Dear reader,

You may wonder why this issue is much thinner than previous issues. It is my aim as new editor-in-chief to improve the quality and the international impact of the Belgian Journal of Zoology. As a consequence, our rejection rate has increased drastically in 2010 to more than 80%, and there were fewer manuscripts that could be accepted for publication. For 2011 and beyond, this new strategy leaves room for more interesting and stimulating papers. I am currently looking for such good-quality manuscripts, and encourage you and your colleagues to submit any suitable manuscripts to the editorial office of the Belgian Journal of Zoology.

I hope that you will enjoy reading this and future issues of the Belgian Journal of Zoology.

Isa SCHÖN

Brussels, January 2011

Interuniversity Master Day Biology (Saturday, March, 12th 2011)
Royal Belgian Institute of Natural Sciences

Event organized by the Royal Belgian Society for Zoology.

Aim

To provide a national forum for 3rd year bachelor students to inform themselves about the different master programs in Biology currently organised at all Belgian universities.

Partners

• Organizing committee: Governing Board of the Royal Belgian Society for Zoology

(Co-ordinator Dominique Adriaens, dominique.adriaens@ugent.be,

Phone 09/264.52.19; Fax 09/264.53.44).

• Universities with master program in Biology: all Belgian universities that are organising a Biology master, and associated master programs focussing strictly on Biology (for reasons of making this event focussed, other master programs that may also be directly accessible from the Bachelor in Biology but are focusing on for example applied biological studies will not be included). The following universities have confirmed their participation and agree to communicate their master program with an oral presentation:

1. VUB (Brussel)
2. ULB (Bruxelles)
3. KULeuven
4. UA (Antwerpen)
5. UGent
6. Ulg (Liège)
7. FUNDP (Namur)
8. UCL (Louvain-la-Neuve)
9. UMH (Mons)

• Other universities: universities that have master programs to which Bachelor students in Biology can have directly access to, but are not strictly Biology Masters, will be asked to present their master program with a poster presentation:

1. UHasselt – Master in 'Environment and Public Health'
2. UA – Master in Environmental Sciences
3. UGent – Master in Nematology
4. UGent – European Master of Science in Nematology
5. VUB-UA-UGent – Master in Marine and Lacustrine Sciences and Management
6. UGent - Erasmus Mundus Marine Biodiversity and Conservation
7. ULg – Master in Environmental Science and Management
8. ULg - Master in Oceanology
9. ULg - Master in Bio-informatics and Modelling
10. ULg – Master in Biochemistry and Molecular Biology
11. ULB – Master in Bioinformatics and Modelling
12. UMons-ULB - Biochemistry and Molecular and Cell Biology
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Belg. J. Zool., 140 (2) : 203-211 July 2010

Food Composition of the Little Owl *Athene noctua* in Farmland Areas of South East Poland

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ABSTRACT. The feeding ecology of the Little Owl (*Athene noctua*) was studied in farmlands of southeast Poland, which is dominated by monocultural farms. 3065 prey dissected from pellets were collected at 13 pellet stations between 1999 and 2001 through 7 seasons in total. Mammals were found to dominate both in total number (54.3% of caught prey) and total biomass (93.0% of caught prey) while insects comprised 43.0% of the number, but only 1.1% of the prey biomass. However, the proportion of insects reached up to 62% in certain seasons. Coleopteran beetles dominated the insect fraction representing 98.3% of the number and 87.9% of the insect biomass. Our study also illustrated that in some seasons, the prey composition included taxa related to intensive agricultural management. The food composition of the Little Owls from the examined Polish sites is more similar to Eastern and Southern populations than to Northern and Western populations of Little Owls in Europe.

KEY WORDS: Little Owl, diet, farmland, Poland

INTRODUCTION

Populations of the Little Owl *Athene noctua* have recently been decreasing in many European countries (MANEZ, 1994; VOGRIN, 1997; VORONETSKY, 1998; HEATH et al., 2000). The species has therefore become a high conservation priority, of increasing interest as a research subject, which in turn has resulted in the study of many aspects of its biology, ecology and distribution (ZERUNIAN et al., 1982; GENOT, 1994; ANGELICI et al., 1997).

Also in Poland, a drop in the number of little owls has been observed during the last 10 to 20 years, seemingly more evident in Western and Central Poland (TOMIALOJC & STAWARCZYK, 2003; ZMIHORSKI et al., 2006; GRZYWACZEWSKI, 2006a). Southeast Poland is slightly more densely populated by *A. noctua* (TOMIALOJC & STAWARCZYK, 2003), where it can be observed in towns and farms (KITOWSKI, 2000; 2002; KITOWSKI & GRZYWACZEWSKI, 2003; KITOWSKI & KISIEL, 2003; GRZYWACZEWSKI, 2006a). The obviously greater numbers of owls at sites in agricultural landscapes allows data collection from a large number of pellet stations for the study of food composition. In Central and East European countries, there is a lack of new data on the food for the Little Owl in contrast to Western Europe, the Mediterranean and Middle East, where several interesting studies were conducted (ZERUNIAN et al., 1982; GOTTA & PIGOZZI, 1997; ANGELICI et al., 1997; OBUCH & KRISTIN, 2004; ALIVIZATOS et al., 2005; VAN NIEUWENHUYSE et al., 2008). A good knowledge of the food ecology of Little Owls that also considers seasonal changes is needed for the development of appropriate conservation strategies. This is especially relevant because some authors (GENOT & VAN NIEUWENHUYSE, 2002; ZMIHORSKI et al., 2006) pointed out that the cause of the decline of Little Owls might be food related. New data on the food composition of Little Owls from Poland are largely lacking with the exception of two studies (BACIA, 1997; GRZYWACZEWSKI et al., 2007), where only the former considered seasonal changes in food. The aim of the presented research was to analyse the food composition of Little Owls and its seasonal changes, in farmland of southeast Poland dominated by large monoculture fields.

MATERIALS AND METHODS

Study area and methods

The study was carried out between 1999 and 2000 in the agricultural landscape of the southeast part of the Lublin region (the surroundings of the towns: Hrubieszow, Tomaszow Lubelski, within the triangle: 50°48'N, 23°55'E; 50°27'N, 24°00'E; 50°27'N, 23°25'E; SE Poland). Lands in this region used to be owned by the State from the Second World War till the early 90's. They were formed into state farms (in Polish: PGR – Panstwowe Gospodarstwo Rolne) or collective farms (in Polish: RSP – Rolnicza Spoldzielnia Produkcyjna). Presently, most of the state farms belong to private farmers or workers' associations. Infrastructure not owned by the new owners has progressively deteriorated. In order to collect pellets, the recesses, garrets, air channels, air holes, chimneys, and other niches in locations such as barns, cowsheds, dovecotes, granaries, corn hop bins, fertiliser store houses, sheds, and flat blocks were searched in 13 villages: Dutrow, Kornie, Przewodow, Mycow, Machnow Stary, Krzewica, Machnow Nowy, Kosciaszyn, Cichoborz II, Nowosiolki Kardynalskie, Dolhobyczow PGR, Szczepiatyn PGR and Zurawce PGR (elevation: 250-300m.a.sl.).

The distribution of meadows and cultivated fields was mapped and their surface areas calculated with a digital planimeter from high-resolution aerial photos for a radius of 2km around each pellet station. In the first five villages, meadows covered more than 30% of the total area within a 2km radius, while in the other eight villages

meadows were less than 30% of the total area within a 2km radius.

All villages were situated closely together (within 50km) in agricultural land. The infrastructure of the farms and their dwellings mostly formed "islands" surrounded by large field monocultures (fields with an average size of 25ha).

The food of Little Owls was studied in seven seasons, starting in summer 1999 until spring 2001. Prior to searching for pellets in the summer of 1999, all the places mentioned above were cleaned of "old pellets." The last collection of pellets of this study was performed on the day of the end of the astronomical winter 2001 (Tables 1 & 2). The overall number of pellets amounted to 608. The pellets were prepared for analysis by standard methods (RUPRECHT et al., 1998). The number of vertebrate prey species was determined on the basis of skulls, mandibles, teeth and other important key remains following several authors (BOHME, 1977; ARNOLD & BURTON, 1980; PUCEK, 1984; CUISIN, 1989; DIESENER & REICHOLF, 1997) The following keys were used to note insects (MROCZKOWSKI, 1954; MROCZKOWSKI, 1955; SMRECZYNSKI, 1966; STEBNICKA, 1978; DAHLGREN, 1979; STEBNICKA, 1991; CHINERY, 1993; WARCHALOWSKI, 1993; HURKA, 1996; BURAKOWSKI et al., 1973; 1974). In some cases, we used our own collections of insects from the study area for comparisons with insect parts in the pellets. In the case of undetermined beetles Coleoptera n. det., the average biomass of specimens was estimated for the most numerous species or genus found in the analysed pellets. Some prey were grouped in different categories (for example as Aves n.det. Arvicolidae n.det., *Sylvaemus,* Muridae*, Pterostichus* n.det., Coleoptera n.det. itp. in Table 1), because sometimes a high degree of fragmentation did not allow them to be identified to the genus or species level. To estimate the prey biomass, data for invertebrate and vertebrate prey biomass were used as in PUCEK (1984), ROMANOWSKI (1988), KRUUK (1989), BACIA (1997) and JEDRZEJEWSKA & JEDRZEJEWSKI (2001). In a few cases, independent weighing of captured insects was performed because data were not available from the literature. Biomass of the earthworm *Lumbricus terrestris* and their number on the basis of chaetae was calculated following KRUUK & PARISH (1981). The breadth of food niches of owls was estimated with the formula by LEVINS (1968): $B=1/Sp_i^2$, where p_i is the proportion of the prey category i in the total biomass of the owl's diet.

Species richness (S) and Shannon-Wiener (H) indices were calculated to measure how similar the abundance of different prey categories was, while the evenness index calculated as E=H/log(S) measured the abundance of different prey categories. In the formula provided, H is the sum $[P_i\log(P_i)]$, S is the number of prey categories and P_i is the proportion of prey category i in the total number of prey (KREBS, 1997). The breadth of the food niche was calculated by the B index: $B=1/\Sigma p_i^2$ where p_i is the amount of the biomass of the i-th prey (LEVINS, 1968).

The means and standard deviation (SD) were provided for parametric data while non-parametric data were presented as medians and standard errors (SE) (FOWLER & COHEN, 1992).

RESULTS

General Food Composition of Studied Little Owls

From all collected pellets, a total of 3065 prey items could be distinguished with a total biomass of 32800.07g (Table 1). Among the analysed prey, vertebrates were dominant and comprised 56.7% of the number of prey captured and 98.8% of its biomass (32393.3g) (Table 1). Amphibians were represented only by a single order and species (Table 1), while one order and two species of birds were found, the sparrows *Passer montanus* and *Passer domesticus*. Avian prey was strongly dominated by the house sparrow *P. domesticus* and they comprised as much as 80.0% of the number and 85.6% of the mass of all birds (Table 1).

Among the vertebrate prey, mammals were most common in both number and biomass (95.7% of prey number and 94.1% of prey biomass – see Table 1). Mammalian prey belonged to three orders (Insectivora, Chiroptera, Rodentia), four families and 15 species, and were dominated by rodents (52.7% of prey number and 91.9% of prey biomass – see Table 1).

Two phyla, namely Annelida and Arthropoda, were represented in the invertebrate prey, totalling 406.8g altogether. Among the invertebrates, the highest numbers (99.4%) and biomass (95.6%) belonged to insects of which three orders with 13 families were identified: the order Orthoptera (families: Gryllotalpidae, Tettigoniidae), the order Coleoptera (families: Carabidae, Chrysomelidae, Curculionidae, Dynastidae, Dytiscidae, Elateridae, Geotrupidae, Scarabaeidae, Silphidae, Tenebrionidae) and the order Dermaptera (family Forficulidae).

Little Owls mostly caught beetles, which comprised as much as 98.3% in number and 87.9% of the biomass of all hunted insects (Table 1). Among the beetles, the mealworm *Tenebrio molitor* was especially frequent, contributing 29.1% (in number) and 48.9% (in biomass) of all beetles sampled in this study that could be identified to genus level.

The average mass of all prey caught by Little Owls in the study area was 10.63±11.06g per prey, ranging from 0.1-166g. The average prey biomass was not significantly different between the 13 sites where pellets were collected (ANOVA: $F_{11, 3053}=0.2197$, p=0.9964). However, highly significant differences were observed in the median mass of prey caught in the villages with less than 30% meadows within a radius of 2km from the pellet stations (8.0±0.236g, 0.1g-166g) as compared to villages with more than 30% meadows (19.0±0.314g, range: 0.1g-166g) (Mann-Whitney U-test: Z=-18.32, $n_1=2032$, $n_2=1033$, p<0.001).

Seasonal Changes of Food Composition

The median of prey mass ranged from 0.55±0.574g to 19±0.520g per study period and was significantly different between the seven study periods (Kruskal-Wallis ANOVA: H=156.1, df=6, P<0.00001) (Table 1). In certain seasons, the prey comprised species related to intensive agricultural management, as for example the

synantropic mammals *Mus musculus* and *Rattus rattus* as well as insects related to storing and processing the harvest. The observed biomass distribution of all synantropic mammals deviated from the expected during the seasons (χ^2=1922.5, df=6, P<0.0001) and they were most often captured in spring 2000, when they comprised 13.7% of the number and 21.4% of the mass of all captured mammals (Table 1). In the same spring, Little Owls captured as much as 44.2% of the total mass (m=2331.5g) of this prey category from the entire study period. Also, the observed distribution of biomass of captured *Mus musculus* deviated from the expected distribution (χ^2=392.0, df=6, P<0.0001), because as much as 25.1% of this prey was captured in winter 2000. Altogether for the autumn-winter periods, *Mus musculus* comprised only 5.0% of 16993g biomass of all captured mammal species. However, during the spring-summer periods, *Mus musculus* contributed 9.3% of 8772g, a difference that was statistically significant: (χ^2=174.2, df=1, P=0.0001).

Also *Micromys minutus* played an essential role as food for Little Owls, mainly in the autumn-winter periods, comprising 5.3% of 16993g mammal prey biomass, whereas in the spring-summer period, this species contributed less than half, namely only 2.1% of 8772g. Also these differences were highly significant (χ^2=145.2, df=1, P<0.0001). Testing the significance for total prey biomass according to the different seasons found *M. minutus* was most important in spring (Table 1).

As shown above, among the insects, beetles were most numerous as food for Little Owls. Depending on the season, beetles composed an average of 98.5 ± 1.5% of the total number and 96.5±3.9% of total mass of captured insects, whereas in summer 2000 and winter 2001, they contributed 100% of insect food for Little Owls. One of the most numerous food components was *Tenebrio molitor*, comprising 62.5% in spring 2000 of all identified beetles. *T. molitor* was also caught in winter, when it contributed 29.5% of all (112) identified beetles. *T. molitor* contributed significantly more to beetle biomass in the spring-summer period than in autumn and winter (32.3% of 134g. *vs* 20.6% of 180g.) (χ^2=6.08, df=1, P=0.014).

Other beetles identified in this study as prey of Little Owls were related to cattle breeding as for example *Geotrupes vernalis, G. stercorarius* and *Copris lunaris*. Beetles typical for grasslands and meadows such as *Zabrus tenebrioides* were also found. In summary, they comprised as much as 12.7% of the 581 beetle individuals identified to the genus level in this study. Sometimes (for example in summer 2000), their proportions reached up to 29.5% of 61 identified beetles (Table 1).

Seasonal changes in the food for the Little Owl were tested for mutual relationships of particular prey categories (Table 2). It was e.g. noticed that an increase of biomass of *Mus musculus* resulted in a significant reduction in biomass of the mammalian prey species *Microtus arvalis* and *Micromys minutus*. Simultaneously, a statistically significant growth in the biomass of insects was observed (Table 2). Similarly, an increase in the biomass of *Apodemus agrarius* reduced the biomass of Amphibia and Insectivora significantly (Table 2). Seasonal changes were also obvious from the breadth of food niche index B. B showed its greatest values in summer and its lowest in winter (Table 1). An increase in the proportion of *Microtus arvalis* in the total biomass of caught prey reduced the size of the food of Little Owls, while an increase of insects and *Mus musculus* in the biomass of caught prey expanded the food niche. Other food categories did not have any influence on the size of the food niche (Table 2). The estimated evenness E=0.68 for pooled data indicated that prey categories were not evenly distributed among the studied prey samples. The E index had its lowest value in both winters (Table 1) and its highest value in summer 1999.

DISCUSSION

Previous studies in the farmland of SE Poland showed that the hunting areas of Little Owls were on average about 20.2ha (Grzywaczewski, 2006b). The data presented here allow testing for correlations between particular prey categories and their seasonal changes, and thus provide deeper knowledge on foraging of Little Owls in monocultural farms of SE Poland.

Our study confirmed that Chiroptera was a rather rare component of the diet of Little Owls as only one specimen was found of the bat *Vespertilio murinus*, which is very rare in Poland (Wołoszyn, 2001). This result fits with observations from other authors (Laiu & Murariu, 1997; Genot & Van Nieuwehuyse, 2002; Obuch & Kristin, 2004; Alivizatos et al., 2005) and is different to diets of other owls such as Tawny Owls *Strix aluco* or Barn Owls (Mikkola, 1983; Ruprecht, 1990; Bekasinski et al., 1996).

Despite the fact that the studied region is unusually poor in wetland areas, 4.4% of the number and 3.3% of the overall mass of hunted prey were *Micromys minutus*. This proportion clearly exceeds data from a nearby, more northern site in central Poland (Romanowski, 1988) where only 1.3% and 0.34%, respectively, were observed and from the Central Lublin region (Grzywaczewski et al., 2007) with 1.5% and 1.57%, respectively. In Southern Europe, for example in the rice fields of Northern Italy and wetlands in Greece, *M. minutus* contributed up to 24.8% and 9.3%, respectively (Gotta & Pigozzi, 1997) and 17.9% and 10.8%, respectively (Alivizatos et al., 2005).

During our study, only a small amount of *Lumbricus terrestris* appeared in the diet of studied owls, exclusively during autumn. As mentioned above, the study region is characterized by intense agricultural management such as multiple annual ploughing and intensive fertilization and pest control (GUS, 1975; 1988; 2008; Nowak & Nowak, 1996). These practices most likely cause low earthworm densities (Genot & Van Nieuwenhuyse, 2002).

