSARIO J., FELIZ-CARRASCO L. MONTOYA-OSPINA R., VELASCO M., 2009, *Tilapia Fry Perform Similarly Under Varied Photoperiods*. Global Aquaculture Advocate, **12** (2): 70-72.

APPELBAUM S., KAMLER E., 2000, *Survival, growth, metabolism and behaviour of Clarias gariepinus (Burchell 1822) early stages under different light condition*s. Aquacultural Engineering, **22**: 269–287

ASHLEY P.J., 2007, *Fish welfare: Current issues in aquaculture.* Applied Animal Behaviour Science, **104** (3-4): 199-235.

BARLOW C.G., PEARCE M.G., RODGERS L.J., CLAYTON P., 1995, *Effects of photoperiod on growth, survival and feeding periodicity of larval and juvenile barramundi Lates calcarifer (Bloch)*. Aquaculture, **138** (1-4): 159-168.

BERG A., HANSEN T., STEFANSSON S., 1992, *First feeding of Atlantic salmon (Salmo salar L.) under different photoperiods*. Journal of Applied Ichthyology, **8** (1-4): 251-256.

BEZERRA K.S., SANTOS A.J.G., LEITE M.R., SILVA A.M.D., LIMA M. R.D., 2008, *Growth and survival of tilapia chitralada submitted to different photoperiods*. Pesquisa Agropecuária Brasileira, **43** (6): 737-743.

BISWAS A.K., TAKEUCHI T., 2003, *Effects of photoperiod and feeding interval on food intake and growth rate of Nile tilapia Oreochromis niloticus*. Fisheries Sciences, **69** (5): 1010-1016.

BISWAS A.K., MORITA T., YOSHIZAKI G., MAITA M., TAKEUCHI T., 2005, *Control of reproduction in Nile tilapia Oreochromis niloticus (L.) by photoperiod manipulation*. Aquaculture, **243** (1-4): 229-239.

BJORNSSON B.T, THORARENSEN H, HIRANO T., OGASAWARA T., KRISTINSSON J.B., 1989, *Photoperiod and temperature affect plasma growth hormone levels, growth, condition factor and hypoosmoregulatory ability of juvenile Atlantic salmon (Salmo salar) during parr-smolt transformation*. Aquaculture, **82** (1-4): 77-91.

BOUJARD T., LEATHERLAND J.F., 1992, *Circadian rhythms and feeding time in fishes*. Environmental Biology of Fishes, Kluwer Academic Publisher, **35**: 109-131.

BRITZ P.J., PIENAAR A.G., 1992, *Laboratory experiments on the effects of light and cover on the behaviour and growth of African catfish, Clarias gariepinus (Pisces Clariidae)*. Journal of Zoology, **227** (1): 43–62.

BRUMMET R.E., 1995, *Environmental regulation of sexual maturation and reproduction in Tilapia.* Reviews in Fisheries Science, **3** (3): 231-248.

BURKE M.G., KIRK M.R., MACBETH N.A., BEVAN D.J., MOCCIA R.D., 2005, *Influence of Photoperiod and Feed Delivery on Growth and Survival of First-Feeding Arctic Char*. North American Journal of Aquaculture, **67**: 344-350.

CAMPOS-MENDOZA A., Mc ANDREW B.J., COWARD K., BROMAGE N.R., 2004, *Reproductive response of Nile tilapia (Oreochromis niloticus) to photoperiodic manipulation; effects on spawning periodicity, fecundity and egg size*. Aquaculture, **231** (1-4): 299-314.

DANIŞMAN-YAĞCI D., YIĞIT M., 2009, *Influence of increased photoperiods on growth, feed consumption and survival of juvenile mirror carp (Cyprinus carpio Linnaeus, 1758)*. Journal of FisheriesSciences.com, **3** (2): 146-152.

DONALDSON E.M., FAGERLUND H.M, HIGGS D.A., Mc BRIDE, J.R., 1979, *Hormonal enhancement of growth*. Fish Physiology, **8**: 455-697, Academic Press, New York.

EL-NADY M.A., EL-NAGGAR G.A, KAMAR M.G., AL-KOBABY A.I., 1999, *Effect of photoperiod, crude protein level and water temperature on reproduction performance and egg hatchability of Nile tilapia (Oreochromis niloticus)*. Bull. Fac. Agric., Cairo Univ., **50**: 591-608.

EL-NAGGAR G.O., EL NADY M.A., KAMAR M.G., AL-KOBABAY A.I., 2000, *Effect of photoperiod, dietary protein and temperature on reproduction in Nile tilapia (Oreochromis niloticus).* In: FITZSIMMONS K., J.C. FILHO (Eds), *Tilapia culture in the 21st Century*. Proceedings of the Fifth International Symposium on Tilapia Aquaculture, Rio de Janeiro, Brazil. American Tilapia Association, Charles Town, West Virginia and ICLARM, Penang, Malaysia, pp.: 352-358.

ELNWISHY N., SABRI D., NWONWU F., 2012, *The effect of difference in environmental colors on Nile tilapia (Oreochromis niloticus) production efficienc*y. International Journal of Agriculture & Biology, **14**: 516-520.

EL-SAYED A.F.M., KAWANNA M., 2004, *Effects of photoperiod on the performance of farmed Nile tilapia Oreochromis niloticus: I. Growth, feed utilization efficiency and survival of fry and fingerlings.* Aquaculture, **231** (1): 393-402.

EL-SHERIF M.S., A.M.I. EL-FEKY, 2009, *Performance of Nile tilapia (Oreochromis niloticus) fingerlings. I. Effect of pH.* International Journal of Agriculture & Biology, **11**: 297-300.

FSBI, 2002, *Fish Welfare*. Briefing Paper 2, Fisheries Society of the British Isles, Granta Information Systems, 82A High Street, Sawston, Cambridge, UK.

GHOMI M.R., SOHRABNEJAD M., ZAREI M., 2011, *Growth rate, proximate composition and fatty acid profiles of juvenile kutum Rutilus frisii kutum under light/dark cycles*. Jordan Journal of Biological Sciences, **4** (1): 37-42.

GIRI S.S., SAHOO S.K., SAHU B.B., SAHU A.K., MOHANTY S.N., MUKHOPADHYAY P.K., AYYAPPAN S., 2002, *Larval survival and growth in Wallago attu (Bloch and Schneider): effects of light, photoperiod, and feeding regimens*. Aquaculture, **213** (1-4): 151-161.

GWINNER E., 1986, *Circannual rhythms, Zoophysiology,* vol. **18**, Springer, Heidelberg, 154 pp.

HANDELAND S.O., PORTER M., BJORNSSON B.T., STEFFANSON S.O., 2003, *Osmoregulation and growth in wild and selected strain of Atlantic salmon on two photoperiod regimes*. Aquaculture, **222** (1-4): 29-43.

HOSSAIN M.A.R., BEVERIDGE M.C.M., HAYLOR G.S., 1998, *The effects of density, light and shelter on the growth and survival of African catfish (Clarias gariepinus Burchell, 1822) fingerlings*. Aquaculture, **160** (3-4): 251-258.

HUNTINGFORD F., ADAMS C., BRAITHWAIT V.A., KADRI S., POTTINGER T.G., SANDØE P., TURNBULL J.F., 2006, *Current issues in fish welfare.* Journal of Fish Biology, **68** (2): 332-372.

LOWE-Mc CONNELL, 1999, *Estudos ecológicos de comunidades de peixes tropicai*s. São Paulo: EDUSP, 535 pp.

McCORMICK S.D., BJORNSSON B.T., SHERIDAN M., EILERTSON C., CAREY J.B., 1995, *Increased day length stimulates plasma growth hormone and gill Na+,K+-ATPase in Atlantic salmon*. Journal of Comparative Physiology, **165** (B): 245-254.

MESKE C., 1982, *Futterung von Aalen in Dunkeln.* Informationen für die Fischwirtschaft, **2**9 (3): 136-138.

MUSTAPHA M.K., OKAFOR B.U, OLAOTI K.S., OYELAKIN O.K., 2012, *Effects of three different photoperiods on the growth of juveniles of African cat fish Clarias gariepinus (Burchell)*. Archives of Polish Fisheries, **20**: 55-59.

ONUMAH E.E., WESSELS S., WILDENHAYN N., BRÜMMER B., SCHWARK G.H., 2010, *Stocking Density and Photoperiod Manipulation in Relation to Estradiol Profile to Enhance Spawning Activity in Female Nile Tilapia*. Turkish Journal of Fisheries and Aquatic Sciences, **10**: 463-470.

PAPOUTSOGLOU S.E., KARAKATSOULI N., CHIRAS G., 2005, *Dietary l-tryptophan and tank colour effects on growth performance of rainbow trout (Oncorhynchus mykiss) juveniles reared in a recirculating system*. Aquacultural Engeneering, **32** (2): 277-284.

PIAIA R., BALDISSEROTTO B., TOWNSEND C., 1999, *Growth and survival of fingerlings of silver catfish exposed to different photoperiod*s. Aquaculture International, **7**: 201-205.

RAD F., BOZAOĞLU S., GOZUKARA S.E., KARAHAN A., KURT G., 2006, *Effects of different long-day photoperiods on somatic growth and gonadal development in Nile tilapia (Oreochromis niloticus L.).* Aquaculture, **255** (1-4): 292-300.

RIDHA M.T., CRUZ E.M., 2000, *Effect of light intensity and photoperiod on Nile tilapia (Oreochromis niloticus L.) seed production.* Aquaculture Research, **31** (7): 609-617.

ROTLLANT J., TORT L., MONTERO D., PAVLIDIS M., MARTINEZ S., WENDELAAR B., BALM B., 2003, *Background colour influence on the stress response in cultured red porgy Pagrus pagrus.* Aquaculture, **223** (1-4): 129-139.

RUCHIN A.B., 2007, *Effect of photoperiod on growth physiological and hematological indices of juveniles Siberian sturgeon Acipenser baerii*. Biology Bulletin, **34** (6): 583-589.

SAWHNEY S., GANDOTRA R., 2010, *Effects of photoperiod on growth feed conversion, efficiency and survival of fry and fingerlings of Mahseer, Tor putitora.* Israeli Journal of Aquaculture-Bamidgeh, **62** (4): 266-271.

SIMENSEN L.M., JONASSEN T.M., IMSLAND A.K., STEFANSSON S.O., 2000, *Photoperiod regulation of growth of juvenile Atlantic halibut (Hippoglossus hippoglossus L.)*. Aquaculture, **190** (1-2): 119-128.

SONMEZ A.Y., HISAR O., HISAR S.A., 2009, *The effect of photoperiod regimes on growth, feed conversion rate and survival of rainbow trout (Oncorhynchus mykiss) fry*. Journal of Animal and Veterinary Advances, **8** (4): 760-763.

STRAND Å., ALANÄRÄ A., STAFFAN F., MAGNHAGEN C., 2007, *Effects of tank colour and light intensity on feed intake, growth rate and energy expenditure of juvenile Eurasian perch, Perca fluviatilis L.* Aquaculture, **272** (1-4): 312-318.

VALENZUELA A, SILVA V., KLEMPAU A., 2008, *Effects of different artificial photoperiods and temperature on haematological parameters of rainbow trout (Oncorhynchus mykiss)*. Fish Physiology and Biochemistry, **34** (2): 159-167.

Received December 20, 2012

Department of Zoology,
University of Ilorin, Ilorin,
Nigeria
e-mail: moonstapha@yahoo.com

NEW MORPHOMETRIC DATA OF WILD BOAR (*SUS SCROFA* LINNAEUS, 1758) FROM THE MINOO ISLAND (IRAN)

MOHAMMAD REZA ASHRAFZADEH[*], MARYAM BORDKHANI[**]

We considered morphometry of *Sus scrofa* in Minoo Island, Khuzestan Province, Iran. Twenty wild boars were collected in the Island during mid February 2010 till end of August 2010. Age of the specimens was determined considering the patterns of growing and eruption of teeth. Eleven body and thirty six skull characters were considered for all specimens. Linear regression test showed that there is a significant correlation between age and all body and skull measurements. The result of t-test indicated that three out of eleven body measurements and eleven out of thirty six skull measurements had significant differences between male and female groups. The Discriminant Analysis classified the males and females in different groups.

Key words: wild boar, morphometry, age, Minoo Island, Khuzestan, Iran.

INTRODUCTION

Wild boars are mammals with most widely geographic distribution in the world (Nowak, 1991). Eurasian wild boar (*Sus scrofa* Linnaeus, 1758) has the most distribution among all wild boars. This species who lives in wide areas of Asia, Europe and North Africa (Herre & Rohrs, 1977; Harrison & Bates, 1991; Ruvinsky & Rothschild, 1998; Wilson & Reeder, 2005; Albarella *et al*., 2009) with at least 16 endemic subspecies, is the ancestral species of domestic pigs (Ruvinsky & Rothschild, 1998; Wilson & Reeder, 2005). *S. scrofa* has occupied an extremely wide range of different habitats and they feed on a very wide range of plant and animal materials including fruits, onions, roots, buds, fungus and some vertebrates and invertebrates, animal corps, crops and sometimes eggs of birds and even fish and domestic animals (Diong, 1973; Pauwels, 1980; Graves, 1984; Spitz, 1986; Harrison & Bates, 1991; Campos, 1993; Choquenot *et al*., 1996; Massei *et al*., 1996; Schley & Roper, 2003; Desbiez *et al*., 2009).