TABLE 1

Food composition of Little Owls from south east Poland [g] – grams, n – number of prey, m –prey biomass

Prey	m	summer 1999		autumn 1999		winter 2000		spring 2000		summer 2000		autumn 2000		winter 2001		Total	
category	[g]	%n	%m	%n	%m	%n	%m	%n	%m	%n	%m	%n	%m	n%	%m	n%	%m
Pelobates fuscus	20	2.1	3.78	0.6	1.32	0.4	0.71	2.8	6.66			0.9	1.37	0.6	0.75	1.14	2.13
Amphibia	**-**	**2.1**	**3.78**	**0.6**	**1.32**	**0.4**	**0.71**	**2.8**	**6.66**			**0.9**	**1.37**	**0.6**	**0.75**	**1.14**	**2.13**
Passer domesticus	32	1.0	2.68	1.6	5.62	1.5	4.16	1.0	4.0					0.8	1.8	1.04	3.12
Passer montanus	23	0.2	0.48	0.2	0.51	0.4	0.82									0.16	0.35
Aves n.det	18	0.2	0.38	0.2	0.39	0.1	0.21									0.10	0.16
Aves	**-**	**1.4**	**3.54**	**2.0**	**6.52**	**2.0**	**5.19**	**1.0**	**4.0**					**0.8**		**1.3**	**3.63**
Sorex araneus	8	1.0	0.7	0.2	0.18	0.1	0.09	0.7	0.67	1.5	1.63	0.9	0.55	0.6	0.3	0.59	0.44
Sorex minutus	3.5	0.2	0.07			0.3	0.08	1.0	0.44					0.6	0.13	0.36	0.12
Crocidura leucodon	8	0.5	0.34			0.4	0.28	0.7	0.67	1.1	1.2	0.4	0.27	1.4	0.75	0.59	0.44
Insectivora	**-**	**1.7**	**1.11**	**0.2**	**0.18**	**0.8**	**0.45**	**2.4**	**1.78**	**2.6**	**2.83**	**1.3**	**0.82**	**2.6**	**1.18**	**1.54**	**1.00**
Vespertilio murinus	14	0.2	0.29													0.03	0.04
Chiroptera	**-**	**0.2**	**0.29**													**0.03**	**0.04**
Clethrionomys glareolus	17					0.1	0.2									0.03	0.05
Microtus subterraneus	17	1.0	1.43	0.6	1.12	0.5	0.8	0.7	1.42	0.8	1.73	1.3	1.75	0.3	0.32	0.69	1.1
Microtus œconomus	26	0.7	1.64	0.2	0.57	0.4	0.92	0.5	1.6	0.4	1.33			0.3	0.49	0.39	0.95
Microtus agrestis	23					0.3	0.54									0.07	0.14
Microtus arvalis	19	**20.1**	**34.27**	**14.7**	**30.05**	**21.3**	**35.10**	**13.8**	**30.84**	**13.2**	**33.92**	**31.7**	**46.93**	**36.4**	**45.84**	**20.49**	36.40
Arvicolidae n.det.	23	11.1	23.16	5.7	14.14	7.8	15.51	6.0	16.28	10.5	32.85	6.6	11.84	10.4	15.92	8.06	17.32
Mus musculus	15.5	4.2	5.85	3.0	4.77	3.7	4.95	4.1	7.61	4.1	8.7	2.0	2.13	2.8	2.9	3.49	5.1
Rattus norvegicus	166							0.7	13.82							0.13	2.02
Micromys minutus	8	4.4	3.19	6.1	5.45	4.0	2.74	0.7	0.67			10.1	6.30	8.2	4.34	4.40	3.3
Apodemus agrarius	17	3.0	4.28	11.5	20.91	9.7	14.28	1.6	3.18	2.3	5.2	10.1	13.41	10.5	11.76	6.98	11.1
Apodemus flavicollis	31	0.2	0.65	0.2	0.68	0.3	0.73							0.3	0.58	0.16	0.47
Apodemus silvaticus	20	1.0	1.68	0.2	0.44	0.9	1.66	0.5	1.25					1.4	1.87	0.65	1.22
Sylvaemus n.det.	23	2.5	5.31	1.4	3.54	1.8	3.27	1.0	2.87	1.1	3.52	2.2	3.95	2.5	3.87	1.73	3.72
Muridae n.det.	18	5.6	9.06	4.5	8.70	7.6	11.93	2.7	5.62	3.0	7.34	7.5	10.5	6.8	8.08	5.42	9.11
Rodentia	**-**	**53.8**	**90.52**	**48.1**	**90.36**	**58.4**	**92.61**	**32.3**	**85.18**	**35.4**	**94.59**	**71.5**	**96.81**	**79.9**	**95.97**	**52.69**	**91.94**
Mammalia	**-**	**55.7**	**91.87**	**48.3**	**90.53**	**59.2**	**93.07**	**34.7**	**86.94**	**38.0**	**97.44**	**72.8**	**97.63**	**82.5**	**97.14**	**54.26**	**92.98**
Vertebrata	**-**	**59.2**	**99.24**	**50.9**	**98.55**	**61.6**	**98.98**	**38.5**	**97.60**	**38.0**	**97.44**	**73.7**	**99.00**	**839**	**99.70**	**56.70**	**98.80**
Lumbricus terrestris	2.5			0.6	0.16							2.0	0.34			0.23	0.05
Myriapoda n.det.	0.01											0.4	+			0.03	+
Gryllotalpa gryllotalpa	2.0					0.4	0.07	0.4	0.08			0.4	0.07			0.20	0.04
Tettigoniidae n.det.	0.7	0.2	0.01													0.03	+
Orthoptera	-	0.2	0.01			0.4	0.07	0.4	0.08			0.4	0.07			0.23	0.04
Forficula auricularia	0.07	0.7	+	2.0	0.01	0.4	+									0.49	+
Dermaptera	**0.07**	**0.7**	**+**	**2.0**	**0.01**	**0.4**	**+**									**0.49**	**+**
Dytiscus marginalis	0.6	0.7	0.04	1.0	0.07	0.3	0.01					3.1	0.14	1.1	0.04	0.69	0.04
Carabus granulatus	0.54	0.2	0.01	0.6	0.04	0.3	0.01	1.0	0.07	0.8	0.06	0.9	0.04	0.3	0.01	0.55	0.03
Carabus cancellatus	0.58					0.1	0.01	0.2	0.01					0.3	0.01	0.10	0.01
Amara aenea	0.01	0.2	+	0.2	+											0.07	+

TABLE 1

Food composition of Little Owls from south east Poland [g] – grams, n – number of prey, m –prey biomass

Prey category	m [g]	summer 1999		autumn 1999		winter 2000		spring 2000		summer 2000		autumn 2000		winter 2001		Total	
		%n	%m	%n	%m	%n	%m	%n	%m	%n	%m	%n	%m	n%	%m	n%	%m
Pterostichus niger	0.05	3.3	0.01	0.2	+			0.9	0.01	1.1	0.02	0.9	0.01	0.8	0.01	0.91	0.01
Pterostichus vulgaris	0.1	3.5	0.03					0.7	0.01					0.3		0.65	0.01
Harpalus aeneus	0.06			0.2	+	0.1	+	0.4	+							0.13	+
Zabrus tenebrioides	0.25	0.2	+		0.01	0.1										0.07	+
Silpha obscura	0.06									0.4	+					0.03	+
Necrophorus humator	0.21	0.5	0.01													0.07	+
Necrophorus vespilloides	0.20	0.7	0.01	0.2	+			0.5	0.01							0.23	+
Geotrupes vernalis	0.5			0.4	0.02	1.6	0.07			1.1	0.08	0.4	0.02			0.59	+
Geotrupes stercorarius	0.7	0.7	0.04	2.5	0.18	0.5	0.03	1.8	0.15	5.6	0.53	0.9	0.05	0.6	0.03	1.57	0.1
Oryctes nasicornis	0.8							0.7	0.07	0.4	0.04					0.16	+
Melolontha melolontha	0.55	0.5	0.02	0.2	0.01	0.3	0.01	1.0	0.07	0.8	0.06					0.42	0.02
Cetonia aurata	0.5	0.5	0.02			0.1	0.01									0.10	+
Copris lunaris	0.7					0.1	0.01	0.9	0.07							0.20	0.01
Agrypnus murinus	0.28							0.2	0.01							0.03	+
Tenebrio molitor	0.6	4.4	0.24	5.3	0.34	3.4	0.18	13.0	0.91	5.6	0.46	1.3	0.06	2.3	0.09	5.51	0.31
Leptinotarsa decemlineata	0.15	0.7	0.01	0.2	+	0.1		0.5	0.01							0.26	+
Hypera zoilus	0.15	0.5	+	0.4	0.01	0.8	0.01	0.4	0.01	1.1	0.02	1.3	0.02	1.1	0.01	0.72	0.01
Agabus n.det.	0.18											0.4	0.01			0.03	+
Amara n.det.	0.015			1.2	0.02	0.3	+	0.4	0.01			0.9	0.01	0.3		0.42	0.01
Pterostichus n.det.	0.05	11.2	0.05	4.1	0.02	1.9	0.01	7.8	0.05	4.9	0.04	2.2	0.01	2.8	0.01	5.02	0.03
Harpalus n.det.	0.06	0.2	+	1.0	0.01	0.3		0.4	+	1.1	0.01					0.42	+
Carabidae n.det.	0.23	7.2	0.15	27.4	0.68	22.0	0.44	25.7	0.69	33.8	1.06	8.4	0.15	4.0	0.06	19.35	0.42
Carabidae larvae n.det.	0.2											1.3	0.02	1.1	0.01	0.23	+
Dytiscidae	0.6	0.2	0.01													0.03	+
Staphyllinidae n.det.	0.06			0.6	+			0.2	+	0.8	0.01	0.4	+			0.23	+
Elateridae n.det.	0.05	0.7	+					0.2	+							0.13	+
Curculionidae n.det.	0.15	0.5	+							0.4	0.01					0.10	+
Coleoptera n.det.	0.32	3.3	0.1	1.0	0.03	5.3	0.15	4.2	0.16	4.1	0.18	2.2	0.05	1.1	0.02	3.30	0.1
Coleoptera		**39.9**	**0.75**	**46.7**	**1.44**	**37.6**	**0.95**	**61.1**	**2.38**	**62.0**	**2.58**	**24.6**	**0.59**	**16.1**	**0.3**	**42.32**	**1.00**
Insecta		**40.1**	**0.76**	**48.4**	**1.45**	**38.5**	**1.20**	**61.9**	**2.4**	**62.0**	**2.58**	**24.9**	**0.66**	**16.1**	**0.3**	**43.03**	**1.15**
Total number of prey		428		490		733		565		266		229		354		3065	
Total biomass of prey [g]			4767.69		4552.95		8452.25		4804.75		1960.68		2915.03		5346.72		32800.07
B Levins index			5.22		5.82		5.33		6.31		8.07		3.71		3.83		5.25
Shannon-Wiener H index		4.21		3.72		3.70		3.87		3.50		3.64		3.42		4.02	
Evenness E index		0.780		0.720		0.695		0.737		0.744		0.756		0.698		0.684	
Median prey biomass ± SE [g]		15.5±0.473		8.0±0.437		17.0±0.351		0.6±0.683		0.55±0.574		17.0±0.520		19.0±0.410		15.0±0.199	

TABLE 2.

Correlation coefficients among food components and Levins (1968) B index. P<0.05*, P<0.01**, P<0.001***

	Amphibia	***Passer sp***	**Aves**	**Insectivora**	***M. arvalis***	***Microtus* spp**	***M. musculus***	***M. minutus***	***A. agrarius***	**Rodentia**	**Insecta**	**B**
Amphibia	-	-0.506	-0.104	0.520	-0.411	-0.344	0.749	-0.680	-0.869*	-0.744	0.573	0.505
		n=5	n=5	n=5	n=6	n=6	n=6	n=6	n=6	n=6	n=6	n=6
Passer sp.		-	0.869	-0.960*	-0.532	-0.576	-0.01	0.291	0.668	-0.04	0.165	0.359
			n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5
Aves			-	-0.879*	-0.866	-0.901*	-0.388	0.096	0.471	-0.458	0.549	0.770
				n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5
Insectivora				-	0.230	0.278	0.367	-0.452	-0.789*	0.05	0.234	0.201
					n=7	n=7	n=7	n=6	n=6	n=7	n=7	n=7
M. arvalis						0,994***	-0.767*	0.548	0.145	0.796	-0.701	-0.756*
						n=7	n=7	n=6	n=7	n=7	n=7	n=7
Microtus spp.							-0.717	0.505	0.059	0.788	-0.666	-0.720
							n=7	n=6	n=7	n=7	n=7	n=7
M. musculus							-	-0.862*	-0.624	0.560	0.888**	0.944**
								n=6	n=7	n=7	n=7	n=7
M. minutus								-	0.710	0.766	-0.652	-0.662
									n=6	n=6	n=6	N=6
A. agrarius									-	0.328	-0.394	-0.385
										n=7	n=7	n=7
Rodentia										-	-0.514	-0.395
											n=7	n=7
Insecta											-	0.920**
												n=7
B index											-	

However, on the other hand, the data above fit with the view point of other researchers, who show that owls as generalist predators reveal an adaptability to local sources for their diet. Consequently, a high proportion of earthworms in Great Britain and in the North and West parts of Europe (LIBOIS, 1977; ALTRINGHAM et al., 1994; GENOT & VAN NIEUWENHUYSE, 2002, HOUNSONE et al., 2004) varies considerably from the prevalence of Arthropoda and Reptilia in the Mediterranean and desert areas of Asia (AL-MELHIM et al., 1997; OBUCH & KRISTIN, 2004; SHAO et al., 2007). The studied Little Owls in SE Poland reflect this NW-SE gradient of diet changes to some extent. Although an unusually small quantity of earthworms was observed in their prey, no reptiles were discovered, despite the fact that they are so characteristic for south-eastern breeding sites of the species (OBUCH & KRISTIN, 2004).

In our study area, we found a positive correlation between the prey biomasses of *Mus musculus* and insects. It illustrates the importance of farm buildings including grain warehouses, barns etc. and grassy areas among them for hunting, and corresponds with the observed negative correlations between the prey biomass of *M. musculus* and *Microtus arvalis* as well as between *M. musculus* and *Micromys minutus*. It shows that Little Owls trade off hunting between farm buildings and nearby fields and meadows. In spring, Little Owls select large numbers of *Micromys minutus* (Table 1) dwelling in meadows, sedges (*Carex* sp.) and buildings (PUCEK, 1984), where they become alternative prey to *M. musculus*, which is also found in buildings. In summer, owls abandon the vicinity of buildings and prey in open fields and meadows, where they cannot acquire large amounts of *M. minutus*.

The analysis of Little Owls' food during spring and summer pointed to a considerable proportion (54.4%) of insects. This percentage is much higher than in Central Poland (ROMANOWSKI, 1988) with 25% but is rather similar to the results from the central part of the Lublin region located about 90km NW from the studied places, where insects comprised 60.5% of prey from April to July (GRZYWACZEWSKI et al., 2007). However, insects form a much higher proportion of Little Owls' diets in Southern Europe, the Middle East or desert areas of Central Asia (ZERUNIAN et al., 1982; GOTTA & PIGOZZI, 1997; ANGELICI et al., 1997; OBUCH & KRISTIN, 2004; SHAO et al., 2007). This gradient in the percentage of insects as prey items from Central to Southern Europe can be explained by a lower availability of microtines in the Mediterranean (MIKKOLA, 1983; ANGELICI et al., 1997; OBUCH & KRISTIN, 2004). Also the proportion of Dermaptera is much lower in our study area than for example in Italy and Greece where they can be an essential food element (in terms of percentage of prey number) (ZERUNIAN et al., 1982; MANGANARO et al., 2001; ALIVIZATOS et al., 2005).

Our study confirmed that, similar to other sites in Central Europe (LAIU & MURARIU, 1997; ILLE & GRINSCHGL, 2001), *Microtus arvalis* are very important prey items for Little Owls. This can be very profitable for the owls in "vole years" – on the other hand, it can cause poor breeding results in those years when vole populations decline (GENOT & VAN NIEUWENHUYSE, 2002). One would expect that most *M. arvalis* should be caught in spring, the time of reproduction and rearing of the young, which was confirmed by GRZYWACZEWSKI et al. (2007) for other parts of southeast Poland. However, in other studies, most *M.*

arvalis in terms of biomass were caught in winter, which is a critical time for all owls including the Little Owl (MIKKOLA, 1983; GENOT & VAN NIEUWENHUYSE, 2002). Our results correspond with the latter data because the highest contribution of *M. arvalis* to prey biomass was found during winters, when other food resources are depleted.

Significant amounts of insects were found in Little Owls' winter diets. This can probably be explained by the fact that Little Owls hunt inside buildings where loose grain is stored that provides good habitats for certain insects such as *Tenebrio molitor.* Hunting inside buildings is undoubtedly attractive for Little Owls for at least three reasons. Firstly, there is no snow, which, similarly to high and dense vegetation, strongly reduces hunting efficiency (EXO, 1992). Secondly, owls avoid exposure to wind and low temperatures, which would require additional energy when hunting in the natural environment. Thirdly, hunting inside buildings offers easily accessible perches such as stored agricultural facilities, tools etc., providing owls with exquisite opportunities for their basic hunting technique, namely attacking from perches (MANEZ, 1994; GENOT & VAN NIEUWENHUYSE, 2002). These arguments are supported by the finding that hunting efficiency is low in high snow and during winter temperatures due to restricted food resources, causing winter mortality of owls (MARTI & WAGNER, 1985; EXO, 1992; MASSEMIN & HANDRICH, 1997). A second group of beetles found in the food of studied Little Owls whose presence can be explained by intense agricultural management are *Geotrupes vernalis*, *G. stercorarius* and *Copris lunaris* (WEGOREK, 1966; SANDNER, 1989). Their appearance is related to intensive cattle breeding and cow excrements in grazed and trampled areas providing easy access for Little Owls to this prey category.

The prey biomass of Little Owls differed significantly between the seasons, which can be explained by changes in availability and abundance of prey resources. Thus, Little Owls in the study area show a strong ability to adapt their diet according to available prey. This is in agreement with the results of other studies (ALTRINGHAM et al., 1994**;** GENOT & VAN NIEUWENHUYSE, 2002). There were no differences in biomass of prey between the studied sites, probably because the distances between them were too small (ALIVIZATOS et al., 2005). Our studies showed an evenness index of 0.68 for all collected data indicating an unequal distribution of prey categories. However, the categories are still more evenly distributed than in Greece (ALIVIZATOS et al., 2005), where the evenness index was between 0.12 and 0.58.

In conclusion, the food composition of the studied Little Owl populations in South-Eastern Poland conforms with gradients in food compositions between northern and southern as well as eastern and western European populations of Little Owls. This study also showed that prey items of Little Owls in South-Eastern Poland are closely related to intense agricultural management. Due to this, changes in agricultural practice might affect the prey and also the population size of Little Owls. However, seasonal changes in the diet components showed that Little Owls seem to be able to adapt to the prey available.

ACKNOWLEDGEMENTS

We are greatly indebted to Grzywaczewski Grzegorz PhD (Department of Zoology, Life Sciences University, Lublin) for creative discussions on the first version of the manuscript. Our particular thanks also go to Krzysztof Kasprzyk PhD (Department of Vertebrate Zoology, Copernicus University, Torun) for his assistance in determining the remains of bats and other mammals.

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Received: July 22, 2007
Accepted: September 1, 2010
Branch editor: Lens Luc

Belg. J. Zool., 140 (2) : 212-215 July 2010

Efficiency of live trapping protocols to assess small mammal diversity in tropical rainforests of Sri Lanka

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ABSTRACT. Live trapping is used extensively for small mammal studies in both temperate and tropical ecosystems. The effectiveness of such studies is dependent on several factors. This paper attempts to investigate how one of these factors, namely the trapping intensity, affects the assessment of species richness and abundance of small mammals in rainforest ecosystems in southwest Sri Lanka. Eight-day live trapping surveys were conducted in seven selected forests yielding a total of 5600 trap days with a total of 186 individuals belonging to nine species being captured. It was evident that, using 100 traps with a trap density of 140 traps per ha, over 90% of the species recorded from each of the seven forests were captured within the initial four days of live trapping after which the rate of capture of new species sharply declined. The results also show that the more common species were captured sooner than the more rare ones. Considering these trends, a four-day trapping protocol could be recommended to broadly compare small mammal communities between forests or habitat types. The number of individuals captured, on the other hand, probably attracted by the bait, increased as trapping progressed; this very likely leads to overestimation of species abundance. Since such projects in developing countries are subject to budgetary constraints, costs incurred are also addressed.

KEY WORDS: Duration, live trapping, rainforest, small mammals, Sri Lanka

INTRODUCTION

Live trapping is the most widely used method employed to investigate the diversity and distribution of small mammals in both tropical and temperate environments. The effectiveness of a live trapping protocol is, however, dependent on several factors such as the number and density of traps, type of bait, trap spacing and the duration of the trapping protocol (e.g. FRANCI et al., 2002; O'BRIEN et al., 2006; CONARD et al., 2008). These factors especially apply to tropical rainforests, which harbour low densities of most species (SHANKER, 2000; WIJESINGHE & BROOKE, 2004). In attempting to obtain reliable data on species richness and abundance in such habitats, one may be led to believe that sampling must cover ever larger areas and/or be extended over a long duration. These ecosystems are found mostly in developing countries where projects of this nature are often subject to budgetary constraints thus prompting the use of short trapping protocols. Apart from budgetary restrictions, lengthy live trapping protocols may prove to be cumbersome given the thick vegetation, difficult terrain and extremely wet conditions that characterize these forests. Thus there is a need to recommend a suitable trapping protocol – one that optimizes capture probabilities whilst minimizing costs in terms of time and money.

Previous studies using live trapping protocols have used four-day (REXTAD & DEBEVEC, 1999; JENKINS et al., 2005; EDALGO & ANDERSON, 2007), five-day (SHANKER, 2000; FRANCI et al., 2002; SOLARI et al., 2002), six-day (O'BRIEN et al., 2006), and seven-day (YÁÑEZ et al., 1999) regimes to survey small mammals. Using diverse trapping intensities not only limits data comparison between studies but also raises concerns about their adequacy and accuracy. This paper attempts to examine the influence of trapping intensity in terms of duration, on the assessment of species richness and abundance of small mammals in rainforest ecosystems in southwest Sri Lanka.

MATERIALS AND METHODS

A survey of small mammals was conducted in seven scattered rainforests in Sri Lanka during April 2007 and February 2008. The selected forests were Masimbula, Walankanda, Sinharaja, Yagirala, Kalubowitiyana, Dellawa and Delgoda forests in the three districts of Kalutara, Matara and Ratnapura, in the southwest, wet zone of Sri Lanka. In each of these forests, two trapping grids were marked each consisting of 50 Sherman's live traps that were laid at 10m intervals. A trap spacing of 10m has been consistently selected as the ideal density for Sri Lanka's rainforests (e.g. WIJESINGHE & BROOKE, 2005; KOTAGAMA et al., 1986). Trapping did not commence on days of heavy rainfall.

The traps were baited with partially roasted coconut kernel. Coconut was found to be the ideal bait for trapping small mammals in wet forests (WIJESINGHE, 2001). Traps were checked each morning and the bait was renewed. Trapping was conducted for eight consecutive nights yielding a total of 800 trap days per forest, totalling 5600 trap days for the seven forests. On each of the days over which trapping was conducted, the captured individuals were identified and their capture/recapture status was recorded. Each of the captured individuals was fur-clipped to enable the identification of the recaptured individuals.

RESULTS

The small mammal survey in the seven rainforests resulted in the capture of 186 individuals belonging to nine species of rodents and shrews. The captured species were the rats *Rattus rattus* and *Srilankamys ohiensis*, mice *Mus mayori* and *M. booduga,* a tree mouse *Vandeleuria oleraceae,* the squirrels *Funambulus layardi, F. sublineatus* and *F. palmarum*, and the shrew *Crocidura miya.* The mean cumulative numbers of species and new individuals captured in the seven forests during each of the eight days of sampling are shown in Figure 1 (a) and (b). Table 1 shows the total species richness and abundance of small mammals captured in each of the individual forests during an eight-day sampling regime. The day on which a particular species was captured for the first time during the eight day trapping protocols in the seven forests is also provided in Table 1. These results show that the rate of species accumulation steadily increased until the fourth day of trapping after which the rate of capture of additional species sharply declined. In fact it is apparent that over 90% of the species recorded during the eight days in a particular forest were captured by day four (Table 1). On the other hand, the number of new individuals captured continued to increase as trapping progressed. In fact the Trend Analyses conducted using the Minitab Statistical software, with the cumulative number of species/individuals captured on each day; indicate that an asymptote is reached at four days for species richness whilst abundance reaches a plateau at 28 days.

TABLE 1

The initial day of capture of the different species of small mammals recorded during eight-day sampling sessions (with 100 traps at 10m spacing) in seven rainforests in southwest Sri Lanka.

Forest	Sampling day								Total Species richness	Total Abundance
	1	2	3	4	5	6	7	8		
Masimbula	*RR*		*SO*						2	32
Walankanda	*RR*			*MM*					2	16
Sinharaja	*MM* *RR*			*FL*					4	28
Yagirala	*MM* *RR*	*FP*		*VO*			*MB*		5	27
Kalubowitiyana	*RR*		*MM*	*FP*				*CM*	4	32
Dellawa	*MM*		*RR*						2	31
Delgoda	*MM* *RR*								2	20

RR – Rattus rattus, SO – Srilankamys ohiensis, MM – Mus mayori, FS – Funambulus sublineatus, FL – Funambulus layardi, FP – Funambulus palmarum, VO – Vandeleuria oleraceae, MB-– Mus booduga, CM – Crocidura miya

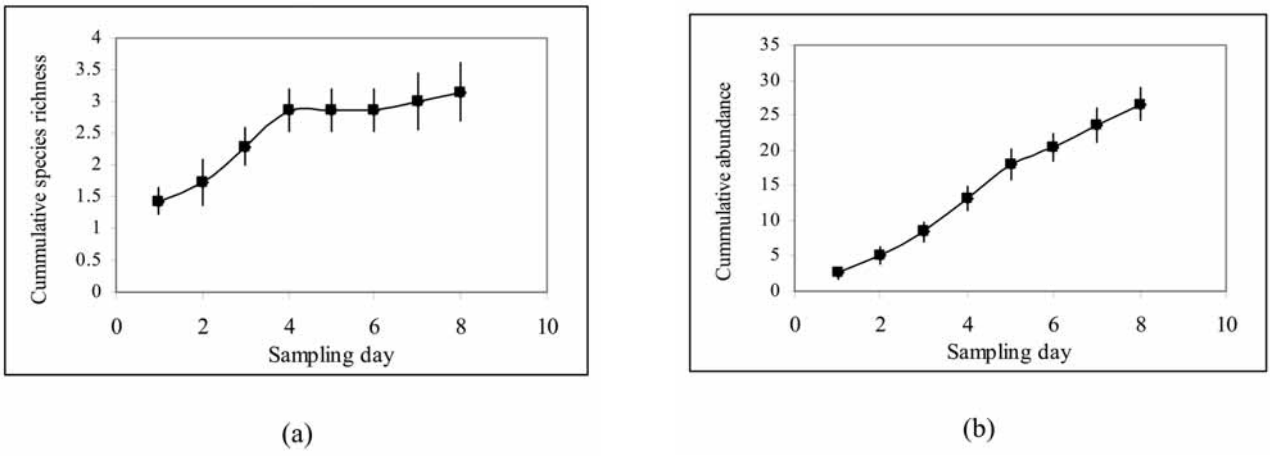

Fig. 1. – Accumulation curves of (a) species richness and (b) abundance of small mammals on each of the eight days of the live trapping. The values shown are means (± standard errors) for seven rainforests.

To investigate whether the abundance of a particular species within a forest influenced its initial day of capture during a given trapping session, the Spearman Rank Correlation test was applied using the abundance of a species in a given forest and the first day on which this species was encountered in the forest. Interestingly, there was a significant negative correlation ($r=-0.55$; $P<0.02$) between the capture day and abundance indicating that species having a higher abundance are captured sooner (Fig. 2).

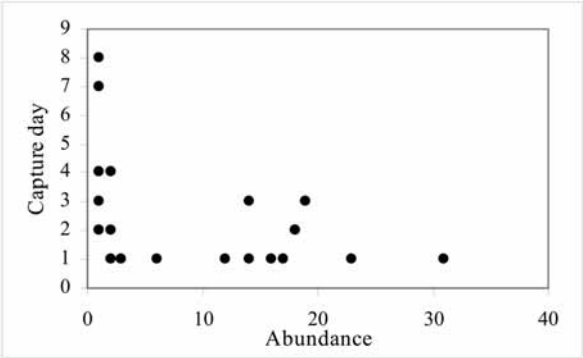

Fig. 2. – Relationship between abundance on the first day of capture of small mammals in the seven rainforests. Each value represents the abundance of a species in relation to the day of capture in the forest concerned.

DISCUSSION

The main objective of this study was to examine to what extent prolonged trapping protocols in tropical rainforests increase efficiency in terms of the capture of species and individuals so that a suitable time frame could be recommended for small mammal studies in these ecosystems. In the present study where 100 traps at 10m spacing (trap density of 140 traps per ha) were used, it was evident that a large fraction of the diversity of small mammals in the seven forests surveyed was captured within the initial four days of trapping. In fact trapping beyond this period in the seven forests only resulted in the capture of one additional species each in two of the seven forests. In a previous study conducted in 1999-2000 in the same locations within two of the forests Sinharaja and Yagirala, one more species was recorded in Sinharaja while two species less were recorded in Yagirala (WIJESINGHE, 2001). In the present study, commencing trapping on days of heavy rainfall was avoided since capture rates of animals on such days are less due to lower mobility (WIJESINGHE, 2001). Varying densities of animals present throughout the year would no doubt affect the capture rates, but no other factors have been noted to affect changes in capture rates.

Small mammal communities may vary greatly between rainforests due to size and shape of forests (RICHARDS, 1969; RENATA, 2004), nature of the surrounding matrix (RENATA, 2004) and the degree of isolation (KOZAKIEWICZ & JURASINSKA, 1989). Even within a particular forest, due to habitat heterogeneity, species are not evenly distributed. Hence, the small mammal communities in one area may be quite different to those of another area within the same forest (ALDER, 1994). This is especially so in the case of Sri Lanka's rainforests, which are heterogeneous ecosystems (GUNATILLEKE & GUNATILLEKE, 1981). Consequently, in the absence of results from previous surveys, it is not possible to make predictions about the expected diversity of small mammals in particular rainforests.

The present study most importantly revealed that the capture probability of a species was greatly influenced by its abundance at a particular site. It was evident that species with higher abundance were captured earlier. It should also be recognized, however, that rare species may not be captured during short trapping protocols. It is noteworthy that extended trapping protocols with a pre-baiting period do not necessarily increase the likelihood of capturing "trap shy" species (see EDALGO & ANDERSON, 2007). From the point of view of conservation, if a species that has been known to exist in a habitat is not captured during a survey, it could be taken as an indication of rarity or low population density. Such a species could then be the target of further investigations.

The results of the present survey show that the intensity of trapping affects the effectiveness of the protocol for recording species richness. In using a four-day trapping regime (using 100 traps and a trap density of 140 traps per ha) a researcher could record the predominant small mammal community in a given rainforest, which is adequate to broadly compare diversity between different forests or habitats. Such a four-day trapping protocol may also be used to compare the relative abundance of species between forest patches. On the other hand, if one's objective is to make an inventory of the small mammals of a selected forest, more effort would be required in terms of trapping intensity and the number of traps.

With regard to abundance, it was apparent that each additional day of trapping resulted in the capture of new individuals, a plateau being reached only after 28 days. SHANKER (2000) has also demonstrated that estimates of density increase with trapping intensity. It has been shown that food enrichment in a given habitat usually results in an increase in the estimates of densities of animals at a particular site (e.g. KOEKEMOER & VAN AARDE, 2000). RATNAWEERA & WIJESINGHE (2007) investigating such effects in the Kanneliya rainforest in Sri Lanka have in fact reported up to five fold increases in the estimation of densities of small mammals after 14 days of food addition. Such a phenomenon might also occur, to a certain extent, when the bait provided attracts animals. This evidence suggests that prolonged trapping may lead to overestimation of the actual population sizes of species due to immigration of individuals from the surrounding areas. MARES & ERNEST (1995), probably for this reason, reported that lengthy trapping durations often lead to less accurate data in terms of individuals in well-defined areas. While it may be difficult to determine an exact trapping intensity that would yield accurate population estimates of individual species, the results of the present survey supported by findings of other studies clearly suggest that it would be preferable to avoid unduly long trapping protocols.

Lengthy trapping protocols also entail numerous other disadvantages. Extended trapping protocols may result in

trap mortality of the recaptured individuals (SHANKER, 2000). Furthermore, curtailing costs by reducing the duration of trapping protocols is of primary importance particularly in developing countries where biodiversity surveys are often subject to budgetary constraints. The costs incurred in terms of labour increase with each additional sampling day. Therefore curtailing costs without unduly affecting the objective of the protocol would be desirable. Conducting live-trapping in rainforests is also tedious due to the excessively wet conditions, presence of hoards of leaches and the lack of accommodation facilities at most sites. Additionally, lengthy field sessions increase the probability of trap theft.

CONCLUSIONS

Considering the drawbacks in extending trapping protocols in developing countries, a four-day regime using around 100 traps at 10m spacing would be useful to assess the predominant small mammal community in a rainforest and to broadly compare the diversity of small mammals between different forest patches. A detailed inventory of species within a forest may require longer trapping protocols. In the case of abundance, unduly lengthy trapping protocols result in less accurate estimates of populations in defined areas.

ACKNOWLEDGEMENTS

I am very grateful to the National Science Foundation for granting financial assistance (2004/Zoo/b2) for this project. I am also thankful to the Forest Department and the Department of Wildlife Conservation for granting me permission to work in the selected forests.

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Received: May 13, 2008
Accepted: March 15, 2010
Branch editor: Hendrickx Frederick

Belg. J. Zool., 140 (2) : 216-224 July 2010

The development of chicken cerebellar cortex and the determination of AgNOR activity of the Purkinje cell nuclei

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ABSTRACT. In this study, the embryonic and post-hatching development of the chicken cerebellar cortex, and the number, size and area of the silver-staining nucleolus-organizer regions (AgNORs) were determined in cerebellar Purkinje cells in layer-hen (Babcock-B380) embryos and chickens. The mean transverse diameter of the Purkinje cells and their nuclei, and the mean area of the Purkinje cell nucleus and AgNOR area increased during the entire experimental period. The mean ratio of the AgNOR area to the Purkinje cell nucleus area reached its highest value at the 15th day of incubation. There was no difference between the mean AgNOR numbers per nucleus. These findings provide reference data for future studies in this and related research topics, for example for establishing AgNOR parameters and we also hope that our data will be supplemented with other techniques such as PCNA and TUNEL in the near future.

KEY WORDS: KEY WORDS: AgNOR, Cerebellum, Purkinje cell.

INTRODUCTION

The cerebellum is an important organ, both because its dysfunction leads to pronounced disturbances in movement, posture and balance and because it is a relatively massive structure in higher vertebrates. In humans, it represents about 10% of the volume of the brain, and even more striking, it has been estimated to hold more than half of all the neurones in the central nervous system (MIAL & RECKESS, 2002).

The cerebellum develops from the dorso-lateral part of the alar plates of the metencephalon (NICKEL et al., 1977; MIAL & RECKESS, 2002). FEIRABEND (1990) first observed that the cerebellum develops from the neuro-epithelium of the dorsal part of the 4th ventricle and recognized the constriction, called "isthmus", formed between mesencephalon and rhombencephalon at the 4th day of incubation in chicken embryos.

Similarly to in mammalian species, the cerebellar cortex in avian species also arises from two different germinal zones called the ventricular germinal zone (VZ) and the external granular layer (EGL) (ESPINAR et al., 1997). According to FEIRABEND et al. (1985), the cerebellar mantle layer arises from the ventricular neuro-epithelium and soon becomes subdivided into an inner (IML) and outer mantle layer (OML) at the early (4-8th day of incubation) embryonic period. The OML subdivides into the deep (OML-d) and superficial (OML-s) parts. The OML-d develops into the central cerebellar nuclei whereas the OML-s develops into the EGL. The inner cortical layer, which gives rise to the future Purkinje cells, is derived from the IML.