Additionally this species has the highest reproductive rates in a way that the annual increase of their population in the area can be two times per year (Massei & Genov, 2004) or more than 150% (Massei & Tonini, 1992). Therefore, because of widespread increase in number and geographical range of this species, there can be considerable impacts on many plant and animal species, habitat structure and crops and livestock production (Massei & Genov, 2004). Wild boars play an important

role in the shaping of many different environments, but this is their relation with humans which outstands specifically their importance (Albarella *et al*., 2009). Eurasian wild boar is a pest of agriculture and substantial damages to agricultural crops are reported by local people and related organizations during population increase of this species in different areas of Iran. In forested areas also, destroy of sapling and seedling and effects on recruitment of certain species are from other items. Lay (1967) has pointed to some damages to crops by wild boars in Iran. Besides, boars are an important potential source of some diseases (Herrera *et al*., 2005; Corner, 2006; Ruiz-Fons, 2007).

The large geographic range occupied by wild boar populations is reflected in the great morphological and size variability that characterizes this species (Albarella *et al*., 2009). Cranial characteristics, especially size and form of skulls, are defined as one of the best ways for classifying the vertebrates by taxonomists (Mayer & Brisbin, 1993). Several studies have analyzed the body and skull, measurements of *Sus scrofa* (Nichols, 1962; Henry, 1970; Sweeney, 1970; Barrett, 1971; Kozlo, 1975; Hell & Paule, 1983; Mayer & Brisbin, 1991; Endo *et al*., 2002; Markina *et al.*, 2004; Lucchini *et al*., 2005). Besides, several studies have determined the age of boars based on growth pattern, wear and eruption of teeth (Sweeney, 1970; Bull & Payne 1982; Sáez-Royuela *et al*., 1989; Clarke *et al*., 1992; Choquenot & Saunders, 1993; Rolett & Min-Yung 1994; Tuen *et al*., 1999; Magnell, 2006; Oroian, 2010; Krapinec, 2011). Age determination is a key factor in understanding the natural history (Tuen *et al*., 1999) and ecological studies of wild species (Larson & Taber, 1980; Lundervold *et al*., 2003). Also, the dentition age estimation at this species is extremely important for establishing correlations between it and aspects of the body conformation and trophy value (Oroian *et al*., 2010).

Ecology and biology of wild boar population are very important because of conservation and economic reasons. This species has wide distribution in Iran (Ziaie, 2008), but very few studies have been done about biology, ecology and systematic position of populations of wild boars in this country (*e.g*., Goshtasb Meigoni *et al*., 2002). We reported new morphometric data of wild boars from Minoo Island, Khuzestan Province, Iran.

MATERIAL AND METHODS

From mid February 2010 till end of August 2010, twenty wild boars have been collected from different habitats of Minoo Island (Fig. 1), with authorization from Department of Environment (DOE) of Iran. Each specimen was weighed by 300 kg balance with precision of 100_{gr}. Morphometry studies have been performed in two groups of body and skull measurements. Eleven body characters were measured using a flexible meter with precision of 1 mm (Table 1; Fig. 2). Also, 36

characters of the skull were measured with vernier calipers of 0.02 mm precision (Table 2; Figs. 3–4).

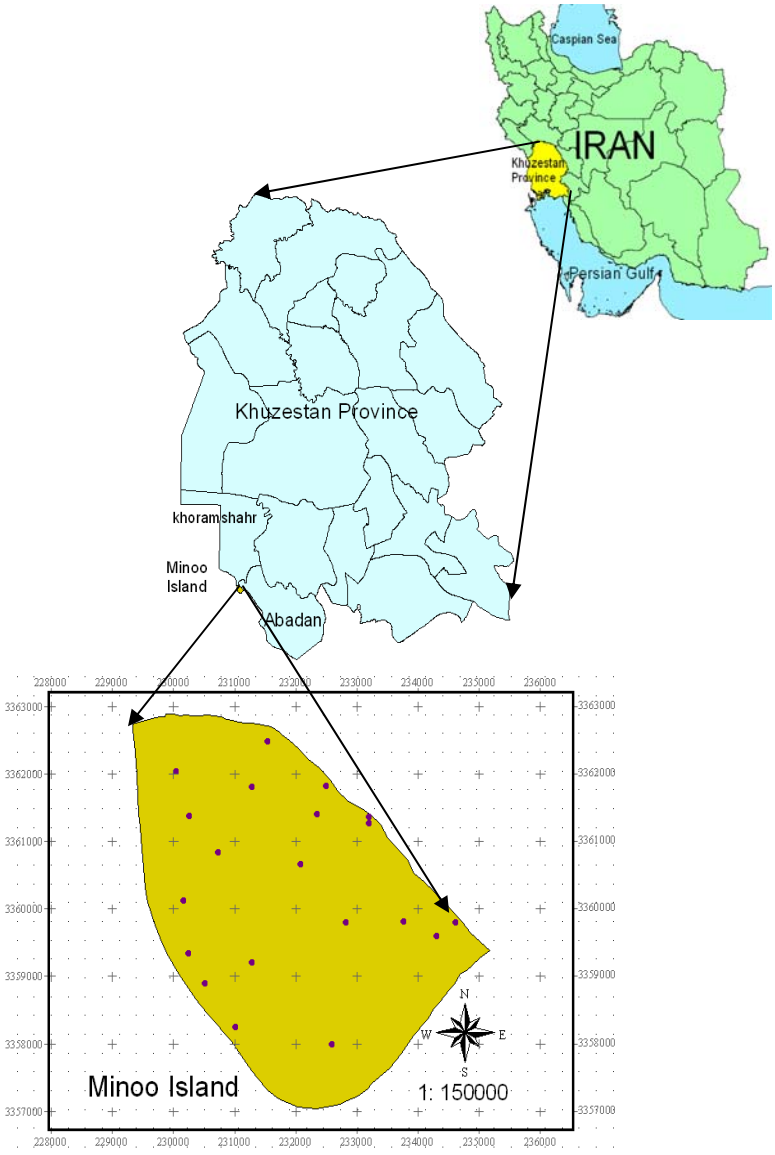


Fig. 1. Location of Minoo Island in Khuzestan province and Iran and hunting places of wild boars.

Table 1

Body characters taken in the present study

Rows	Description	Measurements
1	Head-body length	HBL
2	Length of tail	LT
3	Total length	TL
4	Shoulder height	SH
5	Back foot length	BFL
6	Weight $_{(kg)}$	W
7	Ear length	EL
8	Heart Girth	HG
9	Head Length	HL
10	Snout–ear distance	SE
11	Ear–shoulder distance	ES

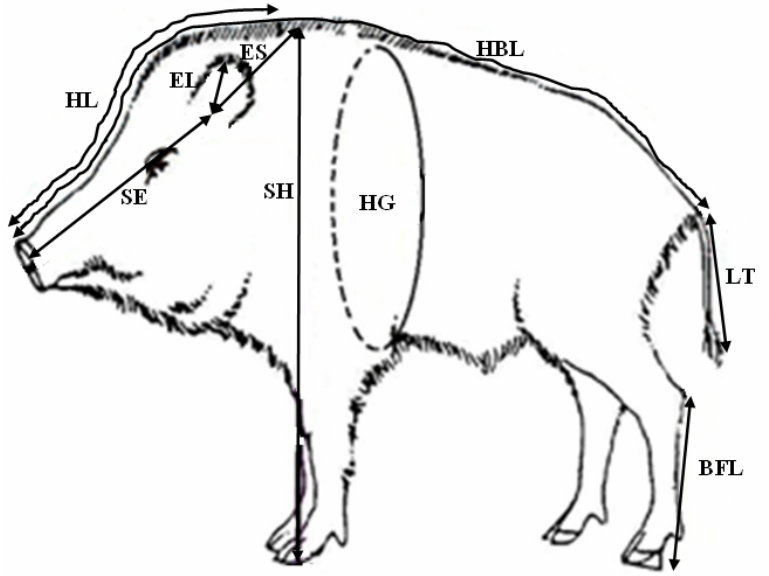


Fig. 2. Body characters taken in the present study.

Among techniques of age determination for mammals, dentition (tooth eruption pattern, tooth wear and tooth sectioning) is particularly useful in ungulates (Brown & Chapman, 1991; Langvatn & Meisingset, 2001). Matschke (1967) and Rowley-Conwy (1993) have estimated the age of wild boars based on tooth eruption and wear of down jaw. We have estimated the age of wild boars based on tooth eruption and wear and teeth formula in different ages (Matschke, 1967; Rowley-Conwy, 1993; Rolett & Min-Yung, 1994; Oroian *et al*., 2010).

Table 2

Skull characters taken in the present study

Description	**Measurements**	**Description**	**Measurements**
Jaw Length	JL	Middle Height of the vertical Ramus	MHR
Jaw Height	JH	Height of the mandible at M1	HM1
Suture Jaw Length	SJL	Height of the mandible at Gnathion	HG
Greatest Skull Length	GSL	Length of the Ramus between the angle and M3	LR
Condylobasal Length	CL	Length of Canine Alveolus	LCA
Basal Length	BL	Breadth at caudal point of I3	BI
Bizygomatic Width	BW	Breadth at Canine alveori	BC
Occipital Breadth	OB	Least Breadth of the Mandible	LBM
Palate Length	PL	Breadth of 2 halves between the most Lateral Points of the 2 angles	BLP
Length of Upper Tooth row	LUT	Breadth of 2 halves between the Condylar Processes	BCP
Length of Upper Molar row	LUM	Breadth between Medial and Lateral points of the condylar process	BML
Post-orbital Breadth	PB	Thickness between rostral and caudal points of the condular process	TC
Width Across Post-orbital processes	WAP	Breadth of the mandible at M1	BM
Length of Frontal + Parietal	LFP	Thickness of the mandible at middle point of M1	TM
Nasal Length	NL	Palatal Length	PL
Length from the Condyle to anterior-most point of symphysis	LC	Premaxillary Rostral Width	PRW
Aboral Height of the vertical Ramus	AHR	Palatal Constriction	PC
Oral Height of the vertical Ramus	OHR	Rostral Length	RL

Descriptive statistics was used for all of the body and skull measurements. Kolmogorov–Smirnov test was used for checking normality of data. Then, all specimens were divided in sex groups of male and female and T test was used to evaluate the differences between male and female groups based on body and skull measurements. The linear regression test was used to evaluate the existence of a

significant correlation between age and morphometry measurements. Discriminant Analysis (DA) was utilized to classify the sex groups based on morphometry measurements.

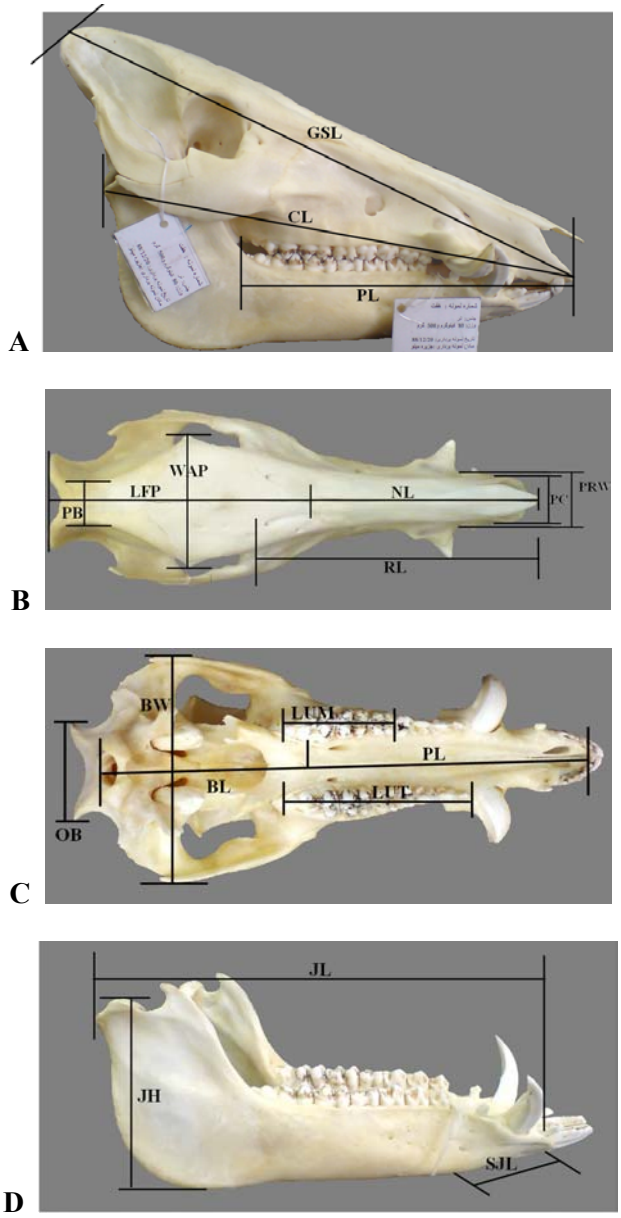


Fig. 3. Skull characters taken in the present study.
A – lateral view; B – dorsal view; C – ventral view; D – mandible lateral view.