Purkinje cells, unique neurons in the central nervous system, originate from the VZ. These cells are the largest neuronal cell type in the cerebellar cortex and have the most elaborate synaptic interactions in the cerebellum (LEE et al., 2001). The myelinated axons of the Purkinje cells terminate on neurons of the cerebellar nuclei and certain brainstem nuclei (NICKEL et al., 1977; VOOGD & GLICKSTEIN, 1998; LEE et al., 2005). Because the Purkinje cells establish morpho-functional and synaptic connections, that demand high metabolic activity, they synthesis different amounts of protein at different developmental stages during the embryonic period (BERTOSSI et al., 1986).

Nucleolar-organizer regions (NORs) are loops of DNA containing ribosomal RNA genes (ROBERT-FORTEL et al., 1993). These regions can be easily stained with silver methods to appear as black dots (AgNORs) in the cell nucleus since they are argyrophilic. NORs are used by cytogeneticists for studying chromosomal disorders. This staining technique is very simple and does not require any special instruments or costly reagents (KHANNA et al., 2005). Additionally, the size, number and dispersion of the silver deposits on the NOR reflect the degree of transcriptional, nucleolar and proliferative activity of the cells (SUR et al., 2003; BUKHARI et al., 2007).

The nervous system is extremely sensitive to several factors that could affect the developing embryo. The cerebellum is often chosen for experimental studies on central nervous systems because of its well-defined cytoarchitecture and pathways of cerebellar cell migration during neurogenesis (FULTS et al., 1985). The chicken embryo has proven to be one of the most valuable study objects for developmental biology, providing several advantages: eggs are easy to obtain; the development of the embryo is well known; the development takes places outside of the maternal organism and is thus easily accessible for experimentation and observation on subsequent development (KUCERA & BURNAND, 1987). Because of their independent development from any maternal influence, chicken embryos also facilitate specific experimental manipulation, whereas determining the effects of teratogenic or an embryotoxic chemicals agent on the central nervous system of mammals is very difficult because of the placenta

(JELINEK et al., 1985). Here, we investigated the embryonic and post-hatching development of the chicken cerebellum and determined some AgNORs parameters of Purkinje cells, a cell type used as a model in similar studies, to obtain basic data for further studies on this system and related research.

MATERIALS AND METHODS

Materials

Animals

For the experiment, 100 fertilized eggs of laying hens (Babcock B-380) were used. Eggs were fumigated (80g potassium permanganate in 130mL 40% formaldehyde solution per m^3 for 20min) and subsequently placed in an incubator, at 37°C and 65% humidity.

Methods

Histological investigations

On the 7th, 9th, 11th, 13th, 15th and 18th days of incubation, six randomly-selected whole embryos were fixed in buffered 10% formal-saline solution (pH 7.4) for a week, dehydrated in alcohol, cleared in xylene and embedded in paraffin blocks. On the day of hatching, subsequent 10th day, 3rd week and 4th week post hatching, six randomly-selected chickens were decapitated. The cerebellum was removed and tissue samples processed according to the histological methods mentioned above. For routine histological examinations, tissue sections of 4-6µm taken from paraffin blocks were stained with Crossman's trichrome staining and hematoxylin & eosin staining (CULLING et al., 1985).

Staining the AgNORs

From the 11th day of incubation onward, tissue sections were stained with a solution containing one volume of 2% gelatine in 1% aqueous formic acid and two volumes of 50% silver nitrate (Merck). The staining was performed at 37°C in the dark for 20-30 minutes (AYDIN & CELIK, 2005).

The histological preparations were examined with a light microscope (Leica DM-2500 attached to a DFC-320 digital camera). In each cerebellum of an embryo or chicken, 25 Purkinje cells having nuclei were analyzed. The transverse diameter of Purkinje cells and of their nuclei, the nuclear area, the AgNOR area and AgNOR counts were analysed with an image analysis programme (IM-50). Also, the percentage of the AgNOR area relative to the whole nuclear area was calculated.

Statistical analyses

All statistical analyses, in particular ANOVA and DUNCAN tests, were conducted with the Statistical Package for the Social Sciences (SPSS 9.0, SPSS Inc. Corp. Chicago, IL., USA) (TEKIN, 2003).

RESULTS

Embryonic development of the Cerebellum

On the 7th day of incubation, the cerebellum developed from the dorsal metencephalic region at the roof of the fourth ventricle. In this stage, the cerebellar anlage consisted of ventricular neuroepithelium lining the ventricle lumen, inner mantle layer and outer mantle layer (Fig. 1). On the 9th day of incubation, the inner cortical layer, which would give rise to the future Purkinje cells, was recognized at the superficial part of the inner mantle layer (Fig. 2). On the same day, cell accumulation was distinct in the superficial part of the outer mantle layer, called "external granular layer (EGL)" (Fig. 2). At the same incubation period, the dark granule cell groups (granule cell raphe) that migrate from the EGL to the inner granular layer were observed in the marginal layer, localized beneath the EGL (Fig. 2). On the 11th day of incubation, the cerebellar folia were observed for the first time, following advanced fissuration of the cerebellar cortex (Fig. 3). Purkinje cells appeared arranged as small clusters beneath the marginal layer. In the cerebellum of a 13-day-old embryo, all nine primary folia were recognizable and a secondary foliation was just beginning in folium V (Fig. 4). Also by the 13th day of incubation, the marginal layer had increased in thickness, whereas the primitive cortex consisted of an external granular layer. A primitive molecular layer, an inner cortical layer and an inner granular layer were very distinct by the 15th day of incubation, at which time the Purkinje cells appeared to be arranged in one or two rows (Fig. 5). By the 18th day of incubation, the molecular layer and the inner granular layer had increased in thickness whereas the inner cortical layer had decreased in thickness. The Purkinje cells were arrayed in a manner similar to that in adult cerebellum (Fig. 6).

Post-hatching development of the Cerebellum

The cerebellar cortex completed its development and the Purkinje cells were then typically located in a single row at the border of the granular and molecular layer by the day of hatching. However, our observations revealed that the EGL gradually decreased in thickness during the four weeks after hatching (Fig. 7).

AgNORs parameters of the Purkinje cells

In general, AgNORs were observed as black patches having irregular shapes in the cell nuclei (Fig. 8). Although AgNORs were identified by the 9th day of incubation, they were too small to be measured reliably before the 11th day of incubation. The mean transverse diameter of the Purkinje cells and their nuclei, the mean area of the Purkinje cell nucleus and the AgNOR area increased through the entire experimental period. The mean percentage of AgNOR area relative to Purkinje cell nucleus area reached its highest value at the 15th day of incubation (25±3.80%). There were no differences between the AgNOR counts on different days (Table 1).

TABLE 1

AgNORs parameters of the Purkinje cells

Time	Mean transverse diameter of Purkinje cell bodies ±SE (µm)	Mean transverse diameter of Purkinje cell nuclei ±SE (µm)	Mean Purkinje cell nucleus area (µm^2)±SE	Mean AgNOR area(µm^2)±SE	Relative (%) AgNOR area ±SE	Mean AgNOR number per nucleus±SE
11th incubation day	4.77±0.09^a	3.34±0.03^a	10.02±0.11^a	1.96±0.2^a	19±2.12^a	1.48±0.06^a
13th incubation day	5.25±0.07^a	3.55±0.09^a	11.60±0.38^a	2.64±0.44^a	20±1.96^a	1.52±0.08^a
15th incubation day	7.58±0.11^b	4.23±0.07^b	17.17±0.51^b	4.45±0.63^b	25±3.80^b	1.46±0.11^a
18th incubation day	10.69±0.38^c	5.44±0.18^c	25.99±2.1^c	4.77±0.49^b	17±5.42^a	1.54±0.03^a
Day of hatching	11.77±0.15^d	5.59±0.04^c	32.44±0.81^d	2.25±0.17^a	6.5±4.28^c	1.37±0.04^a
10th day post-hatch	15.85±0.55^e	8.04±0.26^d	61.87±3.6^e	4.67±0.26^b	7.1±3.07^c	1.35±0.04^a
3rd week of post-hatch	17.75±0.33^f	8.96±0.15^e	70.65±1.7^f	5.94±0.68^{bc}	8±1.12^c	1.54±0.06^a
4th week of post-hatch	18.46±0.44^f	9.10±0.18^e	69.38±1.9^f	7.19±0.84^c	10±1.52^c	1.37±0.06^a

a—f Values within a column with no common superscripts are significantly (P<0.05) different.

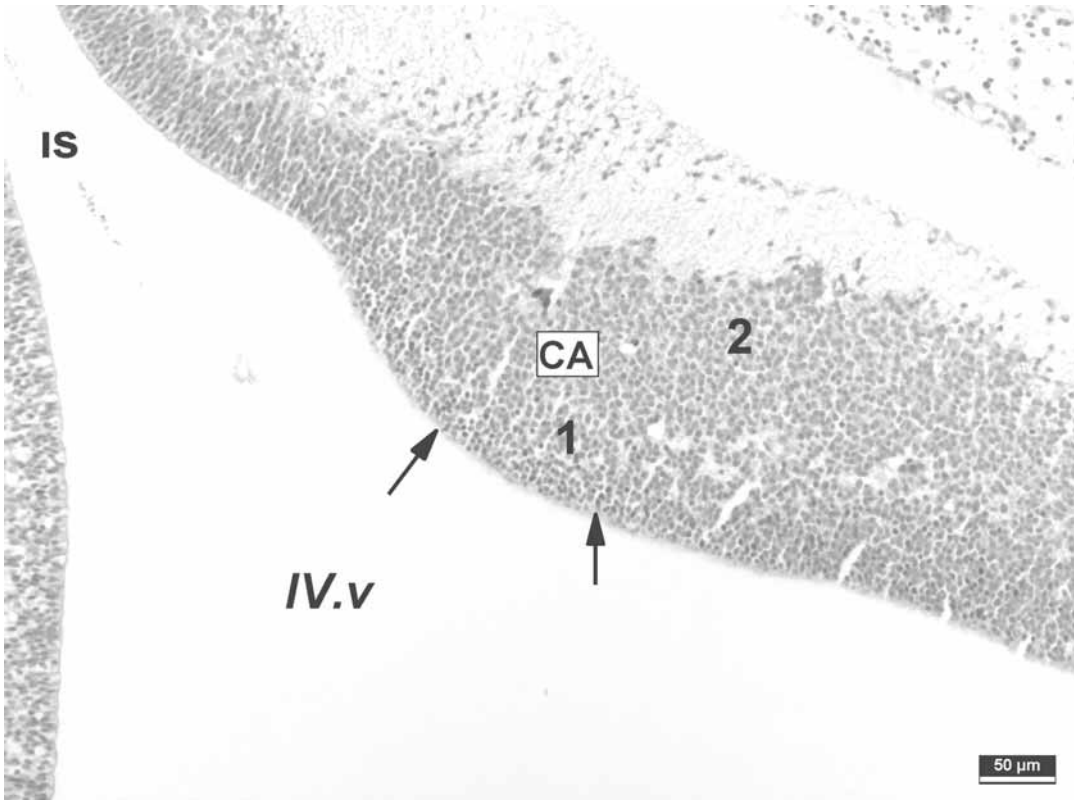

Fig. 1. – A section from the developing cerebellar primordium of chicken embryo on day 7 of incubation. CA: Cerebellar anlage, IS: Isthmus, IV. v: Fourth ventricle, 1: Inner mantle layer, 2: Outer mantle layer, Arrows: Ventricular neuroepithelium. Hematoxylene and Eosin staining.

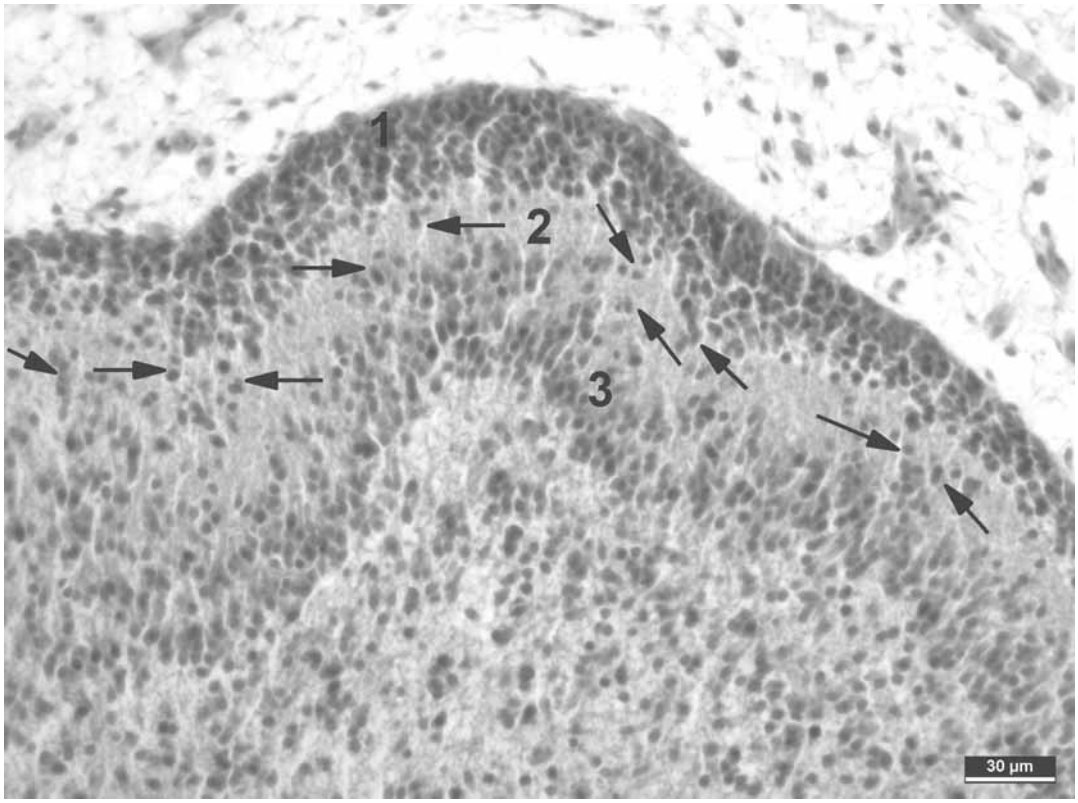

Fig. 2. – A section from the developing cerebellar primordium of chicken embryo on the 9th day of incubation. 1: External granular layer (EGL), 2: Marginal layer, 3: Inner cortical layer, Arrows: Granule cells migrating from EGL to the internal granular layer. Trichrome staining.

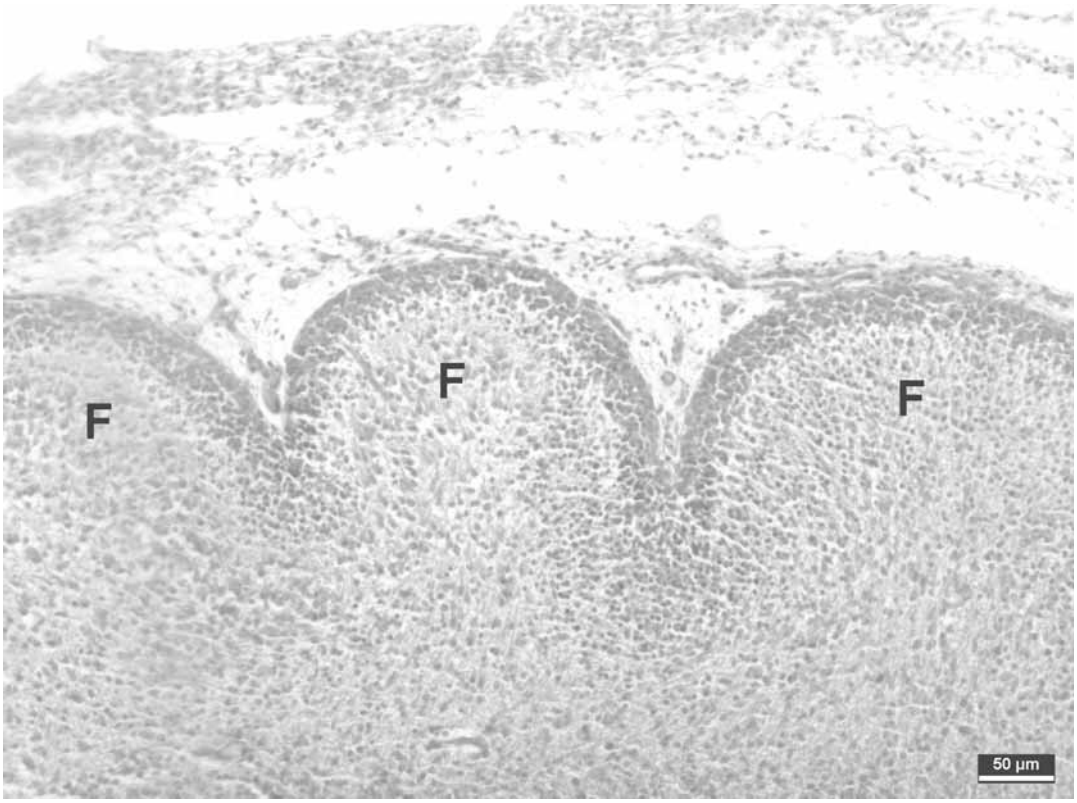

Fig. 3. – A section from the developing cerebellum of chicken embryo on day 11 of incubation. F: Cerebellar folia. Trichrome staining.

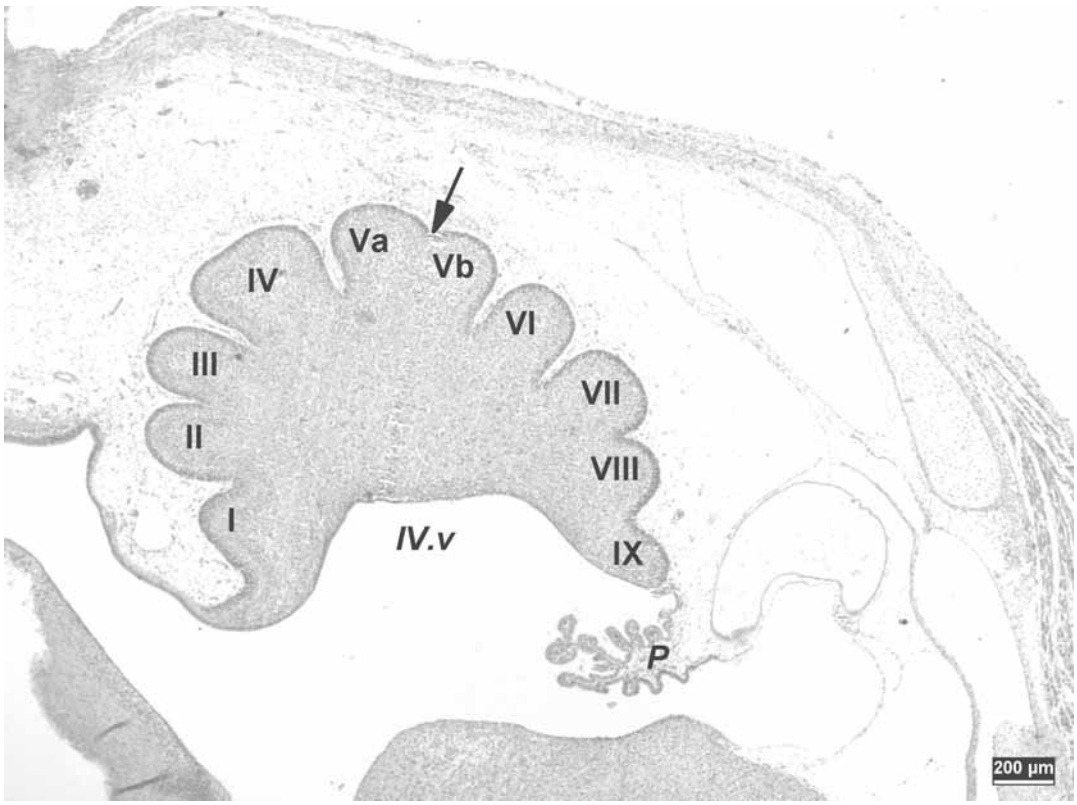

Fig. 4. – A section from the developing cerebellum of chicken embryo on day 13 of incubation. All 9 primary folia are distinct. I-IX: Primary folia, a, b: Secondary folia, Arrow: Secondary foliation, P: Plexus choroideus, IV.v: Fourth ventricle. Trichrome staining.

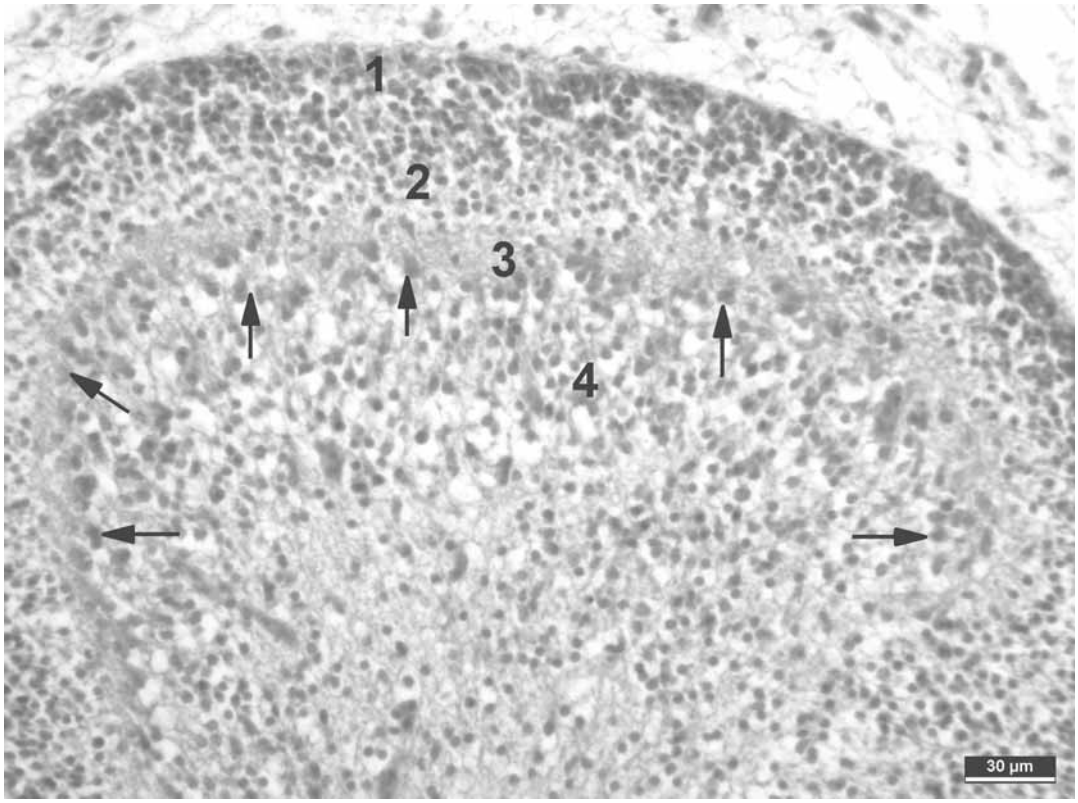

Fig. 5. – The primitive cortex of developing cerebellum on day 15 of incubation in chicken embryo. 1: External granular layer (EGL), 2: Primitive molecular layer, 3: Inner cortical layer, 4: Internal granular layer, Arrows: Future Purkinje cells. Trichrome staining.

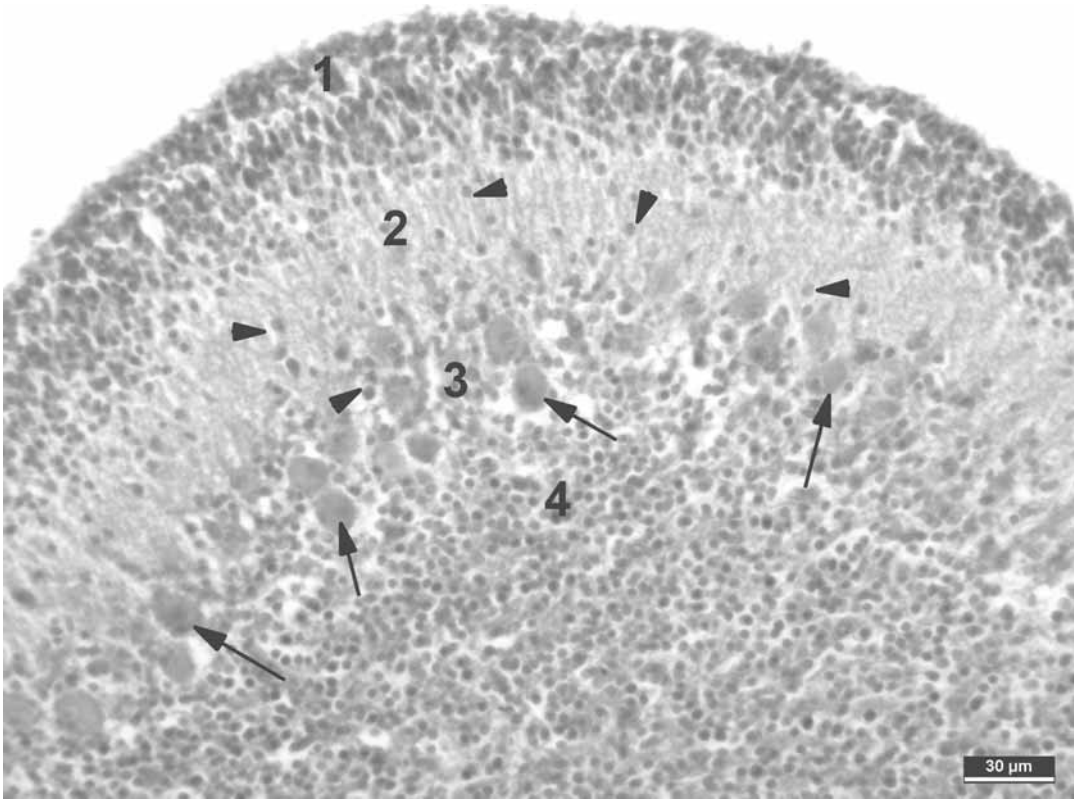

Fig. 6. – Histological sections from the cerebellum of chicken embryo on the 18th day of incubation. 1: External granular layer (EGL), 2: Molecular layer, 3: Inner cortical layer, 4: Internal granular layer, Arrows: Purkinje cells, Arrow heads: Granule cells still migrating from the EGL to the internal granular layer. Trichrome staining.

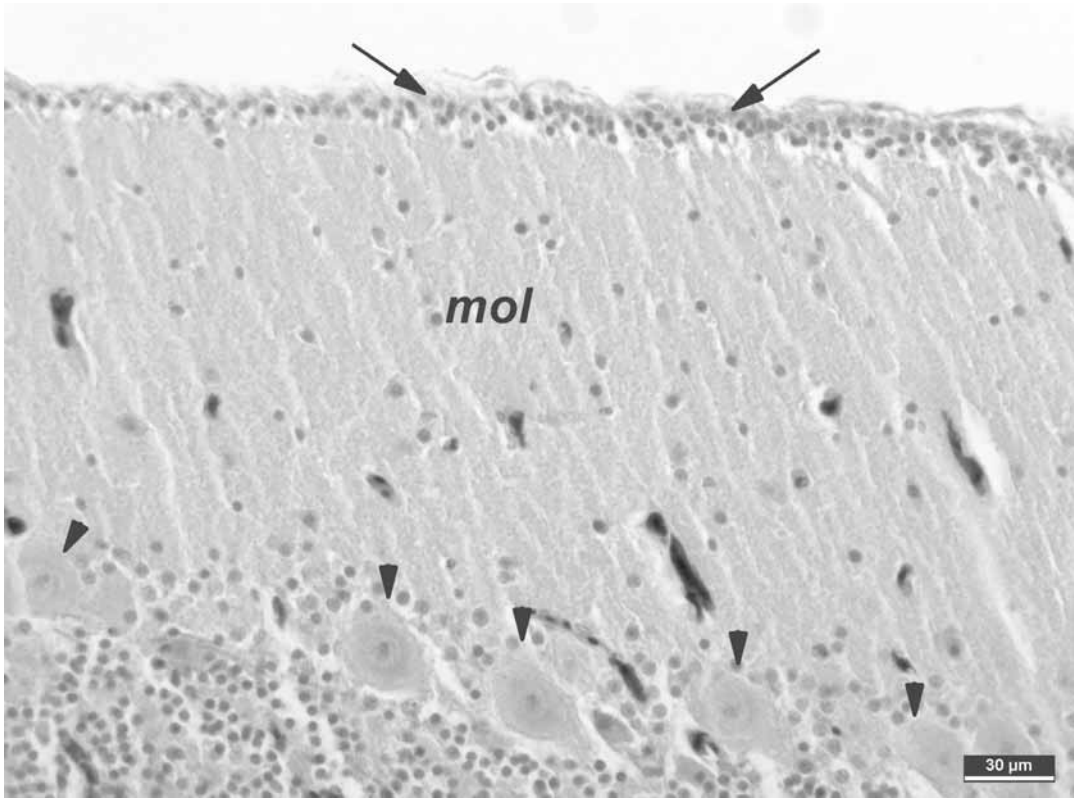

Fig. 7. – Histological sections from the cerebellum of 10-day-old chicken. The external granular layer is decreased in thickness (Arrows). mol: Molecular layer, Arrow heads: Purkinje cells arrayed in a single row. Hematoxylene and Eosin staining.

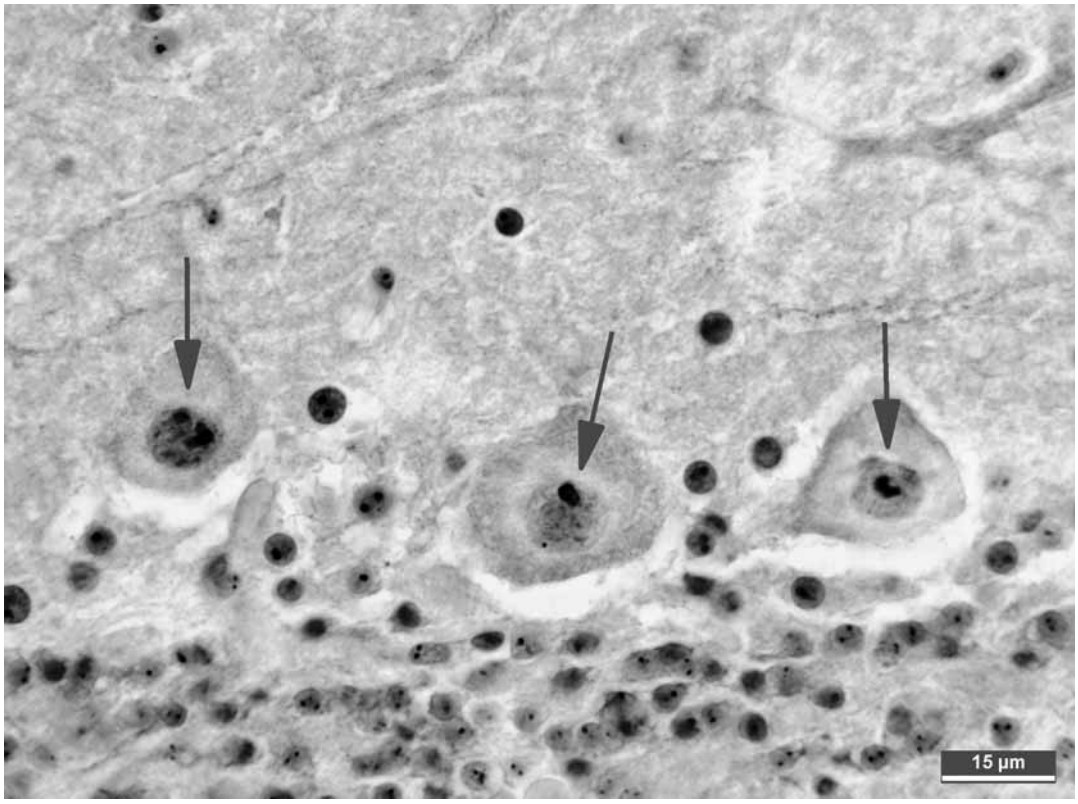

Fig. 8. – Histological sections from the cerebellum of 28-day-old chicken. Arrows: AgNORs in the nuclei of Purkinje cells. AgNOR staining.

DISCUSSION

The cerebellum differs from most of the other brain regions in that it contains two distinct germinal layers, the ventricular zone (VZ), which is most active during embryonic development, and the external granular layer (EGL), which contributes to neurogenesis after birth or hatching (FEIRABEND et al., 1985). FEIRABEND (1990) has reported that the 9th day of incubation is especially important in the histogenesis of the cerebellum, because a complete and distinctive longitudinal pattern in the arrangement of the cells of the inner mantle layer (IML) then appears for the first time. The inner cortical layer, which gives rise to the future Purkinje cells, is derived from the IML. Also in our study, the inner cortical layer was firstly recognized on the 9th day of incubation at the superficial part of the IML giving rise to the future Purkinje cells. At incubation day 9, cell accumulation was distinct in the superficial part of the outer mantle layer (EGL) (Fig. 2). This transient external layer is very important for cerebellar development because, in birds as in mammals, it produces the granule cells, which migrate through the maturing molecular and Purkinje cell layers to reach their final location in the inner granular layer (BOUVET et al., 1987). REDIES et al. (2002) found that cell raphes consist of migrating dark granule cells (Granule cell raphe) between the Purkinje cells clusters at intermediate stages of chicken embryonic development. These cells have been assumed to migrate from the EGL towards their definitive position in the inner granular layer (FEIRABEND, 1990). In our study, these dark granule cell groups (granule cell raphe) that migrate from the EGL to the inner granular layer were observed on the 9th day of incubation (Fig. 2).

The developing cerebellum is characterised by marked folding of its surface, giving rise to closely packed transverse folds referred to as "folia" (MCGEADY et al., 2006). The primary folia are the main folia whereas the secondary folia are folia that are formed after the subfoliation of the primary folia during embryonic development of the cerebellum (FEIRABEND, 1990). Primary folia are individually numbered and secondary folia alphanumerically numbered. Differences in the number, size and morphology of the folia are associated with distinct behavioural differences (IWANIUK et al., 2006; 2007). Most comparative neuroanatomists have accepted that there are ten main folia in the avian cerebellum and the mammalian lobules I-X are homologous, respectively, with the avian folia I-X (RICHARDS, 1972). FEIRABEND (1990) reported that fissuration and foliation of the chicken cerebellar cortex have advanced on the 11-12th days of incubation. He also observed 10 primary folia and recognized the secondary foliation in folia V and IX in a mid-sagittal section through the cerebellum of a 13-day-old chicken embryo. We also observed the first cerebellar folia on the 11th day of incubation (Fig. 3) and 9 primary folia were recognized on the 13th day of incubation when a secondary foliation was just beginning in folium V (Fig. 4).

BOUVET et al. (1987) reported that the cerebellar cortex is composed of four layers at the 16th incubation day in chicken: the outer surface of the cerebellar anlage, the so-

called external germinative layer, the molecular layer, the Purkinje cell layer and the internal granular layer. In our study, the primitive cortex consisting of the external granular layer, a primitive molecular layer, an inner cortical layer and the inner granular layer were distinct a little earlier, namely on the 15th incubation day (Fig. 5).

In human cerebellum, the highest cell proliferation rate in the EGL occurs between the 28th-34th gestational weeks, and the width of the EGL remains unchanged from the 28th gestational week until the end of the first postnatal month, and it completely disappears by the 11th postnatal week (ABRAHAM et al., 2001). In chicken, BOUVET et al. (1987) observed that the EGL consists of a few persistent cells after the first week of hatching whereas the layer disappears almost completely 34 days after hatching. In our study, we observed that the EGL gradually decreased in thickness during the four weeks after hatching.