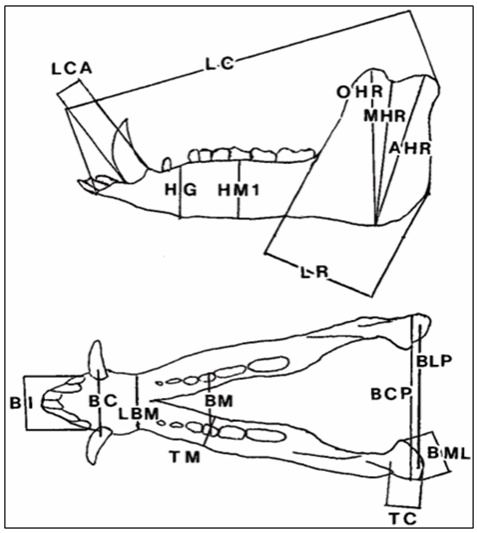


Fig. 4. Characters of the mandible.
Up – lateral view; Low – dorsal view (Endo *et al*., 2002).

RESULTS

In total, twenty wild boars were collected from different habitats in Minoo Island. Fig. 5 shows an adult wild boar in this island. Based on performed studies, this species was observed in all parts of this island including sides of rivers, streams, marshes, farms, palm groves and tamarix woodlands. Besides, they enter the city and residential parts of island after midnight. The ages of wild boars were estimated. Tables 3 and 4 show descriptive statistics of body and skull measurements of wild boars from this area divided by sex.

Fig. 5. An adult *Sus scrofa* in Minoo Island.

Fig. 6. Canine teeth of an adult male *Sus scrofa* in Minoo Island.

Table 3

Measurements (cm) of the body characters for adult *Sus scrofa* in Minoo Island divided by sex

Characters	Female					Male				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
HBL	9	133.33	10.06	117	144	11	148.00	16.77	131	184
LT	9	22.17	5.11	12	31	11	21.89	3.55	13.4	27
TL	9	155.50	14.46	132	175	11	169.89	15.81	153	197.4
EL	9	11.80	0.90	10.2	13.3	11	12.38	1.39	11	14.7
SH	9	70.39	8.12	59	86	11	75.32	6.92	67	89
BFL	9	31.50	1.66	29	34	11	33.09	2.07	29.5	36
W (kg)	9	69.24	20.22	43	102.75	11	109.27	75.78	61.75	260

Among the body measurements of wild boars in this area, just characters of HBL, TL and HL between the male and female had significant differences ($P < 0.05$). Among the skull measurements, the characters of JL, SJL, OB, LEP, LCA, HM1, HG, BI, BC, LBM, BML and PL between male and female wild boars had significant and highly significant differences ($P < 0.05$, $P < 0.01$). These founding shows that males and females have significant and highly significant differences in skull measurements. Figs. 7 and 8 show the comparison between average morphometry measurements of specimens from Minoo Island dividing by sex.

Table 4

Measurements (mm) of the skull characters for adult *Sus scrofa* in Minoo Island

Characters	Female					Male				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
JL	9	253.10	20.82	223.40	281.00	11	279.52	23.89	244.40	319.80
SJL	9	67.75	5.12	60.21	76.38	11	80.35	13.26	64.22	102.40
JH	9	122.44	9.00	107.00	131.68	11	127.93	10.31	114.70	146.10
GSL	9	352.08	23.80	312.70	380.00	11	378.08	34.30	324.50	432.70
CL	9	321.34	22.40	281.35	346.00	11	343.47	28.12	306.10	388.55
OB	9	63.61	7.40	54.52	76.84	11	71.46	8.87	63.80	88.84
BW	9	135.62	10.90	121.60	153.94	11	147.56	15.16	131.52	171.15
LUM	9	61.74	15.20	42.26	80.30	11	69.47	13.86	43.80	82.56
LUT	9	111.89	15.11	92.60	133.20	11	120.96	14.64	95.40	138.20
PL	9	178.69	11.60	163.75	194.40	11	192.71	20.09	167.80	225.60
BL	9	309.66	21.41	271.90	333.00	11	329.83	25.91	296.80	371.20
PB	9	27.66	9.27	14.96	41.78	11	31.70	5.37	23.60	40.50
WAP	9	91.37	7.93	76.74	99.82	11	96.81	11.33	79.00	117.00
NL	9	185.06	16.58	158.10	202.30	11	193.51	34.87	117.50	238.30
LFP	9	167.30	7.41	156.20	177.95	11	176.37	10.73	157.00	196.35
LC	9	274.35	22.78	236.70	299.10	11	297.79	30.80	258.95	347.75
LCA	9	11.56	2.17	8.38	15.20	11	19.71	4.58	13.54	27.20
HM1	9	48.40	4.26	42.90	55.80	11	53.92	6.40	46.16	66.90
HG	9	46.40	3.28	41.00	52.00	11	52.79	7.91	41.62	65.34
OHR	9	122.44	9.00	107.00	131.68	11	127.93	10.31	114.70	146.10
MHR	9	108.62	9.67	92.86	119.26	11	104.31	33.12	11.60	141.12
AHR	9	121.27	7.90	108.24	131.54	11	124.21	11.18	109.30	146.80
LR	9	83.27	3.69	79.44	87.42	11	89.02	5.98	81.38	99.48
BI	9	39.36	2.77	36.00	43.00	11	44.72	5.41	39.72	52.94
BC	9	33.98	1.87	31.12	36.40	11	40.29	6.38	34.00	50.00
LBM	9	39.86	3.54	36.30	45.60	11	44.54	5.00	39.12	53.74
BM	9	55.39	3.78	51.18	61.12	11	59.17	4.56	52.82	65.62
TM	9	11.15	0.70	9.80	11.90	11	11.77	0.77	10.80	13.00
TC	9	22.72	3.60	18.70	28.62	11	26.24	4.20	21.00	34.80
BML	9	27.52	1.56	24.56	29.62	11	29.85	2.93	26.12	34.82
BCP	9	115.19	8.88	102.60	129.76	11	123.22	11.80	112.30	142.86
BLP	9	101.28	10.15	87.66	119.12	11	107.43	13.01	95.00	134.80
PRW	9	35.11	3.95	26.78	40.00	11	38.99	5.79	31.40	50.00
PC	9	36.71	2.56	32.86	40.00	11	39.96	4.40	33.30	47.96
PL	9	226.48	15.55	201.00	244.00	11	246.68	26.17	208.00	288.50
RL	9	222.76	17.49	193.80	242.50	11	241.18	24.80	204.00	279.00

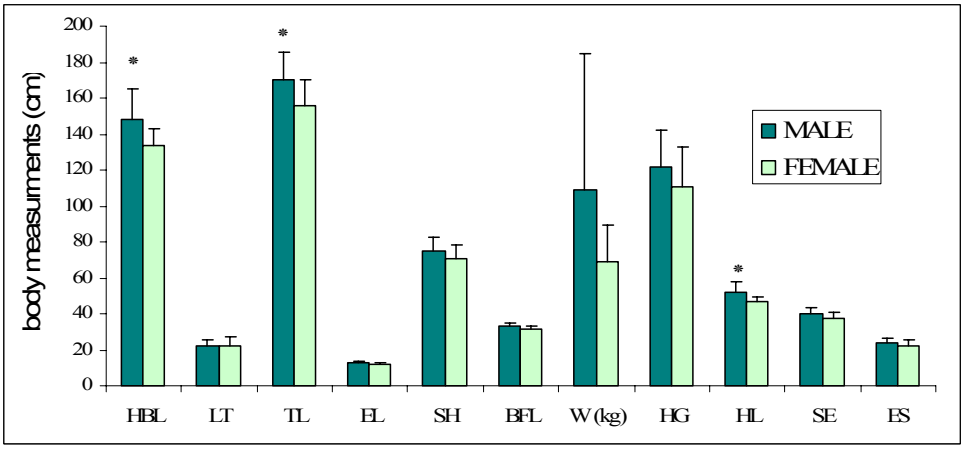


Fig. 7. Comparing average body measurements of *Sus scrofa* in Minoo Island divided by sex.

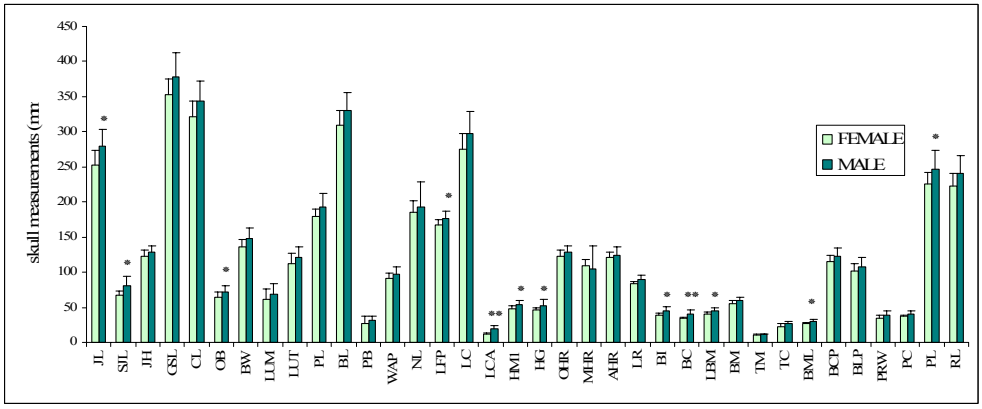


Fig. 8. Comparing average skull measurements of *Sus scrofa* in Minoo Island dividend by sex (**P < 0.01 = *P < 0.05).

Based on the results of linear regression test, there is a positive highly significant correlation between all skull measurements and increase of the age (P < 0/01). Also the founding of this test shows a positive highly significant correlation between body measurements HBL, HL, LT, SE, SH and TL and increase of the age of the animal (P < 0/01) and also there is a positive significant correlation between body measurements EL, BFL, ES, HG and W_{kg} and the increase of the age (P < 0/05). The result of DA test also showed that males and females classification has been done correctly in level of 80%. Table 5 shows a summary of the test results.

Table 5

The result of DA test for classifying the male and female wild boars

Genus			Predicted Group Membership		Total
			F	M	
Original	Count	F	5	4	9
		M	0	11	11
	%	F	55.6	44.4	100.0
		M	.0	100.0	100.0

A 80.0% of original grouped cases correctly classified (F – Female; M – Male).

DISCUSSION AND CONCLUSION

Totally twenty wild boars from different parts of Minoo Island have been hunted. Assessment on the age of specimens showed that the youngest and the oldest hunted wild boars are about 18 and 80 months old, respectively. It seems that there is a relationship between age and body size, although in wild boars this relationship is masked in some age groups because of the dependency of body weight on environmental factors. It is usually accepted that teeth system is not constant in one species and it changes based on items like genetics, feeding, habitat and severity of the seasons (Deniz & Payne, 1982; Hillson, 2005). Also, in many wild ungulates species like red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*), eruption of permanent teeth is complete by 18 to 36 months, so aging beyond this limit is more difficult (Brown & Chapman, 1991; Moore *et al*., 1995; Langvatn & Meisingset, 2001).

Table 6

Measurements (cm) of the body characters for adult *Sus scrofa* in Golestan National Park (North of Iran) divided by sex (Goshtasb Meigoni *et al*., 2002)

Characters	Female					Male				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
HBL	7	204.29	18.75	166	223	9	222.56	23.93	186	250
LT	7	37.43	3.21	32	41	9	39.44	5.75	31	48
TL	7	166.86	19.56	125	185	9	183.11	19.73	153	203
EL	7	19.29	2.43	14	21	9	19.27	2.11	16	22
SH	7	76.00	5.54	69	83	9	72.00	10.68	51	85
BFL	7	31.71	1.38	29	33	9	33.00	1.75	31	37
W (kg)	7	86.57	20.74	50	115	9	107.78	39.25	60	165
HG	7	115.00	7.98	106	128	9	125.00	16.92	96	145

Facts of our data showed that male wild boars are bigger than females in all body measurements except LT. Results of t-test indicated that among eleven body measurements, three of them HBL, TL (total length of head and body and tail length) and HL have significant differences between male and female groups ($p < 0.05$). Based on achieved results, males are heavier than females. The biggest hunted wild boar was a male with 260 kg weight. The smallest hunted specimen in the area was a female wild boar with 43 kg weight. Sjarmidi & Gerard (1988) stated that adult wild boars have weight between 35 and 230 kg. Besides, they said that the smallest individuals occur in Mediterranean countries and the largest individuals are found in the northeastern part of the species range. As Table 6 shows, the average body measurements of wild boars and specially HBL in Golestan National Park (in the North of Iran) (Goshtasb Meigoni *et al*., 2002) is bigger than average of these measurements in wild boars of Minoo Island (in the South West of Iran).