Purkinje cells arise from the ventricular neuroepithelium of the rhombencephalic alar plate (WASSEF et al., 1985) whereas granule cells originate from EGL and migrate to the inner granular layer (REDIES et al., 2002). In chickens, the majority of Purkinje cells are formed on days 3, 4 and 5 of incubation, after which Purkinje cells are arranged as longitudinal cell clusters at either side of the midline around day 9 (FEIRABEND, 1990). The Purkinje cells grow and form a single row between the molecular and the granular layers after 17 days of incubation (ESPINAR et al., 1997). BERTOSSI et al. (1986) reported that the developing Purkinje cell bodies are bipolar at first, because of two processes emerging from opposite poles of the oval cell body. They grow progressively in size and reach mean transverse diameters (mtd) in the chicken cerebellar cortex of 5.58µm, 7µm and 10.29µm on the 10th, 12th, and 14th days of incubation, respectively. BERTOSSI et al. (1986) furthermore observed that the larger (mtd: 12.30µm) Purkinje cell bodies are arranged in a single row parallel to the outline of the folium on the 16th incubation day. We found a similar pattern, namely that Purkinje cells arranged themselves in one or two rows by the 15th incubation day while on the 18th day of incubation, and they were arrayed in a manner similar to that of adult cerebellum (Figs 5-6). However, our measurements of the mtd of Purkinje cells were smaller than those of BERTOSSI et al. (1986; Table 1). Several factors may be responsible for this difference such as the image analysing programme, the numbers of embryos and Purkinje cells examined or the incubation circumstances etc.

LAFARGA et al. (1989) showed that the mean number of nucleoli per granule cell is 1.42 in rat cerebellum. We found mean AgNOR counts of the Purkinje cells of 1.35-1.54 (Table 1) which is slightly lower than the mean counts of AYDIN (2004) (1.40-1.66) in broiler and layer embryos and the average of 1.60 that LAFARGA et al. (1995) found in chicken Purkinje cells.

In our study, the nuclei of Purkinje cells and the AgNOR area increased through the entire experimental period. The mean ratio of AgNOR area to the Purkinje cell nucleus area reached its highest value on the 15th day of incubation. RUSSEL et al. (1991) reported that the occurrence of AgNORs was related to proliferative activities of the cell. However, they claimed that an increase in AgNOR cluster size rather than elevated AgNOR cluster numbers was the major feature. Ultrastructural morphology and size of the nucleolus could be sensitive indicators of cellular activity, particularly of protein synthesis (LAFARGA et al., 1989), and a relationship between transcriptional activity and cell firing rate is known (GARCIA-MORENO et al., 2000). We, therefore, propose that the relative differences that we found between the AgNOR areas might indicate different rates of protein synthesis and functional activity of the Purkinje cells.

As our results illustrate, investigating Purkinje cells as a model by applying the AgNOR technique in chicken embryo offers a good approach to the functional study of structures in the central nervous system. We hope that our findings provide reference data for future studies in this and related research topics, for example for establishing AgNOR parameters. We also hope that our data will be supplemented with other techniques such as PCNA and TUNEL in the near future.

ACKNOWLEDGEMENTS

This study forms part of the Master of Science Thesis of Serdar AKAR, for which we thank the "Selcuk University Scientific Research Projects (BAP) Coordinating Office", Project no: 06202022, for financial support.

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Received: December 26, 2008
Accepted: July 12, 2010
Branch editor: Huybrechts Roger

Belg. J. Zool., 140 (2) : 225-228 July 2010

Habitat use by the endangered Lesser Grey Shrike *Lanius minor* in Central Romania

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ABSTRACT. In this paper we present data relating to nest density and habitat use by the Lesser Grey Shrike *Lanius minor* in the Târnava Mare Valley, Romania, using both nesting tree parameters (microhabitat), and habitat parameters measured in a 100m radius around each nest. The density of nests was 0.96 per km². Average distance between nests was 768.4m. Most of the nests (94.1%) were found in poplars, in the region of the middle third of their trunk, especially at the terminal parts of the branches. The birds preferred open habitats, with extended arable field cover. Moreover, the tree and shrub cover were small in areas used for nesting. As poplars are the preferred nesting habitats of this bird, and are scarcely represented in this area, the protection of these trees is critical for conservation of the Lesser Grey Shrike.

KEY WORDS: *Lanius minor*, nest site selection, habitat preference, Romania

INTRODUCTION

Lanius minor (Gmelin, 1788) is a long distance migrant passerine bird, which overwinters in South Africa, its nesting area extending from south-west Europe to Central Asia (Cramp & Perrins, 1993). This bird is in decline in the majority of the European countries, both the population abundances and the range of the species being affected (Lefranc & Worfolk, 1997; Sanderson et al., 2006). Romania is a stronghold of this species in Europe, nowadays having 364,000-857,000 pairs (Burfield & van Bommel, 2004). The species is protected according to the Birds Directive – Annex I, being one of the species for which Natura 2000 sites are designated in the European Union, and for which compensation measures are offered for land owners in the Member States. In Romania there is a lack of information regarding the density of this species/area unit and its ecology.

In this study we determined the following: (1) nest density per unit surface area (1km²); (2) microhabitat used for nest building (the tree species and tree parameters); (3) habitat characteristics of the areas selected as territories, in comparison with unoccupied (control) sites, in a 100m radius (3.14ha) around each nest, underlining the proportion of habitat elements in the areas where territories were established.

MATERIALS AND METHODS

Study area

The Târnava Mare Valley is located in southern Transylvania, Romania (central coordinates: 46°14'N, 24°48'E). From a landscape perspective, the area is characterized by a hilly relief with elevations roughly between 400 and 700m. Arable fields cover less than 32% of total land cover, and are situated only at the base of gentle hill slopes and in the floodplain of larger valleys. The slopes of the hills are covered with meadows, pastures and various succession stages of forestation. The floodplain is situated at 320-450m altitude, and has a width of 600-1000m (most often 800m). In the floodplain the main land use is represented by arable fields, this having around 70% cover. Several abandoned plots covered by weeds and hedgerows and scattered shrubs and trees are situated in and across these arable fields. Moreover, the narrow adjacent water courses of the Târnava Mare River are bordered by small riparian forest corridors. Compact alluvial galleries stretch along the Târnava Mare River. These galleries are formed by White Willow *Salix alba* and White Poplar *Populus alba*, and are up to 50m in width.

Our study was conducted in the major river bed, represented by the former floodplain. Here the White Willow (Doniță et al., 2005), is the most common tree, with up to 70% cover. The rest of the trees are represented by White Poplar (around 28%) and other species such are Ash (*Fraxinus angustifolia*) and very rarely Black Alder (*Alnus glutinosa*), these covering around 2%. We considered only White Willow and White Poplar in our analysis because these were the most common trees. In the rest of the major river bed, the characteristic riparian vegetation is represented by old trees (almost exclusively by *Salix alba* and *Populus alba*), shrubs, patchily distributed reed *Phragmites australis* and different sedge *Carex* spp. species.

Data collection and analysis

The study was conducted between May 5 and June 28, 2008. We used line transects with unlimited width (Bibby et al., 2000), the maximum length of a transect being 1km, depending on accessibility in the field. The total length of transects was 22km and the average width of the studied floodplain section was 800m, meaning a study area of 17.6km². The observations were done between 9am and 12am always on clear days without strong wind.

Observations were done simultaneously by two people, walking side by side close enough to allow permanent visual contact between them while still covering the entire width of the major river bed. After the identification of *L. minor* individuals, their nests were detected by observing the birds heading towards their nests. Distances between nests were measured with a handheld GPS device.

The trees where the nests were constructed by the birds were considered by us as being microhabitats. In each microhabitat we recorded the following parameters: (i) the tree species in which the nest was found, (ii) the diameter at breast height of the tree (d.b.h.), (iii) the section of the tree in which the nest was built (lower, middle or upper 1/3), and (iv) the exact place where it was built (near the trunk or on a lateral branch). Since only one nest was observed in a willow, the d.b.h. values were compared only in the case of the poplars. In the comparison we considered 16 randomly selected poplars in which no nests were built.

We characterized the surroundings of trees with *L. minor* nests using seven parameters (see below). We consider these variables habitat variables. We chose 100m radius (i.e. 3.14ha) around each microhabitat because this is close to the documented territory size of the studied bird: 3.3ha (Min=1.9, Max=11.2ha) (KRIŠTÍN, 1995), and 6.21ha (Min=2.9ha, Max=14.6) (WIRTITSCH et al., 2001). The following habitat variables (in % cover) were estimated, after the methodology described by CRISTEA et al. (2004): (i) tree cover, meaning the canopy projection cover of all trees in the study plot, and similarly (ii) willow cover (out of total tree cover); (iii) poplar cover (out of total tree cover) and (iv) the total shrub cover. The total open habitat area covered only by herbaceous vegetation and arable field (v) was further split into two categories based on visual estimations: (vi) the herbaceous vegetation cover (grassland and abandoned agricultural plots invaded by weeds out of total open habitat cover) and (vii) agricultural field cover (out of total open habitat cover). Most of the agricultural fields were represented by maize cultures (very rarely and only in small plots we found potato plantations and straw cereals), the plantation distance being 70cm between rows and 35cm in rows, therefore in the study period around 45% of the field was bare ground. In order to determine the position of the nests, and the centre of the bird territories, we considered the distance (measured with GPS) from the tree where the nest was located to the Târnava Mare River, separating two situations: (1) the nest was built in a tree from the main alluvial forest gallery, or (2) in the floodplain, in isolated tree groups or vegetation strips along the tributaries, some of which are dried-out during summer.

On the transects where we did not find the species, we described the same variables in 17 observation points in a 100m radius, selected randomly, in order to include all habitat types from the Târnava Mare floodplain, from the river edge to the first terrace. The minimum distance between the randomly selected study points and the observation points was 500m.

For the comparison of the habitat variables between the observation points in which nests were present (bird territories), with those where no nests were found (control sites) we used the ANOVA parametric *t* test, and the non-parametric Mann-Whitney U test. Data normality was tested with the Levene test.

RESULTS

We recorded 17 *L. minor* nests meaning 0.96 nests per km². The average distance between nests was 768.4m (Median=575, Min=50, Max=2800, SD=692.5). All nests were built separately and had a dispersed distribution in the landscape.

Out of these 17 nests, 16 (94.1%) were found in poplars, and only one nest (5.9%) was built in a willow. The d.b.h. of these poplars (Mean=102.8, Min=35, Max=152.9, SD=36.1), is larger than the d.b.h. of the 16 poplars we used as control sites (Mean=34.1, Min=23.9, Max=66.9, SD=10.6), the difference being statistically significant (Mann-Whitney U test, Z=4.70, P<0.0003). Out of the 16 nests, 12 (75%) were built in the middle 1/3 of the tree trunks, and four (25%) were built in the upper 1/3. The nest built in a willow was located in the upper 1/3 of the tree, the d.b.h. was 100.31cm, and the distance to the river was 150m. 15 nests (88.2%) were built in lateral branches, towards their top, and two nests (11.8%) were built between the main trunk and one of its lateral branches (the case of one nest built in poplar and the only nest built in willow).

The descriptive analysis of the habitat variables recorded in the observation points located in bird territories (nest was present) and in the control sites, and the comparison of the variables between these two categories are presented in Table 1. The average distance between the trees where nests were built and the river is 213.2m (Median=80.00, Min=10, Max=1000, SD=318.7, n=17). Out of the 17 trees where nests were built, six (35.3%) were located in the main alluvial forest gallery of the Târnava Mare, and 11 (64.7%) were located in the floodplain, in isolated tree groups or vegetation strips with few trees.

The average values for the proportion (%) of different habitat elements from the bird territories are presented in Table 1. The largest proportion is represented by open habitats (87.88%), and within these the arable fields (57.6%). The average shrub cover is 9.4% over the entire area, while tree cover is 6.5%. Among trees, poplars are more preferred.

TABLE 1

Descriptive analysis and comparison of habitat variables related to the presence and absence of *L. minor*.

	***L. minor* presence**				***L. minor* absence**				
	Average cover (%)	**Min.**	**Max.**	**SD**	**Average**	**Min.**	**Max.**	**SD**	***P***
Total tree cover	6.52	3.00	10.00	2.06	20.88	5.00	40.00	13.37	0.001[2]
Total willows	2.76	0.00	5.00	1.82	16.88	3.00	35.00	11.87	**<0.0004[2]**
Total poplars	3.64	1.00	6.00	1.45	4.11	0.00	10.00	2.42	0.49[1]
Shrub cover	9.41	2.00	20.00	4.54	23.52	10.00	40.00	10.11	**0.0001[2]**
Open habitat	87.88	75.00	96.00	4.97	61.58	35.00	90.00	18.60	**<0.0004[2]**
Herbaceous vegetation	31.11	5.00	54.00	14.90	17.05	5.00	55.00	12.38	**0.005[1]**
Arable land	56.76	30.00	85.00	16.29	44.52	25.00	85.00	18.32	**0.04[1]**

Note: 1 – *t* test, 2 – Mann-Witney U test.

DISCUSSION

The recorded nest density was 0.96 per km², this being a small to average density compared to figures given in the consulted literature data. KRIŠTÍN (1995) recorded 66 nesting pairs in 50km² (1.32 pairs per km²) in a study conducted in Slovakia, whereas LOVÁSZI et al. (2000), in Hungary recorded 0.05 nesting pairs per km², the largest density being 0.6 nesting pairs per km². In a Slovakian study, KRIŠTÍN et al. (2000), recorded a density of 4.20 pairs/km² in 1996, and 3.85 pairs/km² in 1997, values that are larger than those from KRIŠTÍN (1995) and LOVÁSZI et al. (2000). We found that the nests were isolated, a situation that is different from other studies (CRAMP & PERRINS, 1993; KRIŠTÍN et al.; 2000; WIRTITSCH et al.; 2001). A potential reason may lie in the scattered distribution of the poplars in the landscape, these trees representing key habitat for nesting for the Lesser Grey Shrike in our area.

Although the floodplain of Târnava Mare is rich in willows – trees, potentially suitable as nesting habitats – all nests except one were found in poplars. Possibly, the structure of these trees offers better conditions for nesting than does that of willows. Moreover, we found that the unoccupied poplars from the control plots were significantly smaller (d.b.h.) than those occupied by birds (see results). These results suggest that even within a particular microhabitat type (i.e. poplar trees in this case) larger ones are more preferred. The predominant use of poplars for nest building was noted also by HORVÁTH (1959) and LOVÁSZI et al. (2000). In the study conducted by LOVÁSZI et al. (2000), beside poplars, four other species were also used in nest building, but to a lesser degree. The situation is similar to our findings that nest site selection is not related to the numerical abundance of the tree species in the area. KRIŠTÍN (1995) noted that 97% of the observed nests were built in fruit trees. Similarly, WIRTITSCH et al. (2001), in a study from central Slovakia, observed a large proportion of nests built in fruit trees. The authors did not record a clear preference for one kind of fruit tree; some of the species (i.e. apple) were used according to their availability while others not. The above-mentioned studies suggest that *L. minor* may show a wide preference for microhabitats. In our case, this bird selected almost exclusively large poplars. This may be due to low competition for microhabitats because of, e.g. the low density of individuals. At low density, the competition for nesting sites may be low, and the actual offer (i.e. large poplars) may allow the occupancy of the best nesting sites. This may explain the almost exclusive use of large poplars, even is these are relatively underrepresented compared to willows. At higher population densities the competition for the best nesting places may result in the use of a wider spectrum of microhabitats (i.e. in terms tree species).

In our study, most of the nests (75%) were built in the middle 1/3 of the trees. Although we found no literature data with which we could compare these findings, we mention that in the study of LOVÁSZI et al. (2000), in one of the sites the nests were built especially high, while in the other site they were built at different heights, without a clear tendency. The authors considered that the height at which nests are built is probably influenced in certain situations by anthropic pressure. The average distance between the Târnava Mare River and the trees from the minor river bed where the nests were built is 213.2m, the maximum distance being 1000m. This suggests that nests are preferentially built in trees forming small groups in the narrow alluvial corridors of the floodplain, and not in the main alluvial forest gallery of the Târnava Mare, where tree canopies are more close to each other. The preference of this species for loose tree cover was observed also by KRIŠTÍN (1995). The majority of the nests from our study were built on lateral branches, towards their top, a situation reported also by KRIŠTÍN (1995). A reason might be the avoidance of predators.

We found that Lesser Grey Shrike territories are established preferably in open habitats with large areas of arable fields and herbaceous vegetation, with small shrub and tree cover. Of the small number of trees found in the territories, poplars were the more numerous. In a similar way, LOVÁSZI et al. (2000) found that birds prefer open, steppe habitats, where trees have a loose distribution and the herbaceous vegetation is low. In the study of KRIŠTÍN (1995) all nests were found in extensive orchards surrounded by pastures and arable plots. We remark again the importance of loose tree cover, open habitat with herbaceous vegetation and of arable fields in nesting territory selection. In the same study, nests were often found nearby houses, in the surrounding gardens. In the study of WIRTITSCH et al. (2001), meadows represented the most preferred habitat in the territories of the Lesser Grey Shrikes (in the period of chick feeding, especially the mowed meadows), but also a considerable area of bare

ground. In our study, the largest area is covered by open habitats, and within these, by arable fields. Similar results were reported by WIRTITSCH et al. (2001), where the largest proportion was represented by meadows, followed by arable fields. The same authors showed that bare ground, lacking vegetation, present in large amounts in agricultural fields, was the most used habitat element for feeding by *L. minor*, during nest building and incubation, while during the period of chick feeding food was gathered especially from mowed meadows. WIRTITSCH et al. (2001) explain these preferences for bare ground and low vegetation by the better accessibility to large insects, on which the Lesser Grey Shrike feeds preferentially (KRIŠTÍN, 1995). The same explanation might be valid also in our case, in the preference for agricultural fields, with bare ground. In the study of (KRIŠTÍN, 1995), of the open habitat categories, meadows were represented in the largest amount, while agricultural fields were present in a lesser amount, a situation that is different in our study.

CONCLUSION AND IMPLICATION FOR CONSERVATION

In the central section of the Târnava Mare Valley, *L. minor* prefers open habitats with small shrub and tree cover, with high amounts of arable fields located in the major river bed of the Târnava Mare. Although there are larger numbers of willows (*S. alba*) available for nest building, the Lesser Grey Shrike nests preferentially in large poplars, located at a distance from the main alluvial forest gallery. Our study shows that old poplars are the preferred nesting microhabitats for *L. minor.* Therefore, the maintenance of old, isolated poplars in the floodplain of the Târnava Mare, and of the small tree-groups where these poplars are found, is an essential condition for the conservation of this species, endangered at the European level. The cutting of old trees (both willows and poplars) by locals is a frequent practice in this area (Moga, unpublished data). For this reason, local communities and authorities responsible for environmental protection need to be better informed regarding the importance of old trees (especially White Poplars) and the insurance of their natural regeneration.

ACKNOWLEDGEMENTS

We thank the Mihai Eminescu Trust for the financial support of this study.

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Received: March 22, 2009
Accepted: March 5, 2010
Branch editor: Blust Ronny

Belg. J. Zool., 140 (2) : 229-234 July 2010

Genetic diversity in see-see partridge (*Ammoperdix griseogularis*, Galliformes) populations from sub-Himalayan Mountain ranges of Pakistan

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ABSTRACT. We used Random Amplified Polymorphic DNA (RAPD) markers to investigate the genetic structure of two populations of see-see partridge (*Ammoperdix griseogularis*, Galliformes) from the Suleiman range, in the Pakistani Himalayan region. The see-see partridge is a vulnerable species with a distribution in the Middle East and central Asia. The percentage of polymorphic bands (94.05%), Shannon Index (H=0.455) and Nei's average gene diversity (I_N=0.298) *of A. griseogularis* at species level were rather high when compared with other avian species. 17% of polymorphic loci showed statistically significant differences in their allelic frequencies. The G_{ST} (Nei's coefficient of genetic variation) values indicated low levels of differentiation (G_{ST}=0.08). A genetic distance D of 0.05 indicated that both populations were to some degree in isolation but their differentiation was not significant. Overall, our genetic data can support action plans aiming to locally preserve differentiated genetic resources that, in the future, could potentially result in ecologically and behaviourally differentiated populations. In view of the rapid environmental changes that the Himalayan region has been experiencing in the last decade, this study could help in conservation plans.

KEY WORDS: *Ammoperdix griseogularis,* Genetic differentiation, Genetic variability, Himalayas, RAPD, Suleiman Mountain range

INTRODUCTION

The see-see partridge (*Ammoperdix griseogularis*, Phasianidae, Galliformes) is a bird species of dry and stony terrain listed as "vulnerable" (BIRDLIFE, 2004) This species has a huge distribution range, from southeast Turkey through Syria and Iraq to Iran and Pakistan (BAKER, 1924; ROBERTS, 1992; GRIMMETT et al., 1998). European populations suffer from demographic decline (BIRDLIFE, 2004). In Pakistan, see-see partridges are also present in Sindh Kohistan, the Punjab Salt Range and the North-Western Frontier Province (NWFP). However, in this latter region, the species is largely declining due to strong human persecution for hunting purposes (GRIMMETT et al., 2009). See-see partridge populations further colonize suitable terrains of the Balochistan, e.g. open, dry and hilly areas with limited agriculture. Birds are usually seen in pairs or, at the most, in flocks of two to four specimens. Yet, over fifty birds in a single flock have been counted in the Salt and Suleiman Ranges (KHALIQ, pers. obs., 2008), which are considered the strong hold of the species (ROBERTS, 1992) and present a well protected area due to tribal customs preventing easy access of outsiders.

The wide distribution range of the see-see partridge notwithstanding, no genetic data is available from the literature. To our knowledge, no work has been carried out to investigate the genetic structure of this species using DNA markers. Moreover, precise identification of the population genetic structure and possible natural population subdivisions is necessary to understand demographic and evolutionary patterns within/among populations, thus providing crucial information for future conservation plans (SCHAAL et al., 1991; WEBSTER et al., 2002; ZINK, 2004; HAIG et al., 2006; ALLENDORF & LUIKART, 2007). Hence, we employed Randomly Amplified Polymorphic DNA markers (RAPDs: WELSH & MCCLELLAND, 1990; WILLIAMS et al., 1990), a fingerprinting technique that has proved to be particularly valuable when DNA sequencing information is lacking.

MATERIALS AND METHODS

Sampling and DNA extraction

We collected 23 samples from two geographic populations of *Ammoperdix griseogularis* from the Suleiman range (Fig. 1) where their habitat is still intact and naturally protected due to the tribal restrictions mentioned above. Sampling was conducted between October 2006 and January 2008 (12 samples from the eastern population in October and November 2006, and 11 from the western population in November 2007 and January 2008). Each bird was captured at least four km away from other sampled birds to reduce the chance of sampling birds from the same covey. Only tail feathers were plucked, and plunged in 95% ethanol before they were stored at -50°C. Total genomic DNA from individual feathers was extracted following BELLO et al. (2001) from a fragment (0.5-1cm long) derived from the base of the quill. 500mL of lysis buffer (50mM Tris-HCl at pH 8, 20mM EDTA at pH 8, 2% SDS) was added, followed by 10mL proteinase K (final concentration, 175mg/mL). Each sample was incubated at 55ºC overnight. Then, a common phenol:chloroform protocol for DNA extraction was employed (SAMBROOK et al., 1989) and DNA concentration and purity were determined spectrophotometrically.

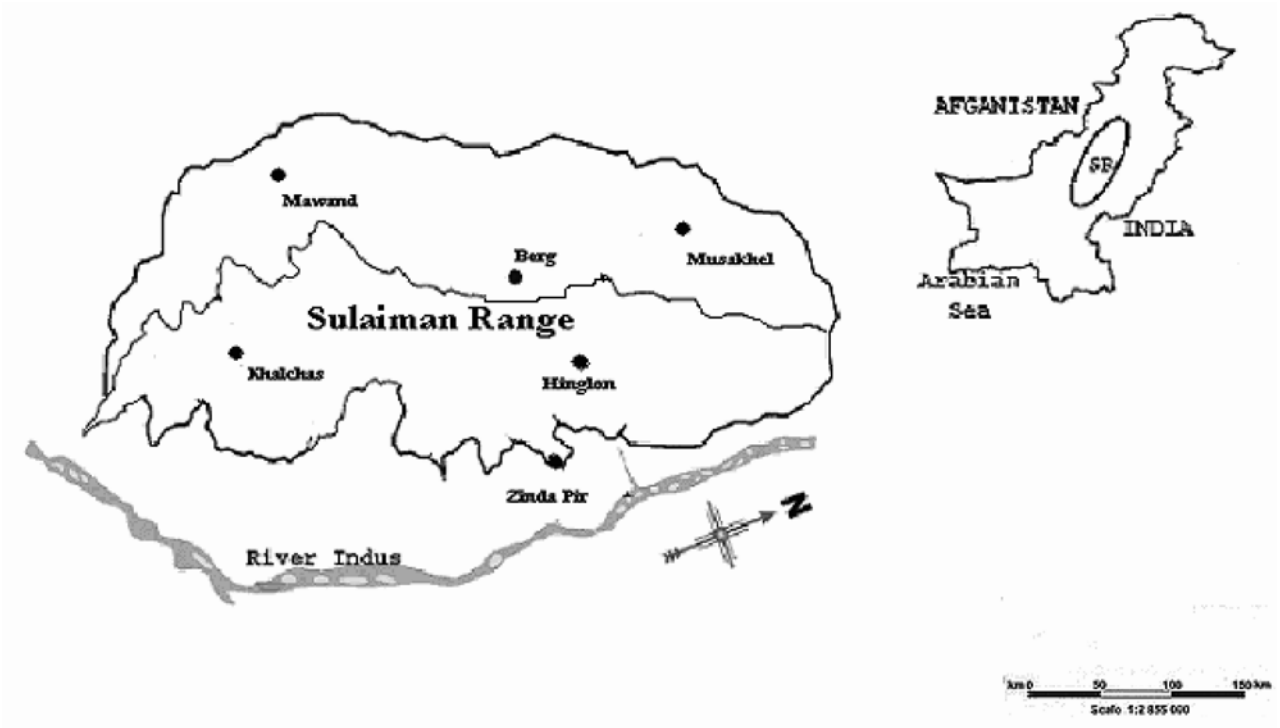

Fig. 1. – Map showing the geographical distribution of the western (Mawand, Berg and Musakhel) and eastern (Khalchas, Hinglon and Zinda Pir) populations of *Ammoperdix griseogularis* in the Himalayan mountains, Suleiman ranges, Pakistan.

RAPD amplification

The RAPD technique can be quickly and easily applied, requires only small amounts of DNA and allows detection of DNA polymorphisms reliably and inexpensively (FRITSCH & RIESEBERG, 1996; HARRIS, 1999). It has been successfully used in many genetic studies of avian population (e.g., BALL & AVISE, 1992; ZINK et al., 2000; HAIG et al., 2004; ZINK, 2004; CHAN et al., 2008; FUNK et al., 2008). For the Galliformes, RAPD markers have for example been successfully applied to detect hybridization among Mediterranean populations of different *Alectoris* partridges (NEGRO et al., 2001; BARBANERA et al., 2005; 2009).

In order to select primers producing only clearly and reliably identifiable polymorphic bands, we applied 25 decanucleotide primers (kits A, B, H, from Genelink, USA; Table 3) to four individuals each from both populations. Each primer was applied thrice to check for reproducibility. Fifteen primers yielded trustworthy band patterns and were subsequently used to screen all samples. PCR reactions (15µL) were prepared as follows: 2.5mM $MgCl_2$, 10xPCR buffer 2.5mM of each dNTP 50ng/µL of each primer, 1.25 unit of *Taq*DNA Polymerase (Fermentas, USA), and 50ng of template DNA. Amplifications were carried out in a thermal cycler GeneAmp® 9700 (Applied Biosystems, USA) with the following thermal profile: 4min at 94ºC, 45 cycles of 45s at 94ºC, 45s at 36ºC and 1min at 72ºC; then, a final extension of 10min at 72ºC. Bands were separated by electrophoresis on 8% denaturing polyacrylamide gel and stained with $AgNO_3$ (HEUKESHOVEN & DERNICK, 1985; BUDOWLE, 1991).

Data analysis

The presence or absence of each band was scored by analyzing the electrophoretic profiles obtained for all individuals using the 15 selected primers. The resulting matrix was imported into different programs for data elaboration. Each locus was treated as a two-allele system, with only one allele per locus being amplifiable by the PCR under the Hardy-Weinberg equilibrium (LYNCH & MILLIGAN, 1994). A G-test was applied to allele frequencies to check for homogeneity of allelic frequency distributions. In order to get a clearer inference from the G-test results, the statistically significant proportion of homogeneous alleles was estimated by dividing the number of total RAPD loci with statistically significant differences in allele frequencies (at least, 5%) by the total number of polymorphic loci. NEI's (1973) average gene diversity (I_N), Shannon Index (H) (LEWONTIN, 1972: $H=-\sum P_i \log_2 P_i$, where P_i is the frequency of a given RAPD band) and Nei's coefficient of gene differentiation (G_{ST}) between populations were calculated using POPGENE v. 1.31 (YEH et al., 1999). To compare the level of genetic diversity between populations, an analysis of variance for both the Shannon Index and the average gene diversity was carried out (randomized block design, two fixed factors: primer and I_N /H). Using the TFPGA software v. 1.3 (MILLER, 1997) with LYNCH & MILLIGAN's (1994) correction, genetic variability and NEI's (1978) unbiased genetic distance (D) were estimated. The effective number of migrants per generation (N_{em}) was obtained from the formula $N_{em}=0.25(1-G_{ST})/G_{ST}$ (SLATKIN & BARTON, 1989). Principal Coordinate Analysis (PCoA:) with Euclidean distance matrix was performed with the MVSP software

v.3.1 5 (KOVACH, 2001). Genetic similarity dendrograms were constructed with the Jaccard (J) coefficient and the UPGMA cluster analysis algorithm in the NTSYS-PC computer program (ROHLF, 1992).

TABLE 1

Numbers and proportion of polymorphic bands generated by RAPD primers

Primers	Number of loci		Polymorphism(%)
	Total	Polymorphic	
GLA-01	13	12	92.30
GLA-02	14	13	92.85
GLA-03	15	14	93.33
GLA-04	15	14	93.33
GLA-07	22	21	95.45
GLA-08	17	16	94.11
GLA-09	20	19	95
GLA-11	16	15	93.75
GLA-13	20	18	90
GLA-14	15	13	86.66
GLB-01	25	24	96
GLH-01	27	26	96.29
GLH-02	21	20	95.23
GLH-03	16	15	93.75
GLH-05	27	25	92.05
Total	**282**	**265**	**94.05**
Average	**18.8**	**17.66**	----------

TABLE 2

Analysis of Variance for I_N/H within populations.

Source	Df	Sum of Squares	Mean Square	F
Population	1	0.138/0.061	0.028/0.012	3.810*/3.465*
Primer	14	0.203/0.098	0.014/0.007	1.997*/1.976*
Error	14	0.507/0.248	0.007/0.004	
Total	29	8.049/3.726		

TABLE 3

Genetic variation within population.

	Species	Population East / West
P	94.05%	85.71/78.57%
I_N	0.298	0.276/0.270
H	0.455	0.420/0.404
G_{ST}	---------	0.083
D	---------	0.05
N_{em}	---------	2.74
G-test	---------	17%

P=Percentage of polymorphic loci;
I_N=average gene diversity;
H=Shannon Index;
G_{ST}=Nei's coefficient of gene variation;
N_{em}=effective number of migrants per generation;
D=Nei's genetic distance,
G-Test=Homogeneity test

RESULTS

The selected 15 primers (Table 1) produced a total of 282 clearly identifiable bands with a size range of 125-1800bp in the two investigated populations. All primers produced polymorphic banding patterns varying from 13 to 27 bands (average 18.9 Table 1). The RAPD primers were found to be statistically significantly different in their ability to detect genetic diversity within populations (P<0.001, Table 2), thus providing sufficient reliability and effectiveness. The percentage of polymorphic bands (P) was 94.05% at species level (Table 1).The G-test showed that 17% of all polymorphic loci were statistically significantly different in their allelic frequencies between the two studied populations (Table 3). The intra-population variation was high in these two populations. In the eastern population, Nei's genetic diversity (I_N) was 0.276 and Shannon Index (H) was 0.420, whereas Nei's average genetic diversity (I_N) equalled 0.270 and the Shannon Index (H) was 0.404 in the western population (Table 3). Also the analyses of variance (ANOVA) for I_N and H showed a high genetic diversity in the studied populations (P<0.001, Table 2). The overall genetic diversity (I_N) at species level was also high; Nei's average genetic diversity reached 0.298 and Shannon Index (H) 0.455, respectively (Table 3). The number of migrants per generation was estimated as N_{em}=2.74 (Table 3). All parameters of genetic differentiation between the two populations showed low levels of differentiation, such as the coefficient of differentiation (G_{ST}) equalling 0.083 (Table 3) and the genetic distance (D) between these populations being D=0.05 (Table 3). In the Principal Coordinate Analysis, 25.26% of variation was represented by the two PCoA (Axis 1 and Axis 2) (Fig. 3).

DISCUSSION

This is the first study ever attempting to analyze the genetic structure of *Ammoperdix griseogularis* (see-see partridge) populations. Genetic data on this widely distributed species is very inadequate and only patchy information about the ecology of this species is available from the literature (BAKER, 1924; ROBERTS, 1992; GRIMMETT et al., 1998). Our analysis showed a high degree of genetic diversity in the species and at the population level. The intraspecific level of genetic polymorphism was around 94.05% (Table 1). This observed high level of genetic polymorphism was also supported by Nei's average genetic diversity (I_N=0.298, Table 3) and the Shannon Index (H=0.455, Table 3). Similar high levels of genetic polymorphism have been reported in other avian species such as Manchurian pheasant and Shiver ring-necked pheasant (GIESEL et al., 1997; KULIKOVA et al., 2002). The selected primers also detected a high degree of genetic polymorphism at the population level and the G-test showed that 17% of polymorphic loci differed significantly in their allelic frequencies (Table 3) between the two populations. In addition, the ANOVA for I_N and H supported high genetic diversity in these two populations (P<0.001, Table 2). This high degree of genetic diversity might be attributed to the sampling area providing undisturbed habitats in the Suleiman Range (due to the Tribal

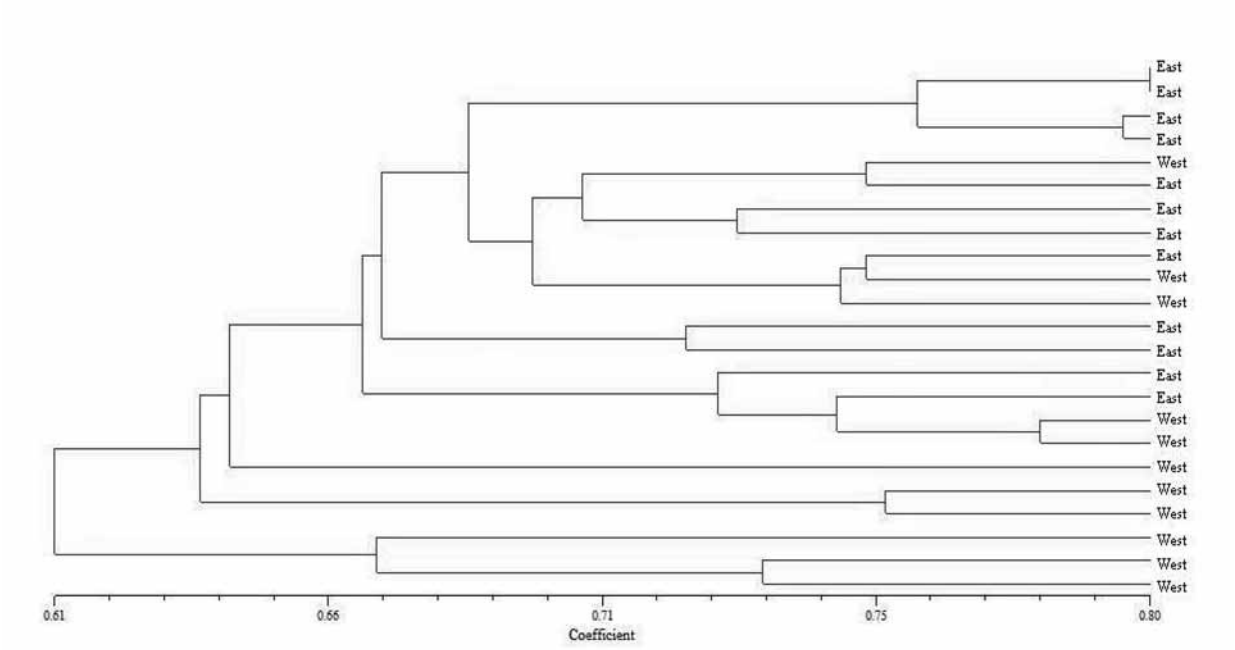

Fig. 2. – UPGMA dendrogram revealing genetic similarities between 23 *Ammoperdix griseogularis* genotypes based on RAPD amplification with 15 selected primers. The scale on bottom is Nei and Li's (1979) coefficient of genetic similarity.