The results show that male wild boars are bigger than females in all skull measurements. Based on results of t-test, among 36 skull characteristics, 12 have significant and highly significant differences between sexes ($p < 0.05$, $p < 0.01$). What is considerable here is that the size of measurements related to teeth, specially canine teeth, for example LCA and BC for males are much bigger than sizes for females with a high difference. In addition, linear regression test showed that there is a significant and highly significant correlation between age and all skull measurements ($p < 0.05$, $p < 0.01$). Markina *et al*. (2004) reported significant correlations between age and body measurements of wild boars. Wild boars have an average weight and height which is increasing from the Atlantic Ocean to central Europe and Asia. Furthermore, there is strong natural selection for large size within Polygamous species such as wild boar that males compete for females. Within females, although having sufficient weight during period of reproduction is necessary (Sáez-Royuela & Telleria, 1987) the pressure of selection to reach to large size is less than in males (Bouldoire & Vassant, 1989). The result of DA test showed that males and females classification has been done correctly in level of 80%.

REFERENCES

ALBARELLA U., DOBNEY K. and ROWLEY-CONWY P., 2009, *Size and shape of the Eurasian wild boar, Sus scrofa, with a view to the reconstruction of its Holocene history*. Environmental Archaeology, **14** (2): 103-136.

BARRETT R.H., 1971, *Ecology of the feral hog in Tehama County, California*. Ph.D. Dissertation. University of California, Berkeley, 368 pp.

BOULDOIRE J.L. and VASSANT J., 1989, *Le Sanglier. Collection Faune Sauvage.* Hatier, Paris, France, 228 pp.

BROWN W.A.B. and CHAPMAN N.G., 1991, *The dentition of red deer, Cervus elaphus – a scoring scheme to assess age from wear of the permanent molariform teeth*. Journal of Zoology, **224**: 519-536.

BULL G. and PAYNE S., 1982, *Tooth eruption and epiphysial fusion in pigs and wild boar*. pp. 55-71. *In*: B. WILSON, C. GRIGSON and S. PAYNE (Eds.), *Ageing and sexing animal bones from archaeological sites*. British Archaeological Reports, Oxford, 268 pp.

CAMPOS Z.M.S., 1993, *Effect of habitat on survival of eggs and sex ratio of hatchlings of caiman, Crocodilus yacare in the Pantanal, Brazil*. Journal of Herpetology, **27**: 127-132.

CHOQUENOT D., McLLROY J. and KORN T., 1996, *Managing vertebrate pests: feral pigs*. Australian Government Publishing Service, Canberra, 163 pp.

CHOQUENOT D. and SAUNDERS G., 1993, *A comparison of three ageing techniques for feral pigs from subalpine and semi-arid habitats*. Wildlife Research, **20**: 163-71.

CLARKE C.M.H., DZIECIOLOWSKI R.M., BATCHELER D. and FRAMPTON C.M., 1992, *A Comparison of Tooth Eruption and Wear and Dental Cementum Techniques in Age Determination of New Zealand Feral Pigs*. Wildlife Research, **19** (6): 769-777.

CORNER L.A.L., 2006, *The role of wild animal populations in the epidemiology of tuberculosis in domestic animals: how to assess the risk*. Veterinary Microbiology, **112**: 303-312.

DENIZ E. and PAYNE S., 1982, *Eruption and wear in the mandibular dentition as a guide to ageing Turkish Angora goats*. pp. 155-205. In: B. WILSON, C. GRIGSON and S. PAYNE (Eds.), *Ageing and sexing animal bones from archaeological sites*. British Archaeological Reports, Oxford, 268 pp.

DESBIEZ A.L.J., SANTOS S.A., KEUROGHLIAN A., BODMER R.E., 2009, *Niche partitioning among whitelipped peccaries, Tayassu pecari, collared peccaries, Pecari tajacu, and feral pigs, Sus scrofa*. Journal of Mammalogy, **90**: 119-128.

DESBIEZ A.L.J. and KEUROGHLIAN A., 2009, *Ageing feral pigs, Sus scrofa, through tooth eruption and wear.* Suiform Soundings, **9** (1): 48-55.

DIONG C.H., 1973, *Studies of the Malayan wild pig in Parak and Johore*. Malayan Nature Journal, **26**: 120-151.

ENDO H., HAYASHI Y., YAMAZAKI K., MOTOKAWA M., PEI J.C.K., LIN L.K., CHOU C.H. and OSHIDA T., 2002, *Geographical Variation of Mandible Size and Shape in the Wild Pig, Sus scrofa, from Taiwan and Japan*. Zoological Studies, **41** (4): 452-460.

GOSHTASB MEIGONI H., JAHANSHAHI M., KAVOUSI K. and HASANZADEH KIABI B., 2002, *A study on herbivorous habits of wild pig, Sus scrofa, in Golestan National Park, Iran*. Iranian Journal of Environmental Studies, **28** (30): 55-65.

GRAVES H.B., 1984, *Behavior and Ecology of Wild and Feral Swine, Sus scrofa*. Journal of Animal Science, **58** (2): 482-492.

HARRISON D.L. and BATES P.J.J., 1991, *The Mammals of Arabia*. 2nd ed. Harrison Zoological Museum, Sevenoaks, United Kingdom, 354 pp.

HELL P. and PAULE L., 1983, *Systematische Stellung des Westkarpatischen Wildschweines Sus scrofa*. Acta Scientiarum Naturalium Brno, **17** (3): 1-54.

HENRY V.G., 1970, *Weights and body measurements of European wild hogs in Tennessee*. Journal of the Tennessee Academy of Sciences, **45** (1): 20-23.

HERRERA H.M., NOREK A., FREITAS T.P.T., RADEMAKER V., FERNANDEZ O. and JANSEN A.M., 2005, *Domestic and wild mammals infection by Trypanosoma evansi in a pristine area of the Brazilian Pantanal region*. Parasitological Research, **96**: 121-126.

HERRE W. and ROHRS M., 1977, *Zoological considerations on the origins of farming and domestication*. pp. 245-279. In: CA Reed (Ed.), Origins of Agriculture. The Hague, Mouton, 1013 pp.

HILLSON S., 2005, *Teeth*. 2nd ed. Cambridge University Press, Cambridge, 388 pp.

KOZLO P.G., 1975, *Dikiy Kaban (Wild Boar)*. Izdatel'stovo Uradzhai, Minsk, U.S.S.R., 224 pp.

KRAPINEC K., KONJEVIĆ D., BREZOVAC I., MANOJLOVIĆ L., SEVERIN K., NJEMIROVSKIJ V., GRUBEŠIĆ M., and TOMLJANOVIĆ K., 2011, *Some morphological characteristics of wild boar tusks and evaluation of aging methods.* Šumarski list br., **1-2**: 7-18.

LANGVATN R. and MEISINGSET E., 2001, *Growth and age in red deer*. Hjorteviltet, **11**: 2-11.

LARSONJ S. and TABER R.D., 1980, *Criteria of sex and age*. pp. 143-202. In: S.D. SCHEMNITZ (Ed.) *Wildlife management techniques manual*. The Wildlife Society, Washington, D.C., 686 pp.

LAY D.M., 1967, *A study of the mammals of Iran, resulting from the Street Expedition of 1962-63*. Fieldiana Zoology, **54**: 1-282.

LUCCHINI V., MEIJAARD E., DIONG C.H., GROVES C.P. and RANDI E., 2005, *New phylogenetic perspectives among species of South-east Asian wild pig (Sus sp.) based on mtDNA sequences and morphometric data*. Journal of Zoology, London, **266**: 25-35.

LUNDERVOLD M., LANGVATN R. and MILNER-GULLAND E.J., 2003, *A comparison of age estimation methods for the saiga antelope Saiga tatatrica*. Wildlife Biology, **9** (3): 219-227.

MAGNELL O., 2006, *Tooth Wear in Wild Boar, Sus scrofa*. pp.189-204. In: U. ALBARELLA, K. DOBNEY and P. ROWLEY-CONWY (Eds.), *Recent advances in ageing and sexing animal bones*. Proceedings of the 9th Conference of the International Council of Archaeozoology. Oxbow Books, Park End Place, Oxford, 264 pp.

MARKINA F.A., SÁEZ- ROYUELA C. and GARNICA R.D., 2004, *Physical development of wild boar in the Cantabric Mountains, Álava, Northern Spain*. Galemys, **16** (n° especial): 25-34.

MASSEI G. and TONINI L., 1992, *The management of wild boar in the Maremma Natural Park*. pp. 443-445. In: F. SPITZ, G. JANEAU, G. GONZALEZ and S. AULAGNER (Eds.), Ongulés/Ungulates, 91. SFEPM-IRGM, Paris, 661 pp.

MASSEI G., GENOV P.V. and STAINES B.W., 1996, *Diet, food availability and reproduction of wild boar in a Mediterranean coastal area*. Acta Theriologica, **41** (3): 307-320.

MASSEI G. and GENOV P.V., 2004, *The environmental impact of wild boar*. Galemys, **16** (n° especial): 135-145.

MATSCHKE G.H., 1967, *Aging European wild hogs by dentition*. Journal of Wildlife Management, **31**: 109-113.

MAYER J.J. and BRISBIN I.L. Jr., 1993, *Distinguishing feral hogs from introduced wild boar and their hybrids: a review of past and present efforts*. pp. 28-49. In: C.W. HANSELKA and J.F. CADENHEAD (Eds.), *Feral swine: A compendium for resource managers*. Texas Agricultural Extension Service, Kerrville, Texas, 169 pp.

MAYER J.J. and BRISBIN I.L. Jr., 1991, *Wild pigs in the United States: their history, morphology, and current status*. The University of Georgia Press, Athens, Georgia, 313 pp.

MOORE N.P., CAHILL J.P., KELLY P.F. and HAYDEN T.J., 1995, *An assessment of 5 methods of age- determination in an enclosed population of fallow deer, Dama dama*. Biology and Environment, Proceedings of the Royal Irish Academy, **95** B: 27-34.

NICHOLS L. Jr., 1962, *Ecology of the wild pig*. Federal Aid in Wildlife Restoration Final Report Project W-5-R-13. Hawaii Department of Land and Natural Resources, Division of Fish and Game, Honolulu, Hawaii, 20 pp.

NOWAK R.M., 1991, *Mammals of the world*. 5th ed. The Johns Hopkins University Press, Baltimore, 1629 pp.

OROIAN T.E., OROIAN R.G., PASCA I., OROIAN E. and COVRIG L., 2010, *Methods of Age Estimation by Dentition in Sus scrofa ferus sp*. Bulletin UASVM Animal Science and Biotechnologies, **67** (1-2): 291-295.

PAUWELS W., 1980, *Study of Sus scrofa vittatus, its ecology and behavior in Ujung Kulon Nature Reserve, Java, Indonesia*. Ph. D. Dissertation, University of Basel, Switzerland, 100 pp.

ROLETT B.V. and MIN-YUNG C., 1994, *Age estimation of prehistoric pigs, Sus scrofa, by molar eruption and attrition*. Journal of Archaeological Science, **21**: 377-386.

ROWLEY-CONWY P., 1993, *Season and reason: the case for a regional interpretation of mesolithic settlement patterns*, pp. 179-188. In: G. PETERKIN, H. BRICKER and P. MELLARS (Eds.), *Hunting and Animal Exploitation in the Later Palaeolithic and Mesolithic of Eurasia*. Archaeological Papers of the American Anthropological Association, 249 pp.

RUIZ-FONS F., VIDAL D., HOFLE U., VICENTE J. and GORTAZAR C., 2007, *Aujeszky's disease virus infection patterns in European wild boar*. Veterinary Microbiology, **120**: 241-250.

RUVINSKY A. and ROTHSCHILD M.F., 1998, *Systematics and evolution of the pig*, pp. 1-16. *In*: M.F. ROTHSCHILD and A. RUVINSKY (Eds.), *The Genetics of the Pig*, CAB International, Oxon, 622 pp.

SÁEZ-ROYUELA C. and TELLERIA J.L., 1987*, Reproductive trends of the wild boar, Sus scrofa, in Spain*. Folia Zoology, **36**: 21-25.

SÁEZ-ROYUELA C., GOMARIZ R.P. and TELLERÍA J.L., 1989, *Age Determination of European Wild Boar*. Wildlife Society Bulletin, **17** (3): 326-329.

SCHLEY L. and ROPER T.J., 2003, *Diet of wild boar, Sus scrofa, in Western Europe, with particular reference to consumption of agricultural crops*. Mammal Review, **33** (1): 43-56.

SJARMIDI A. and GERARD J.F., 1988, *Autour de la systématique et la distribution des suidés*. Monitore Zoologico Italiano, **22**: 415-448.

SPITZ F., 1986, *Current state of knowledge of wild boar biology*. Pig News and Information, **7** (2): 171-175.

SWEENEY J.M., 1970, *Preliminary investigations of a feral hog (Sus scrofa) population on the Savannah River Plant, South Carolina*. M.S. Thesis, University of Georgia, Athens, 58 pp.

TUEN A.A., RAHMAN M.A. and TAJUDDIN M., 1999, *Age classification of bearded pigs (Sus barbatus) from Bario, Kelabit Highlands*. Asean Review of Biodiversity and Environmental Conservation (ARBEC). Abdullah University, Malaysia, Sarawak, 5 pp.

WILSON D.E., REEDER D.M., 2005, *Mammal Species of the World. A Taxonomic and Geographic Reference* (3rd ed). Johns Hopkins University Press, 2142 pp.

ZIAIE H., 2008, *A field guide to the mammals of Iran* (2nd ed). Iranian Wildlife Center, Tehran, Iran, 432 pp.

Received October 3, 2012

[*] *Department of Environment, Faculty of Natural Resources,*
University of Tehran, Karaj, Iran
e-mail: mrashrafzadeh@ut.ac.ir
[**] *Department of Environment, Faculty of Natural Resources,*
Isfahan University of Technology, Isfahan, Iran
e-mail: m.bordkhani@gmail.com

LIVER STRUCTURE OF BROWN BEAR (*URSUS ARCTOS* L.)

PAULA PRUNESCU, CAROL-CONSTANTIN PRUNESCU

The microanatomy of the brown bear liver (the hepatic lobule, portal tracts, hepatic veins, intralobular biliary canals, solitary arterioles and Glisson' capsule vessels) was described. The assertion about the similitude of the bear hepatic lobule with the pig hepatic lobule was invalidated, at least concerning *Ursus arctos* species. The hepatic lobules of the brown bear liver were not separated each other by interlobular connective septa, like in the pig liver. An explanation of the possible source of the erroneous but widespread opinion about this similitude was advanced.

Key words: Brown bear, *Ursus arctos*, hepatic lobule, histology, pig liver.

INTRODUCTION

The recent papers referring to the bear liver considered its histological structure being *a priori* well known and never described it.

With the aim to evaluate the pollution degree in the northern ecosystems, numerous papers studied heavy metals or chlorinated hydrocarbons accumulations in the liver of *Ursus maritimus* (Braune *et al*., 1991; Norheim *et al*., 1992; Bandiera *et al*., 1995; Dietz *et al.*, 2000; Routti *et al.*, 2011) or *Ursus arctos* (Zilincar *et al*., 1992; Medveev, 1999). Other papers described bear liver lesions produced by parasites (Garner *et al*., 1997) or pathogenic bacteria (Dunbar *et al*., 1995). Studies about stellate perisinusoidal cells biology and vitamin A accumulations (Leighton *et al*., 1988; Senoo *et al*., 1999) or about the peripheral position of hepatocytes nuclei in the liver of arctic animals including polar bears (Sato *et al*., 2001) represented other points of interest. None of these papers presented any description of the histological structure of the hepatic lobule, portal tract, hepatic vein, capsule of Glisson, etc. in the bear liver.

In the Grassé's treatise the hepatic lobule of the family *Ursidae* (Dorst, 1973), respectively the polar bear (Gabe, 1973), was considered to be similar with the hepatic lobule of *Sus scrofa.* In the pig liver, the hepatic lobules were separated each other by fibrous septa, clearly delineating the morphological units (Wünsche, 1985; Ekataksin & Wake, 1991; Prunescu P. & Prunescu C.-C., 2002). This similitude between the pig and bear hepatic lobules was evocated by Ekataksin & Wake (1991), following an extensive literature survey.

The present paper was realized using the study of serially histological sections through liver samples from the brown bears (*Ursus arctos*) originating of the Romanian Carpathians Mountains. The affirmation about the similitude of the

bear hepatic lobule with the pig hepatic lobule was invalidated. In the normal brown bear liver, the hepatic lobules were never separated by the fibrous septa towards the adjacent lobules.

MATERIAL AND METHODS

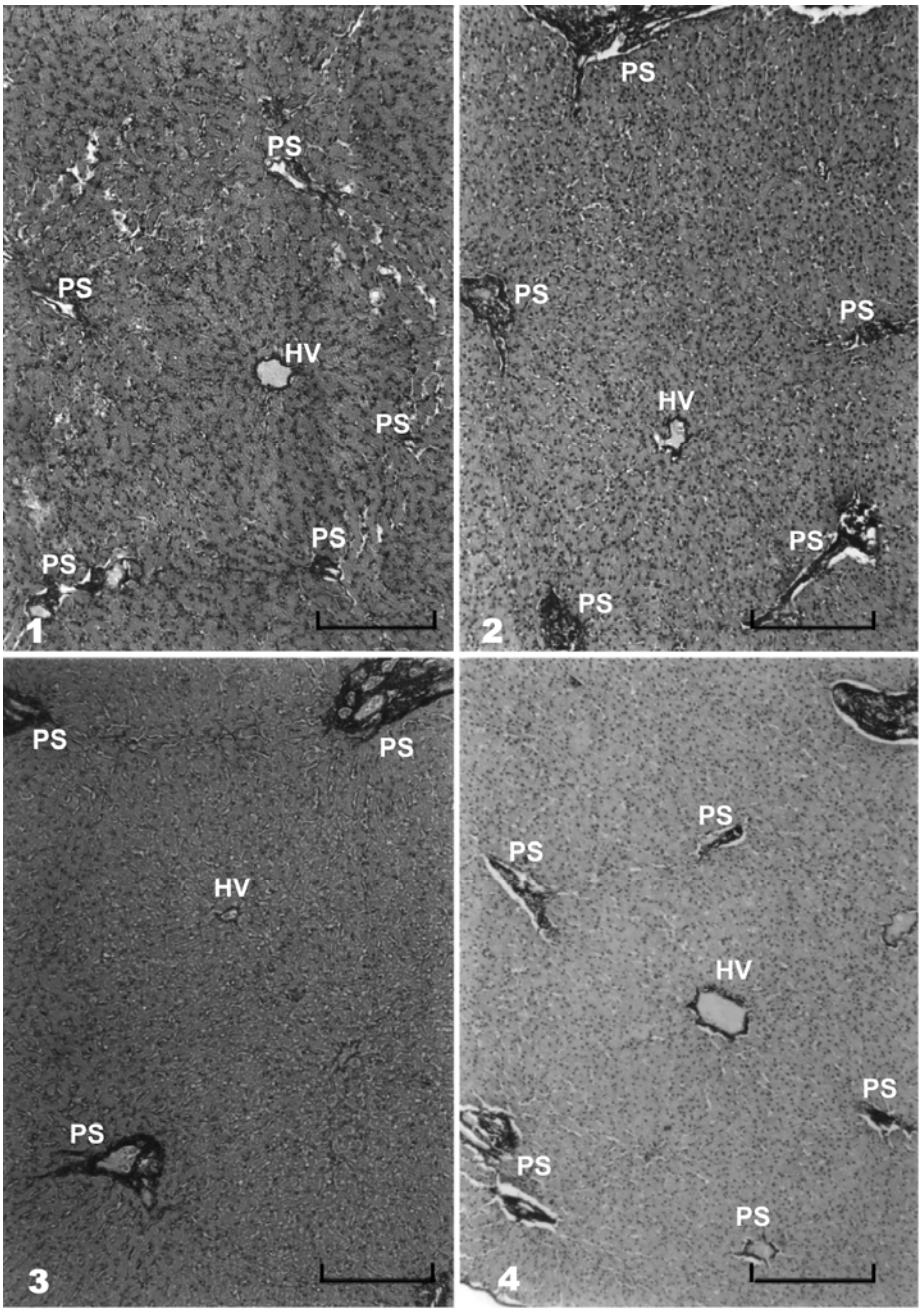
There were studied liver samples from 16 males, adult brown bears (*Ursus arctos)* shot in Carpathians Mountains, during the autumn hunts. It was considered that this period of optimum metabolism must correspond to the normal histological structure of the liver, in comparison with the liver structure observed in early spring, after a long period of hibernation (Prunescu *et al*., 2003). The liver samples were fixed in 8% formaldehyde in saline. Because the difficult accessibility to the hunting places, sometimes the fixation was delayed with few hours after the bear's death. About 70 liver samples were embedded in paraffin and sectioned at 5 μm. From this material 600 slides with serially histological sections were obtained. The most liver samples were serially cut on few slides. From other samples 200–300 serially histological sections were obtained. The histological staining was made with hemalum-eosin (H-E) for observations of the micro-anatomical structure and picro-Sirius red hemalum (PSR-H) (Junqueira *et al*., 1979) for specific collagen fibers staining. Microphotographs were obtained with a camera mounted on the Amplival Microscope (Zeiss, Jena).

RESULTS

Bear hepatic parenchyma was structured in lobules of polygonal shape and various sizes, which were distinguished only by the radial orientation of the sinusoids, respectively of the hepatic cords, within the portal tracts and the terminal hepatic vein, in central position (centrilobular vein) (Figs.1–4).

Great and medium sized portal tracts (Fig. 5) were covered by a stroma of collagen fibers. In this dense collagen network the portal vascular profiles (portal vein, one or more arteries or arterioles and many profiles of biliary canals) were observed. Sometimes, lymphatic canals as narrow spaces lined with rare endothelial cells were noted. The portal tracts of superior dimensions branched into portal tracts of inferior orders, getting up to the subterminal and terminal portal structures (Fig. 6). The terminal portal tracts were formed by the association of a biliary canal, a fine arteriole or capillary and a small branch of the terminal portal vein.

A biliary canal (Fig. 7) always accompanied by a capillary, but without the venous component, was sometimes observed in the territory of a hepatic lobule.



Figs. 1–4. Histological sections through *Ursus* liver. Note the absence of the interlobular septa; portal space (PS); centrilobular hepatic vein (HV). PSR-H (Scale bar = 200 μm).

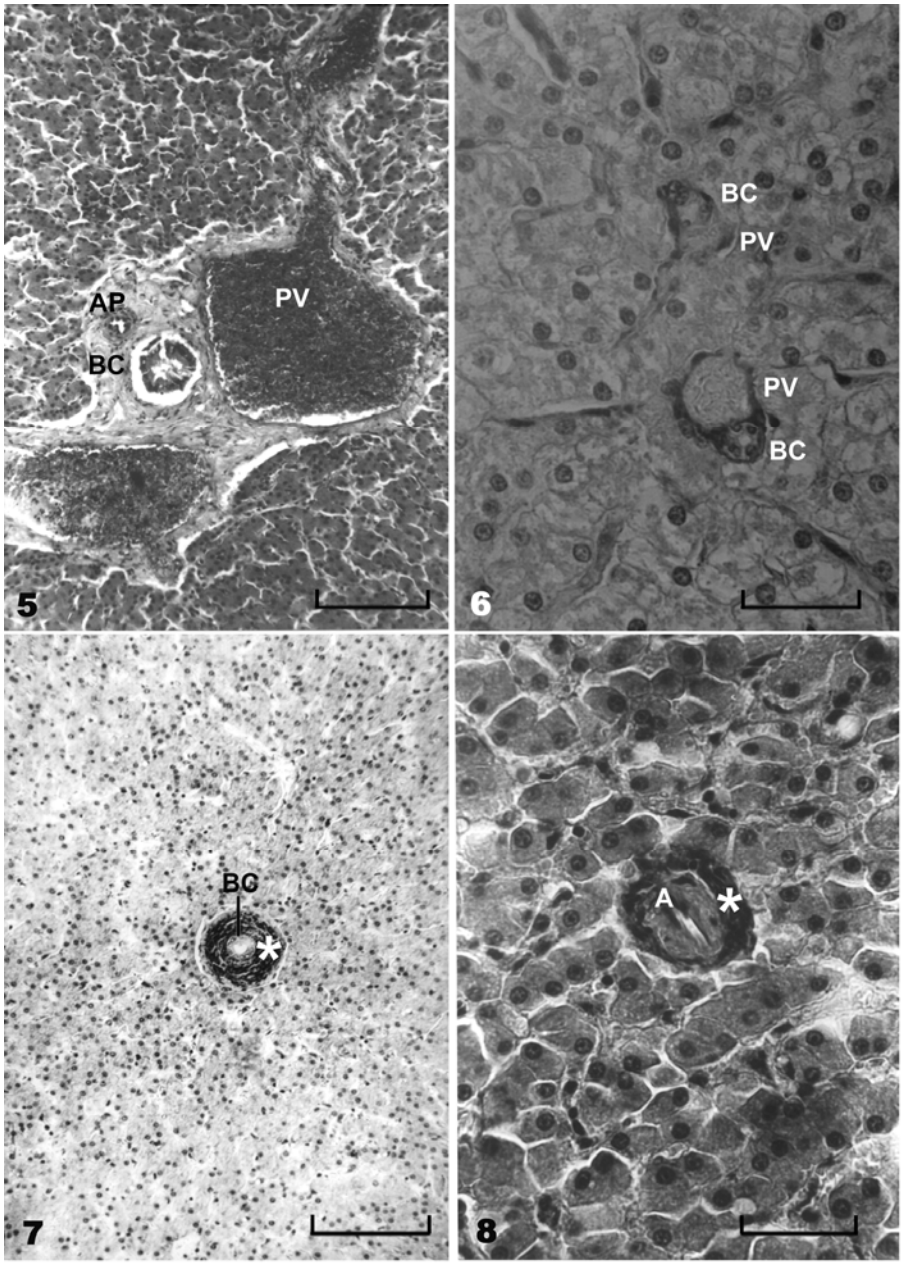


Fig. 5. Medium sized portal space: portal vein (PV), biliary canal (BC), portal arteriole (AP). H-E (Scale bar = 110 μm).
Fig. 6. Two small portal spaces: biliary canal (BC); portal vein (PV). PSR-H (Scale bar = 40 μm).
Fig. 7. Solitary biliary canal (BC); collagen sheath (*). PSR-H (Scale bar = 150 μm).
Fig. 8. Solitary arteriole (A); collagen sheath (*). PSR-H (Scale bar = 40 μm).

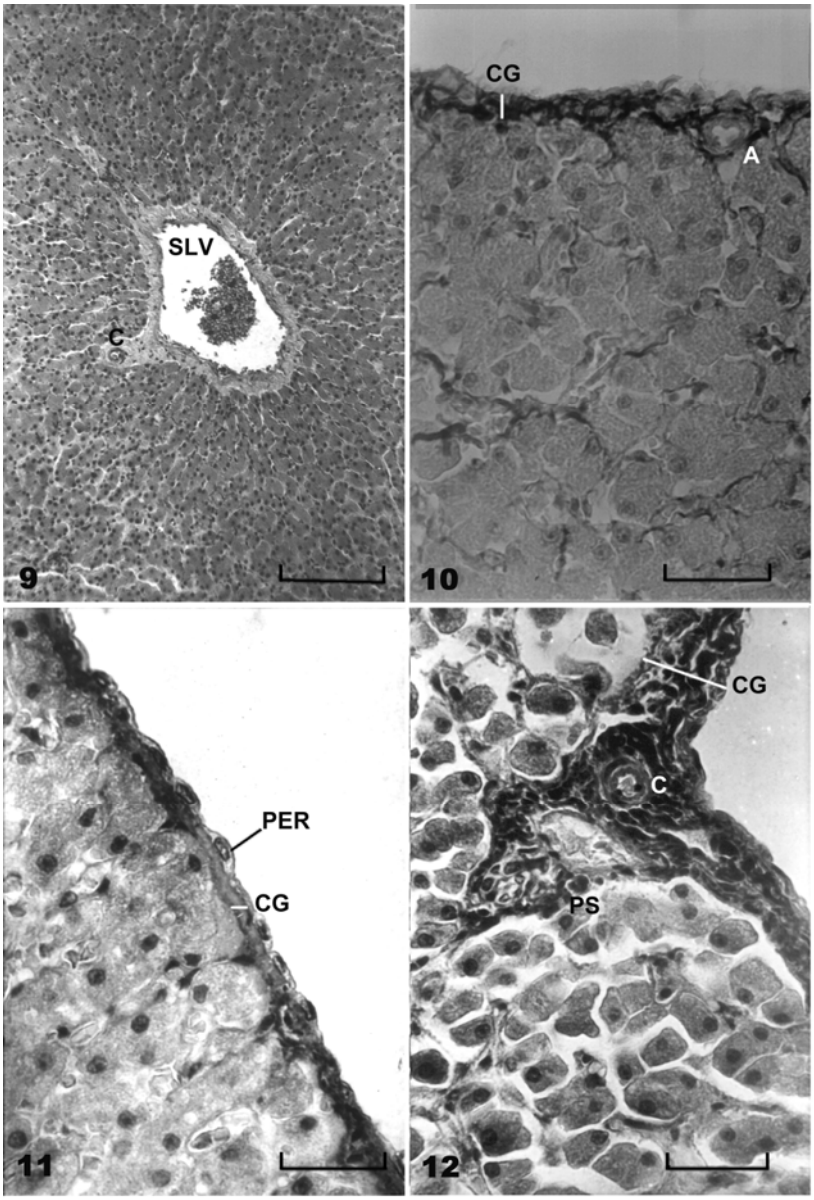


Fig. 9. Capillary (C) of the vasa vasorum system of a sublobar hepatic vein (SLV). H-E (Scale bar = 150 µm).

Fig. 10. Subcapsular zone of a hepatic lobe: thin capsule of Glisson (CG) of about 10 µm and an arteriole (A). PSR-H (Scale bar = 40 µm).

Fig. 11. Thin capsule of Glisson (CG) covered with a layer of mesothelial cells: peritoneal lining (PER). PSR-H (Scale bar = 40 µm).

Fig. 12. Thickened and fibrosed capsule of Glisson (CG). Note a capillary (C) and a portal space (PS). PSR-H (Scale bar = 40 µm).

Free solitary intralobular arterioles isolated towards the hepatic cords by thick picro-sirius red positive sheets were observed (Fig. 8). This type of arterioles was relatively frequent in the liver parenchyma, *e.g.*: on a histological section with the surface of about one square centimeter, three solitary arterioles were noted. The directions of the solitary arterioles were studied by serially histological sections. As a result, different possible destinations of these vessels can be described:

– The solitary arterioles may constitute the vasa vasorum system of some sublobar hepatic veins (Fig. 9). However, in the brown bear liver such arterioles or capillaries feeding the hepatic veins walls were rarely observed.

– Other category of solitary arterioles penetrated the connective tissue of the capsule of Glisson (Fig. 10). Sometimes, the portal subcapsular tracts extended just in the close vicinity of the capsule and the portal arteriole entered directly in the capsular connective wall from the subterminal portal space.

In the areas near the hilum, the capsule Glisson presented the thickness of 35–50 μm and was highly vascularizated. In other zones, the capsule of Glisson was relatively thin (5–20 μm). The outer surface of the capsule was lined by the visceral peritoneal membrane composed of mesothelial flat cells (Fig. 11). Towards the hepatic parenchyma, the connective fibrous layer was rich of arterioles, capillaries and fine lymphatics (Fig. 12).

DISCUSSION

According to histological observations on the *Ursus arctos* liver, each hepatic lobule is delimited from the neighbouring lobules by radial orientation of the sinusoids, respectively hepatic cords towards the hepatic central vein, having in peripheric position some portal spaces. There were never observed connective septa between different hepatic lobules, like in the pig liver.

It should be interesting to know the real source of the widespread affirmation about the similitude between the structure of the hepatic lobules of the polar bear (Gabe, 1973; Ekataksin & Wake, 1991), the black bear (*Ursus americanus*) (Ekataksin & Wake, 1991), all the family Ursidae (Dorst, 1973), the bear (probably *Ursus arctos*) (Braus, 1924; Pfuhl, 1932) with the structure of hepatic lobule of the pig.

As a result of an extensive literature investigation, Ekataksin & Wake (1991) concluded that of about 80 mammals species studied, there were seven species which presented a hepatic lobulation more or less similar with that one of the pig. Among these species the polar and the black bear were enumerated. To support this assertion, seven treatises published between 1924–1990 were cited.

This opinion, which dominated the bear liver knowledge during the 19-th and 20-th centuries, was possible to have the origin in the paper published in 1843 by E.H. Weber and M. Rusconi. An illustration and an annotation with a comparison between the liver blood vessels of the polar bear and those of the pig, was realized by E.H. Weber. This study was performed on liver material preserved in a slight solution of alimentary vinegar. By applying this conservation method, the maceration of both the liver parenchyma and collagenous septa was produced; only the main vessels of the liver remained more or less preserved. In these conditions, Weber (1843) affirmed that the bear liver vessels were alike with the pig liver vessels. This observation has no connection with the presence or absence of the connective septa in the hepatic lobules structure.

In *Ursus arctos* the capsule of Glisson was very thin (5–20 μm). Only towards the hepatic hilum and near the ligaments, the capsule of Glisson was thicker. But even in these zones it was not similar with the thickness of the pig's liver capsule (Ekataksin & Wake (1991), see the footnote of the page 117 which refers to "...the concave capsule appeared to be quite a few times thicker than the convex capsule, the thicknesses being 70–110 μm and 25–30 μm, respectively").

These differences between the brown bear and pig livers might be assigned to their taxonomic position. The pig (family Suidae) is enframed in the order Artiodactyla together with the family Ruminantia (Wilson & Reeder, 2005). The brown bear with whole family Ursidae belongs to the order Carnivora (Garshelis, 2009). It is possible that the bear liver structure as presented here would be characteristic for the order Carnivora. The fact is sure that the liver capsule in *Bison bonasus* (family Ruminantia) is over one hundred micrometers thick (Prunescu *et al*., 2002) having relatively similar thickness with the hepatic capsule of the pig (Ekataksin & Wake, 1991). The fact that both *Ursus arctos* and *Sus scrofa* are secondary omnivorous does not change the differences remarked in their liver histology.

Papers about the isolated arteries and/or arterioles (Braus, 1924; Elias, 1949; Dorst, 1973; Gabe, 1973; Ekataksin & Wake, 1997; Ekataksin, 2000) showed the free course of the solitary arterioles feeding the vascular bed of the portal veins, the vascular plexus of the hepatic veins and of the biliary canals (periductal plexus) and also the capsule of Glisson. Some of these destinations of the solitary arterioles were found again in the brown bear liver structure and were presented in this paper.

The fact is that during the winter, the brown bear hibernated without eating or drinking water for long periods (Hissa, 1997). It seems that during hibernation, the liver metabolism did no more depend exclusively upon the portal venous system (Prunescu *et al*., 2003). In this case, it should be possible that this relative rich system of solitary arterioles might assure the hepatocytes survival by the nutritive supply.

More information is necessary to have an explanation about the free solitary arterioles frequency through the parenchyma of the brown bear liver.

CONCLUSIONS

About 70 liver samples from 16 male brown bears (*Ursus arctos)* were histologically analyzed with the aim to describe hepatic lobule structure: portal tracts, hepatic veins, intra-lobular biliary canals, solitary arterioles, Glisson capsule.

The assertion about the similitude of the bear hepatic lobule with the pig hepatic lobule was invalidated: in the brown bear liver, each hepatic lobule is not separated from the other lobules by interlobular connective septa.

It seemed that at the origin of the erroneous affirmation might be the obsolete techniques used for the biological samples preservation which were in work over the past 150 years.

The differences between brown bear liver and pig liver histology might be assigned to their taxonomic position. It is possible that *Ursus arctos* pattern of the liver structure should be characteristic for ord. Carnivora different from the liver pattern of *Sus scrofa* enframed in the Artiodactyla order, Ruminantia family.

Acknowledgements. We are indebted to Dr. Nicolae Şerban-Pârâu who offered us liver samples of brown bears collected in the period 1998–2001.

REFERENCES

BANDIERA S.M., TOROK S.M., RAMSAY M.A., NORSTROM R.J., 1995*, Catalytic and immunologic characterization of hepatic and lung cytochromes P450 in the polar bears.* Biochem. Pharm., **49**: 1135-1146.

BRAUNE B.N., NORSTROM R.J., WONG M.P., COLLINS B.T., LEE J., 1991, *Geographical distribution of metals in livers of polar bears from Northwest Territories, Canada.* Science Total Environment, **100**: 283-300.

BRAUS H., 1924, *Anatomie des Menschen. Ein Lehrbuch fűr Studierende und Ärzte*, Verlag von J. Springer, Berlin. Die Leber, pp. 306-336.

DIETZ R., RIGET F., BORN E.W., 2000, *Geographical difference of zinc, cadmium, mercury and selenium in polar bears (Ursus maritimus) from Greenland.* Science Total Environment, **245**: 25-47.

DORST J., 1973*, Appareil digestif et annexes*. In: *Traité de Zoologie*, P.P. GRASSE, T. XVI Mammifères, Splanchnologie, fasc. V (vol. 1) par J. Anthony, L. Arvey, J. Dorst, M. Gabe, R. Weill, P. Zeitoun, Masson et Co. Eds, Paris, pp. 354-373.

DUNBAR M.R., WOODING J.B., THOMAS L.A., 1995, *Salmonella Hartford infection in a Florida black bear (Ursus americanus floridanus).* Florida Scientist, **58**: 252-254.

EKATAKSIN W., 2000, *The Isolated Artery: An intrahepatic arterial pathway that can bypass the lobular parenchyma in mammalian livers.* Hepatology, **31**: 269-279.

EKATAKSIN W., WAKE K., 1991, *Liver units in three dimensions. 1. Organization of argyrophilic connective tissue skeleton in porcine liver with particular reference to the compound hepatic lobules.* Am. J. Anat., **191**: 113-153.

EKATAKSIN W., WAKE K., 1997, *New concepts in biliary and vascular anatomy of the liver*. Progress in Liver Diseases, Ed. Saunders, **XV**: 1-30.

ELIAS H., 1949, *A re-examination of the structure of the mammalian liver*. Am. J. Anat., **85**: 379-456.

GABE M., 1973, *Anatomie microscopique de l'appareil digestif des Mammifères*. In: *Traité de Zoologie*, P.P. GRASSÉ, T. XVI, Mammifères, Splanchnologie, fasc. V (vol. 1), par J. ANTHONY, L. ARVEY, J. DORST, M. GABE, R. WEILL, P. ZEITOUN, MASSON et Co. (Eds). Paris, pp. 383-483.

GARNER H.M., BARR B.C., PACKHAM A.E., MARSH A.E., BUREK HUNTINGTON K.A., WILSON R.K., DUBNEY J.P., 1997, *Fatal hepatic sarcocystosis in two polar bears (Ursus maritimus)*. J. Parasitol., **83**: 523-526.

GARSHELIS D.L., 2009, *Family Ursidae (Bears)*. In: *Handbook of the Mammals of the World*, DON WILSON, RUSSELL A. MITTERMEIER (Eds.), Vol. 1, Carnivore. Barcelona, Lynx Edicions.

HISSA R., 1997, *Physiology of the European brown bear (Ursus arctos arctos)*. Ann. Zool. Fennici, **34**: 267-287.

JUNQUEIRA L.C.A., BIGNOLAS G., BRENTANI R.R.. 1979, *A simple and sensitive method for the quantitative estimation of collagen*. Annal. Biochem., **9**4: 96-99.

LEIGHTON F.A., CATTET M., NORSTROM R., TRUDEAU S., 1988, *A cellular basis of high levels of vitamin A in livers of polar bears (Ursus maritimus): the Ito cell.* Canadian J. Zool., **66**: 480-482.

MEDVEEV N., 1999, *Levels of heavy metals in Karelian wildlife (1989-1991).* Environmental Monitoring Assessment, **56**: 177-193.

NORHEIM G., SKAARE J.U., WIIG O. 1992, *Some heavy metals, essential elements and chlorinated hydrocarbons in polar bears (Ursus maritimus) at Svalbard,* Environmental Pollution, **77**: 51-57.

PFUHL W. 1932, *Die Leber*. In: *Handbuch der mikroskopischen Anatomie des Menschen*, W. von Möllendorff, Springer Verlag, Berlin, vol. **5**, Part 2, pp. 235-425.

PRUNESCU C.-C., PRUNESCU P., KRASIŃSKA M., KRASIŃSKI Z.A., 2002, *Liver histological structure in adult European bison. Bison bonasus (Linnaeus, 1758).* Folia Morphol., **61** (3): 137-142.

PRUNESCU C.-C., ŞERBAN-PĂRĂU N., BROCK J.H., VAUGHAN D.M., PRUNESCU P., 2003*, Liver and kidney structure and iron content in Romanian brown bears (Ursus arctos) before and after hibernation.* Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology, Elsevier, **134** (1): 21-26.

PRUNESCU P., PRUNESCU C.-C., 2002, *Liver structural particularities in the adult pig.* Proceedings of the Institute of Biology, **IV**: 489-496.

ROUTTI H., LECHER R.J., BORN E.W., BRANIGAN M., DIETZ R., EVANS T.J., FISK A. T., PEACOCK E., SONNE C., 2011, *Spatial and temporal trends of selected trace elements in liver tissue from polar bears from Alaska, Canada and Greenland.* J. Environ. Monit., **13** (8): 2260-2267.

SATO M., MIURA M., KOJIMA N., HIGASHI N., IMAI K., SATO T., WOLD H.L., MOSKAUG J.Ø., BLOMHOFF R., WAKE K., ROOS N., BERG T., NORUM K., SENOO H., 2001, *Nuclear deviation in hepatic parenchymal cells on sinusoidal surfaces in Arctic animals.* Cell Structure and Function, **26**: 71-77.

SENOO H., IMAI K., WAKE K., WOLD H.L., MOSKAUG J.Ø., KOJIMA N., MATANO Y., MIURA M., SATO M., ROOS N., BERG T., LANGVATN R., NORUM K., BLOMHOFF R., 1999, *Vitamin A storing system in mammals and birds in arctic area. A study in Svalbard Archipelago.* Kupffer Cell Foundation, E. WISSE, D.L. KNOOK, R. DE ZANGER and R. FRASER (Eds.), **7**: 34-35.

WEBER E.H., RUSCONI M., 1843, *Ueber den Bau der Leber des Menschen und einiger Thiere.* Archiv fűr Anatomie, Physiologie und Wissenschaftliche Medicine, Dr. Johannes Műller (Ed.), Berlin, Verlag von Veit et Comp., pp. 338-342.

WILSON D.E., REEDER D M., 2005, *Mammal Species of the World (3-rd. ed.)*, Artiodactyla, Johns Hopkins University Press, pp. 111-184.

WÜNSCHE A., 1985, *Zum vergleich der Leberläppchentypen einschliesslich des Rappaportschen Acinus beim Schwein*. Zentbl. Vet. Med. (Anat. Histol. Embryol.), **14**: 15-32.
ZILINCAR J., BYSTRICA B., KUBIN D., HELL P., 1992, *The presence of heavy metals in brown bears in the West Carpathian Mountains.* Zeitschrift fuer Jagdwissenschaft, **38**: 235-243.

Received August 10, 2012

Institute of Biology Bucharest of the Romanian Academy, 296 Splaiul Independentei, 060031 Bucharest, P.O. Box 56-53, *Romania*
e-mail: paula.prunescu@yahoo.com

HARD TICKS (ACARI: IXODIDAE) – VECTORS FOR TICK-BORNE ENCEPHALITIS VIRUS IN ROMANIA

LUCIA ELENA IONESCU[*], ALEXANDRU VLADIMIRESCU[**], DIANA POPESCU[*], SIMONA BICHERU[*], IRINA TEODORESCU[***], GABRIELA DUMITRESCU[*], MARIUS NECŞULESCU[*], VIOREL ORDEANU[*]

Our research demonstrates that the vector *Ixodes ricinus* has been involved in the transmission of tick-borne encephalitis virus (TBEV) in Romania (Brateiu village and surroundings, Sibiu County). Ixodid specimens were collected from domestic animals in the outbreak area and viral isolates were obtained from them by intracerebral inoculation on suckling mice (BALB/C strain). The resulting viral isolates were examined by enzyme-linked immunosorbent assay (ELISA) followed by reverse-transcription (RT) and polymerase chain reaction (PCR). The serological and molecular analyses demonstrated the presence of TBEV in viral isolates from *Ixodes ricinus* vector. This study has allowed the development of a model for analysis and identification of presumed TBEV isolated from *Ixodes ricinus*. This may be useful for implementing adequate epidemiological surveillance and detection of the virus presence in both the ixodid vector populations and even in the vertebrate hosts in areas where humans could have contact with ticks.

Key words: Ixodidae, *Ixodes ricinus*, tick-borne encephalitis virus, Romania.

INTRODUCTION

Ticks belong to Phylum Arthropoda, Subphylum Chelicerata, Class Arachnida, Subclass Micrura (mites and ticks), Superorder Parasitiformes, Order Ixodida (Metastigmata).

Ixodida order comprises three families: Ixodidae (hard ticks), Argasidae (soft ticks) and Nuttalliellidae (with one species) (Cupp, 1999; Horak, 2002). Ixodidae family includes 14 genera with 702 haematophagous ectoparasite species (feeding on mammals, birds, and sometimes reptiles and amphibians) (Guglielmone *et al.*, 2010).

Ixodes, the largest genus of the Ixodidae family, comprises 243 extant species considered valid, and found worldwide (Guglielmone *et al.*, 2010). *Ixodes ricinus* is considered a complex of 14 species worldwide distributed. The most prevalent species in Europe, *Ixodes ricinus* is a haematophagous ectoparasite of over 100 mammalian, reptilian and bird species. Hosts of the adult ticks are some large mammals such as sheep, cattle, dogs, deer, horses, and even humans. The hosts for larvae and nymphs are many insectivores, rodents, rabbits, bats, birds, reptiles.

The Romanian fauna of hard ticks (Acari: Ixodidae) comprises 25 species (Feider, 1965; Bădescu, 1967; Coipan *et al*., 2010; Mihalca *et al.*, 2012), 13 of these belonging to the genus *Ixodes*. Many of these tick species are important vectors for different human and animal diseases (Dumitrache *et al.*, 2012) produced by pathogens of both medical and veterinary importance (Coipan, 2010). Among them, *Ixodes ricinus* is the most common and widespread species in Romania (Feider, 1965; Coipan, 2010; Coipan *et al.*, 2011). In Romania *Ixodes ricinus* hosts are mammals, birds, lizards, and humans (Feider, 1965; Teodorescu *et al.*, 2002). Recent studies showed 20 new tick-host associations for *I. ricinus* in Romania (Coipan *et al.*, 2011; Mihalca *et al.*, 2012).

Ixodidae species are vectors for many pathogenic organisms: protozoans (*Babesia divergens*, *B. venatorum*, *B. bovis*, *B. bigemina*, *B. duncani*, *B. caballi*, *B. canis*, *B. gibsoni*, *B. ovis*, *Theileria annulata*, *T. parva*, *T. orientalis*, *T. equi*, *Hepatozoon canis*, *Trypanosoma theileri***)**, bacteria (Rickettsiaceae-*Cowdria ruminantium*, *Cytoecetes phagocytophila*, *Ehrlichia canis*, *E. bovis*, *E. ovina*, *Anaplasma marginale*, *Coxiella burneti*, *Rickettsia conori*, *R. sibirica*, *R. australis*, *R. slovaca*, *R. sennetsu*, *R. canada*), Spirochaetes (*Borrelia burgdorferi*, *Francisella tularensis*), viruses (Louping ill, Tick-borne encephalitis viruses, Omsk hemorrhagic fever virus, Kyasanur forest virus, Eyach virus, Nairobi virus) (Mitrovic *et al.*, 2004; Conrad *et al.*, 2006; Bitam *et al.*, 2009).

Tick-borne encephalitis virus (TBEV) is an arbovirus (**Ar**thropod **Bo**rne **Virus**) member of the Flaviviridae family, *Flavivirus* genus. There are three genetic lineages clearly distinguished: the European or Western TBEV, the Far Eastern TBEV and the Siberian TBEV subtypes (Ecker *et al.*, 1999) of which only the first one is present in Romania. *Ixodes ricinus* is both vector and reservoir for TBEV.

TBEV causes human tick-borne encephalitis (TBE), a viral infection endemic in a vast area of Europe and Asia, transmitted by the bite of an infected tick. The virus can produce central nervous system disorders among them being meningitis, encephalitis (irritation and inflammation of the brain), meningoencephalitis (inflammation of the brain and meninges), meningoencephalomyelitis (inflammation of the brain and spinal cord) or meningoradiculoneuritis Bannwart's syndrome (nerve pain radiating out from the spine) (Kaiser *et al.*, 1999; Mansfield *et al.*, 2009, neurological and psychiatric sequels and a significant mortality rate. A postencephalitic syndrome, more severe in older patients, maintains in about 40% of the infected persons.

The increase of ixodid populations and the extension of TBEV geographic spread area correlated mainly with climate change, especially with temperature and humidity increase, and conducted to the rise of TBE incidence in human populations (Randolph, 2004). The increase of the ixodid populations is also a consequence of "vector urbanization", because the ticks are spread not only in

coniferous and deciduous forests, in grassland and rough pastures, but even in parks and other new places for leisure in urban areas more numerous in the last years (Korenberg *et al.*, 1984; Greenfield, 2011; Kriz *et al.*, 2012).

An outbreak of TBE with 39 confirmed cases was registered in 1999 in Brateiu village and surroundings, Sibiu county, Romania and *Ixodes ricinus* ticks were identified in the area (Ungureanu *et al.*, 2001). We have performed this study to demonstrate that *Ixodes ricinus* ticks transmitted the TBEV during the outbreak.

MATERIAL AND METHODS

TICK SAMPLING

Ixodes ticks were collected from domestic animals in the households of patients diagnosed with TBE during the outbreak in Brateiu and they belonged to *Ixodes ricinus* species (Ungureanu *et al.*, 2001). The identification of TBEV in *Ixodes ricinus* ticks has been performed both by serological and molecular methods.

OBTAINING OF VIRAL SAMPLES

The supernatants obtained by centrifugation (2000 rpm for 5 minutes) of *Ixodes ricinus* suspensions from ground ticks were intra-cerebrally inoculated in families of suckling mice (BALB/C strain). Brains of suckling mice (possibly containing TBEV) were collected between the 4th day and the 6th day after inoculation. The same procedure was used with Hypr TBEV reference strain (Central European viral strain) as positive control (Ionescu *et al.*, 2009).

SEROLOGICAL METHODS

Sucrose-acetone extraction method (Clarke & Casals, 1958) was used for the extraction of viral antigens from brains of suckling mice.

Testing of viral antigens to detect the positive samples for TBEV from *Ixodes ricinus* in parallel with antigen from Hypr TBEV reference strain was performed by the technique of the IgM *capture enzyme-linked immunosorbent assays* (*ELISA*) for arboviruses (Martin *et al.*, 2000) in which we used the antigens extracted from brains of suckling mice and human sera confirmed positive for antibodies against TBEV during the TBE outbreak.

MOLECULAR METHODS

The *RN-easy Lipid Tissue Mini Kit* (Qiagen) for the extraction of genomic viral RNA (ribonucleic acid) from tissue samples (brains of inoculated suckling

mice) was used. The reverse transcription PCR was performed with *One Step PCR Kit* (Qiagen) and nested PCR with *AmpliTaq Gold* (Applied Biosystems). In both reactions, two pairs of oligonucleotide primers specific for 5`noncoding region (5 'NCR) of TBEV genome were used. The amplicons were visualized following electrophoresis on 2.5 % agarose gel (stained with ethidium bromide) (Floris *et al.*, 2006).

RESULTS AND DISCUSSION

The main aim of this study was to demonstrate, using both serological and molecular investigations, that *Ixodes ricinus* was involved in the transmission of TBEV in Brateiu (Sibiu County, Romania) in 1999. This study also draws up a model of analysis and identification of TBEV in *I. ricinus* vectors sampled from an epidemic area.

The serological tests revealed similar results for antigen extracted from *I. ricinus* viral isolates and for the antigen extracted from the viral reference strain. We found that both specific antigens positively reacted with the antibodies present in human TBEV positive serum samples. These findings confirmed the presence of the virus in the viral isolates obtained from *Ixodes ricinus*.

Genomic viral RNA extracted from brains infected with virus from *Ixodes ricinus* and with Hypr TBEV reference strain was used for molecular analysis.

Extracted genomic RNA was used in RT-PCR and nested PCR. Using specific primers we obtained 178 bp (base pairs) amplicons, characteristic for the TBEV, confirming the presence of this virus in *Ixodes ricinus* viral isolates.

In this study we designed a model of analysis and identification of TBEV from *Ixodes ricinus*, by serological and molecular methods (Fig. 1).

The confirmation of TBEV presence in *Ixodes ricinus*, by serological and molecular methods, has a great importance in determining the areas where the virus is present in the ixodid vectors and where there is a potential risk for TBE infections.

TBE diagnosis is very important because it can distinguish between the infection with TBEV and infections with other etiologic agents but with the same symptoms. This fact is very important for the implementation of an adequate and supportive epidemiological surveillance.

Furthermore, ticks (Acari: Ixodidae) are vectors for various pathogens, including biological warfare agents with lethal and incapacitating potential (*i.e*. TBEV). Therefore, establishing a standard protocol for analysis and identification of some of those agents represents a major achievement, not only for the national public health, but also for the national protection against biothreats.

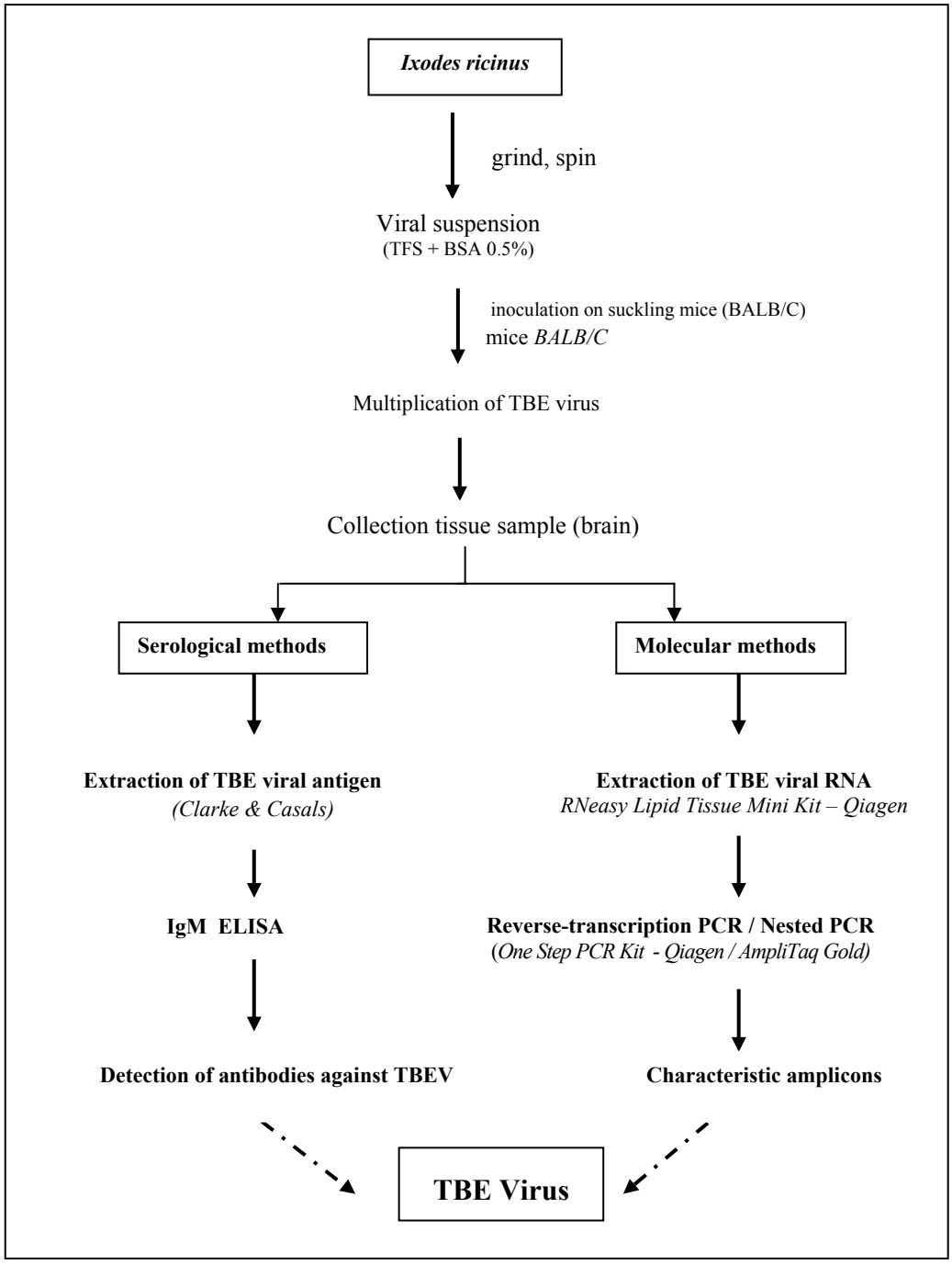


Fig.1. Identification of TBE virus in *Ixodes ricinus* by serological and molecular methods.

CONCLUSIONS

This study showed that TBEV was present in *Ixodes ricinus* collected from domestic animals in the households of patients that have been diagnosed with TBE in Brateiu village (Sibiu County, Romania) during the outbreak in 1999.

Our research led to the development of a protocol for analysis and identification of TBEV isolated from *Ixodes ricinus*.

Positive results for viral isolates analyzed both by serological and molecular methods demonstrate the presence of TBEV virus in *Ixodes ricinus* and the involvement of this tick species in the meningoencephalitis outbreak caused by this arbovirus in one location in Romania.

Using serological and molecular methods for the detection of TBEV in the ixodid vector populations is useful for the implementing of the adequate epidemiological surveillance in the risk areas.

Acknowledgement. The authors are grateful to Scientific Researcher Alexse Aurora for help and support during their research activities.

REFERENCES

BĂDESCU C., 1967, *Ixodes arboricola Schulze şi Schlottke 1929*, *o nouă specie a subgenului Pholeoixodes Sch. 1942 din Republica Socialistă România*. Lucrări Ştiinţifice, Seria C, Zootehie şi Medicină Veterinară, **10**: 329-338.

BITAM I., RAOULT D., 2009, *Other tick-borne diseases in Europe*. Curr. Probl. Dermatol., **37**: 130-154.

ECKER M., ALLISON S.L., MEIXNER T., HEINZ F.X., 1999, *Sequence analysis and genetic classification of tick-borne encephalitis viruses from Europe and Asia*. Journal of General Virology, **80**: 179-185.

CLARKE D.H., CASALS J., 1958, *Techniques for haemagglutination and haemagglutination-inhibition with arthropod-borne viruses*. Amer. J. Trop. Med. Hyg., **7** (5): 561-573.

COIPAN E.C., 2010, *Hard ticks (Acari: Ixodidae) – Vectors for Lyme disease spirochetes in Romania*. Romanian Journal of Biology-Zoology, **55** (2): 177-184.

COIPAN E.C., VLADIMIRESCU A.F., CIOLPAN O., TEODORESCU IRINA, 2011, *Tick species (Acari*: *Ixodoidea) distribution*, *seasonality and host associations in Romania*. Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa", **LIV** (2): 301-317.

CONRAD P.A., KJEMTRUP A.M., CARRENO R.A., THOMFORD J., WAINWRIGHT K., EBERHARD M., QUICK R., TELFORD S.R., HERWALDT B.L., 2006, *Description of Babesia duncani n. sp.* (*Apicomplexa*: *Babesiidae*) *from humans and its differentiation from other piroplasms.* International J. Parasitol., **36** (7): 779-789.

CUPP E.W., 1991, *Biology of Ticks, Veterinary Clinics of North America, Small Animal Practice.* Hoskins J.D. (Ed.), 21: 1 Jan. 1992.

DUMITRACHE M. O., GHERMAN C. M., COZMA V., MIRCEAN V., GYÖRKE A., SÁNDOR A. D., MIHALCA, 2012, *Hard ticks (Ixodidae) in Romania: surveillance, host associations, and possible risks for tick-borne diseases*. Parasitol. Res., **110** (5): 2067-2070.

FEIDER Z., 1965, *Arachnida Acaromorpha*, *Suprafamilia Ixodoidea* (*Căpuşe*), pp.: 89-104. *In*: Fauna R.P.R., Vol. 2, Edit. Acad. R.P.R.

FLORIS R., ALTOBELLI A., BOEMO B., MIGNOZZI K., CINCO M., 2006, *First detection of TBE virus sequences in Ixodes ricinus from Friuli Venezia Giulia (Italy)*. New Microbiologica, **29**: 147-150.

GUGLIELMONE A.A., ROBBINS R.G., APANASKEVICH D.A., PETNEY T.N., ESTRADA-PEÑA A., HORAK I.G., SHAO R., BARKER S.C., 2010, *The Argasidae*, *Ixodidae and Nuttalliellidae* (*Acari*: *Ixodida*) *of the world*: *a list of valid species names*. Zootaxa, 2528: 1-28.

GREENFIELD B.P.J., 2011, *Environmental parameters affecting tick (Ixodes ricinus) distribution during the summer season in Richmond Park*, *London*. Bioscience Horizons, **4** (2): 140-148.

HORAK I.G., CAMICAS J.L., KEIRANS J.E., 2002, *The Argasidae*, *Ixodidae and Nuttalliellidae* (*Acari*: *Ixodida*): *a world list of valid tick names*. Experimental and Applied Acarology, **28**: 27-54.

IONESCU L., ALEXSE A., CEIANU C., NECSULESCU M., POPESCU D., BICHERU S., DUMITRESCU G., CUMPĂNĂŞOIU E., CUMPĂNĂŞOIU C., PASAT L., TÎRZIU E., 2009, *Investigation methods used for identifying the presence of tick-borne encephalitis virus (TBEV) in vector arthropods.* Scientifical Papers Veterinary Medicine, **XLII** (1): 288-293.

KAISER R., 1999, *The clinical and epidemiological profile of tick-borne encephalitis in southern Germany 1994–1998*. Oxford Journals, Medicine, Brain, **122** (11): 2067-2078.

KORENBERG E., CERNÝ V., DANIEL M., 1984, *Occurrence of ixodid ticks-the main vectors of tick-borne encephalitis virus in urbanized territory*. Folia Parasitologica (Praha), **31** (4): 365-370.

KRIZ B., MALY M., BENES C., MILAN D., 2012, *Epidemiology of Tick-Borne Encephalitis in the Czech Republic 1970–2008*. Vector-Borne and Zoonotic Diseases, **12** (11): 994-999.

MARTIN D. M., MUTH D.A., BROWN T., JOHNSON A. J., KARABATSOS N., ROEHRIG J.T., 2000, *Standardization of Immunoglobulin M Capture Enzyme-Linked Immunosorbent Assay for Routine Diagnosis of Arboviral Infections*, J. Clin. Microbiol., **38** (10): 1823-1826.

MANSFIELD K. L., JOHNSON N., PHIPPS L. P., STEPHENSON J. R., FOOKS A. R., SOLOMON T., 2009, *Tick-borne encephalitis virus – a review of an emerging zoonosis*. J. Gen. Virol., **90** (8): 1781-1794.

MIHALCA A.D., DUMITRACHE M.O., MAGDAŞ C., GHERMAN C. M., DOMŞA C., MIRCEAN V., GHIRA I.V., POCORA V., IONESCU D.T., SIKÓ BARABÁSI S., COZMA V., SÁNDOR A.D., 2012, *Synopsis of the hard ticks (Acari: Ixodidae) of Romania with update on host associations and geographical distribution*. Exp. Appl. Acarol., **58** (2): 183-206.

MITROVIC S., KRANJCIC-ZEC I., ARSIC-ARSENIJEVIC V., DZAMIC A., RADONJIC I., 2004, *Human babesiosis – recent discoveries*. Med. Pregl., **57**: 349-353.

RANDOLPH SE., 2004, *Evidence that climate change has caused 'emergence' of tick-borne diseases in Europe?* Int. J. Med. Microbiol., **293** (37): 5-15.

TEODORESCU IRINA, POPA E., 2002, *Ixodidae species in domestic mammals in Romania.* Revue Roumaine de Biologie, Série de Biologie Animale, **47** (1-2): 107-115.

UNGUREANU A., CEIANU C., NICOLESCU G., NIȚESCU L., PAUL F., BALAN M., OLARU C., 2001, *Investigations into the focus of tick-borne encephalitis in Brateiu village, Sibiu district*. Bacteriologia, Virusologia, Parazitologia, Epidemiologia, **46** (3): 125.

Received November 15, 2012

[*] *Military Medical Research Center*
C.A. Rosetti, 37, Bucharest, Romania
e-mail: ionescu.lucia@gmail.com

[**] *"Cantacuzino" National Institute of Research-Development for Microbiology and Immunology*
Splaiul Independenței 103, 050096, Bucharest, Romania
e-mail: alexandruvladimirescu@cantacuzino.ro

[***] *University of Bucharest, Faculty of Biology, Department of Systems Ecology and Sustainability*
Splaiul Independenței 91-95, 050095, Bucharest, Romania
e-mail: Teodorescubiologie@yahoo.com