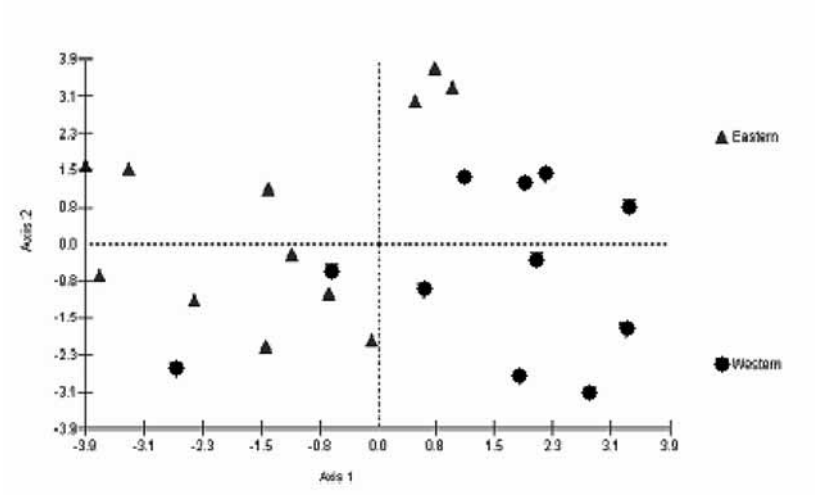

Fig. 3. – Principal Coordinates analysis of *Ammoperdix griseogularis*; Axis 1 comprised 14.99% and Axis 2 10.27% of the total variance.

restrictions, mentioned in the introduction). Morphologically, no distinct differences were observed in the specimens from the two studied populations. Specimens were similar in their size and plumage. The only visible difference between the two populations was the beak colour. Beaks from the western population had a yellowish tinge while birds from the eastern population displayed a red tinge. As far as the possibility of sub-species existence is concerned, no sub-species of *Ammoperdix griseogularis* (see-see partridge) have ever been reported except for a single study from 1950s by Kolez (1950) who suggested *Ammoperdix griseogularis Peraticus* as a new sub-species. This might just be a geographical morph as no other taxonomist or ornithologist ever acknowledged it.

In the past, RAPD identified high diversity at the population level in the Iberian eagle (*Aquilla adalberti*) where average genetic diversity was around 0.267 (Padilla et al., 2000), similar to the results at the population level of our see-see partridge study (I_N=0.276 and H=0.420 for the eastern and I_N=0.270 and H=0.404 for the western population, respectively; see Table 3). Thus, the two populations investigated here showed similar levels of high genetic diversity.

ROBERTS (1992) and GRIMMETT et al. (1998) described the see-see partridge as a sedentary species that does not move long distances as this species prefers walking over flying, but our results do not support their observation. Despite the relatively large geographical distances of up to 150km between some sampling locations, genetic parameters suggested that the two see-see partridge populations were not significantly genetically differentiated. With a G_{ST} (coefficient of differentiation) of 0.083 (Table 3), results are comparable to the Light-footed clapper Rail (*Rallus longirostris levipes*) (NUSSER et al., 1996). Also the low genetic distance (D=0.05) between the two populations supported the low genetic differentiation between the two populations as in other avian species (Grasshopper sparrow (*Ammodramus savannarum*) with D ranging from 0.018 to 0.134 and an average 0.07; DOLMATOVA et al., 2000). The population similarity dendrogram revealed that the similarity coefficient varied from 0.61 to 0.81 (Fig. 2) suggesting that both populations are genetically similar. In the population dendrogram (Fig. 2) and the Principal coordinate analysis (Fig. 3), no separate clustering of the two populations was observed but specimens were rather scattered among clusters. This observation was also supported by the total number of migrants between these two populations that were N_{em}=2.74. This suggests that gene flow between these two populations is high (HEDRICK, 2000). In comparison with the Israeli chukar (*Alectoris chukar*, Phasianidae) populations, which showed very high numbers of migrants per generation N_{em}=6 and strong population structuring, (RANDI & ALKON, 1994), the value estimated in our study is low but high enough to keep the two populations from drifting apart (HEDRICK, 2000). So, on the basis of our genetic data, we do not consider this species as sedentary as previously reported.

CONCLUSIONS

Ammoperdix griseogularis showed a relatively high level of genetic diversity at the species and population levels, with substantial gene flow between populations thus resulting in low levels of genetic differentiation despite long distances between populations. Our study also illustrates that naturally preserved habitats in the Suleiman ranges could be an ideal conservation area because of their limited access due to tribal restrictions, thus providing a natural buffer zone for its wildlife, including *A. griseogularis*. Such areas are becoming very rare in the world due to human expansion and exploitation of natural resources. Our study also illustrates the need for more thorough genetic studies of this bird species with additional molecular markers and DNA sequence analysis to unravel its genetic structure in more detail.

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Received: May 7, 2009
Accepted: April 22, 2010
Branch editor: Schön Isa / Blust Ronny

Belg. J. Zool., 140 (2) : 235-240 July 2010

New records of sessile rotifers (Phylum Rotifera: Floscullariacea, Collothecacea) from Southeast Asia

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ABSTRACT. Recognizing the dearth of information on the biodiversity and biogeography of Southeast Asian micrometazoa, particularly sessile rotifers, we examined two samples of submerged aquatic macrophytes (*Hydrilla verticillata* (L.f.) Royle and *Utricularia* sp.) from different sites in Cambodia. We were able to distinguish a total of 23 taxa, three of which may represent undescribed species. Two more taxa could not unequivocally be ascribed to a known species. We further comment on the distribution of *Octotrocha speciosa* Thorpe, which may be much less widespread than previously thought.

In addition to the three possibly new species, nine are newly recorded for the Oriental region; of these, two are recorded for the second time ever. All represent new records for the fauna of Cambodia. Our results illustrate the need for more detailed and thorough taxonomic and faunistic studies on this group of poorly known organisms.

KEY WORDS: freshwater, diversity, biogeography, Cambodia, epiphyton

INTRODUCTION

Sessile rotifers, which belong to only two families (Collothecidae and Flosculariidae) of Class Gnesiotrocha, Monogononta, are a particularly interesting and attractive group of organisms. They are interesting because they exhibit a wide range of evolutionary adaptations, from solitary to fully colonial with task division between different individuals, and from fixosessile to permanently pelagic, conditions that, apparently evolved in parallel multiple times (WALLACE, 1980; 1987). They are attractive because of their relatively large size and static behavior, which makes them relatively easy objects to study when alive, and their beauty, which gave one of the taxa concerned its name, *Floscularia* or flower animals, referring to its lobed corona resembling flower petals. These features have led early microscopists, starting with VAN LEEUWENHOEK (1703, cited by HUDSON & GOSSE, 1886; and these authors themselves), to study and brilliantly illustrate many such animals. Recently, however, they are receiving much less attention. In particular, hardly anything is known about the Southeast Asian fauna of sessile rotifers, apart from the comprehensive taxonomic and faunistic report by KOSTE (1975), and the same is true for most tropical regions.

Of all Southeast Asian countries, Cambodia has one of the least well-documented rotifer faunas (SEGERS, 2001). The sole relevant paper is by BERZINS (1973), who describes *Anchitestudinella mekongensis* and *Filinia camasecla cambodgensis*, and the same author mentions some Cambodian material in taxonomic revisions of the *Keratella valga* group (BERZINS, 1955) and *Anuraeopsis* (BERZINS, 1962). Finally, MIZUNO & MORI (1970) include a few Cambodian records of rotifers in the results of their survey, and we welcome the recent contribution by MEAS & SANOAMUANG (2010). None of these authors, however, mentions any sessile rotifers.

Considering this lack of information, we took the opportunity to study a limited number of samples of sessile rotifers when the occasion arose to visit Cambodia during early 2010. We believe that the results of this examination, as preliminary as they may be, are sufficiently significant to be formally presented here, especially as they draw attention to an almost completely overlooked taxon in which much remains to be discovered.

MATERIALS AND METHODS

During a short visit to Cambodia from January 30th to February 1st, 2010, we collected samples of submerged macrophytes in two different localities. The first sample consists of a fragment of *Hydrilla verticillata* (L.f.) Royle, collected on January 31st, from the historical pond Sra Srang, in the Ankhor temple complex region near Siem Reap (N 13° 25' 51.7", E 103° 54' 44.4", water temperature 29°C, DO 2.9mg.L^{-1}, pH 8.1, Cond. 6.0µS.cm^{-1}); the second is a strand of *Utricularia* sp., collected February 1st, from the swampy edge of a reservoir near Trapang, along the road from Seam Reap to the Phusing border crossing with Thailand (N 14° 14' 05.5", E 104° 05' 0.30", water temperature 30°C, DO 1.2mg.L^{-1}, pH 7.6, Cond. 16.0µS.cm^{-1}). Plants were kept in sufficiently large containers to avoid crowding during transport and examination in the days following collection. The samples were examined at the Applied Taxonomic Research Center of Khon Kaen University. Small plant fragments were first examined using an Olympus SZ-PT dissecting microscope, and fragments onto which epi-

phytic rotifers had attached were transferred to an Olympus BX51 compound microscope equipped with an image capturing device for detailed examination of their external and trophi morphology. Apart from a limited number of permanent preparations of trophi, no material was deposited due to the difficulty, and, at times, impossibility of obtaining specimens that, in preserved condition, are useful for subsequent morphological study. Identification of the material was done using the works by EDMONDSON (1949) and KOSTE (1978), and papers as indicated below; nomenclature and taxonomy follow SEGERS (2007).

RESULTS

The material reported here consists of the epiphytic rotifers living on two species of submerged macrophyte, *Hydrilla verticillata* (L.f.) Royle, and *Utricularia* sp. (Table 1: "H" and "U", respectively). The number of species observed in the *Hydrilla* and *Utricularia* samples is similar (13 and 14 species, respectively), but only five species are shared. On the *Hydrilla*, rotifers were almost exclusively present on the underside of leaves, and, in many cases (e.g., *Floscularia armata* Segers, 1997) and *Limnias ceratophylli* Schrank, 1803) present as pseudocolonies of up to 5 (*F. armata*) or 20 (*L. ceratophylli*) individuals. On *Utricularia,* rotifers appeared on all parts of the plants, but the most abundant species were concentrated on the *Utricularia* traps, with *Ptygura beauchampi* (Edmondson, 1940) and *P. melicerta* Ehrenberg, 1832 var. *socialis* Weber, 1888 in mixed groups on the trap doors, and individual *Floscularia bifida* Segers, 1997 and *P. crystallina* (Ehrenberg, 1834) on the traps proper.

A total of 23 taxa were observed (Table 1). Of these nine are new records for the Oriental region, while two are being recorded for the second time ever. All are new records for the Cambodian fauna, a result of this fauna being notoriously understudied, as mentioned above.

DISCUSSION

Of the 23 taxa of epiphytic rotifer observed, five could not be positively identified. Three of these may represent undescribed species, and two more belong to a probable species complex (*B.* cf. *crucigera*) or could not be reliably identified. We refrain from formally describing and naming these species here, as we feel more observations are needed. However, we do present some remarks on their taxonomy and biogeography as follows:

Beauchampia cf. *crucigera* (Dutrochet, 1812) – (Figs 1-2) The Cambodian specimens have the autapomorphic and diagnostic feature of prolonged and stiff dorsal antenna of genus *Beauchampia*, however, the length of this dorsal antenna is relatively short when compared to literature records. Only DONNER (1954) records a specimen with a similarly short antenna, but that case concerns a young specimen, as can be judged from the very short tube it inhabits (less than trunk length). The Cambodian specimens are definitely adults, as they inhabit a well-developed tube containing an egg. The case calls for a review of the taxonomy of this purportedly monospecific, cosmopolitan and eurytopic taxon (KOSTE, 1978).

TABLE 1

List of rotifer taxa recorded

Beauchampia cf. *crucigera* (Dutrochet, 1812):	H[1, 6]
Collotheca cf. *ambigua* (Hudson, 1883):	U[6]
Collotheca campanulata (Dobie, 1849):	H, U[2, 6]
Collotheca ornata (Ehrenberg, 1832):	H, U[3, 6]
Collotheca tenuilobata (Anderson, 1889):	H, U[4, 6]
Collotheca trilobata (Collins, 1872):	U[6]
Floscularia armata Segers, 1997:	H[6]
Floscularia bifida Segers, 1997:	U
Lacinularia elliptica Shephard, 1897:	H[3, 6]
Lacinularia flosculosa (Müller, 1773):	U[6]
Limnias ceratophylli Schrank, 1803:	H, U[5, 6]
Octotrocha speciosa Thorpe, 1893:	H[6]
Pentatrocha gigantea Segers & Shiel, 2008:	U
Ptygura barbata Edmondson, 1939:	H
Ptygura beauchampi (Edmondson, 1940):	U
Ptygura crystallina (Ehrenberg, 1834):	H, U
Ptygura melicerta Ehrenberg, 1832 var. *socialis* Weber, 1888:	U[6]
Ptygura sp. near *linguata* Edmondson, 1939:	H
Ptygura sp. near *melicerta* Ehrenberg, 1832:	H
Ptygura pedunculata Edmondson, 1939:	H[6]
Ptygura pilula (Cubitt, 1878):	H
Sinantherina socialis (Linnaeus, 1758):	U[5, 6]
Sinantherina sp. near *triglandularis* Arora, 1963:	U

[1] recorded from Thailand by KOSTE (1975) and HECKMANN (1979),
[2] by SANOAMUANG et al. (1995),
[3] by HECKMANN (1979),
[4] by SANOAMUANG & SAVATENALINTON (2001),
[5] by KOSTE (1975);
[6] recorded from China (ZHUGE et al., 1998)
H: on *Hydrilla verticillata* (L.f.) Royle, U: on *Utricularia* sp.

Collotheca cf. *ambigua* (Hudson, 1883) – We observed one specimen that matched the diagnosis of *C. ambigua*. A ring-shaped stiffening on the basis of the foot, diagnostic for the related *C. ferox* Pénard, 1914 was not observed but this may follow from the difficulty of examining living specimens attached to a substratum.

Floscularia bifida Segers, 1997 – The Cambodian material matches the diagnosis of the species in all aspects (e.g., trophi morphology, long attachment stalk), albeit that the shape of the species-specific pair of bifid dorsal hooks differs slightly from previously described material. This feature is, however, quite variable in the related *F. armata*, which was also observed during this survey and, more recently, from Thuy Tien Lake, Hue city, Vietnam (7 March 2010). The present records are the second and third ever of the species, after its description from South America (Brazil: SEGERS, 1997).

Octotrocha speciosa Thorpe, 1893 – (Figs 3-7). Several specimens of this remarkable species were found attached to *Hydrilla*. When comparing the material with published records of the species, we noted that its trophi morphology matches the original description by THORPE (1893), but is at variance with all subsequent illustrated records, including EDMONDSON (1959), KOSTE (1974; 1978; 1989), SARMA & ELIAS-GUTIEREZ (1998), and SEGERS & SHIEL (2008). In particular, the differentiation of

the unci teeth of the *O. speciosa* we identified here is much less pronounced than reported in these sources. In addition, the corona of the present material again matches the description by THORPE (1893), but differs from that of the material seen by the senior author (SEGERS & SHIEL, 2008) in having more numerous and more developed corona loops. We at present believe that the species reported by the recent authors cited above, as well as that identified as *O. speciosa* by Myers (see JERSABEK et al., 2003) belong to a different species. This would explain how EDMONDSON (1959) and KOSTE (1989) came to question the accuracy of THORPE's (1893) original description. *Octotrocha speciosa*, as recognized here, is known to us from China (THORPE, 1893), and was seen in abundance, but always solitary, in a lake in Southern Thailand (Thale Noi Lake, Phatthalung province, Southern Thailand: P. MEKSUWAN, unpublished) and Vietnam (Thuy Tien Lake, near Hue City, Central Vietnam, January 2009 and March 2010: H. SEGERS, unpublished), and also Cambodia. These observations may imply that the species is restricted to China and Southeast Asia, rather than being widespread (KOSTE, 1978).

Pentatrocha gigantea Segers & Shiel, 2008 – (Fig. 8). We observed several specimens of this unmistakable but only recently described species. The present record expands the known range of the species beyond its type locality in Australia (SEGERS & SHIEL, 2008) and into the Oriental region.

Ptygura sp. near *linguata* Edmondson, 1939 – (Figs 9-12). At first glance, the species belongs to the *P. brachiata* (Hudson in HUDSON & GOSSE, 1886) group, by its elongate lateral antennae and by the presence of a pair of sharp dorsal hooks. The presence of a tongue-shaped process in its buccal region places it closest to *P. linguata* Edmondson, 1939. However, the different corona, buccal process carrying a group of long, immobile cilia, variable peduncle and of the dorsal antenna being situated on a rather prominent, rounded rise anterior and between the dorsal hooks, distinguishes it from that species. It could also be mistaken for *P. brachiata*, on account of the relatively strong rods supporting the corona. The latter species has a short peduncle, and lacks a process in the buccal region (see Table 2) (note: EDMONDSON's (1944) "*P. longicornis* var. *bispicata*" is not implied here as there exists no formal description of the taxon). We at present consider the material to belong to a possibly undescribed species of the *P. brachiata* group, closest to *P. linguata*.

Ptygura sp. near *melicerta* Ehrenberg, 1832 – A single specimen of this species had installed itself in the mucous tube of an *Octotrocha speciosa*. The animal clearly belongs to the *P. melicerta* complex, by its relatively small corona (only ca. 1.5 times the width of the trunk) and presence of diagnostic dorsal spines. However, the latter are unlike any of the known members of the complex (see KOSTE, 1978): in the present animal, there is a pair of quite minute spines, which have a low basis and are pointed dorsally rather than ventrally as in *P. melicerta*. In addition, it has a particularly long foot (ca. 4-5 times the trunk length). Again, we are unable to identify this animal as any described species. The taxonomy of the *P. melicerta* complex is particularly confused, especially, the status of the taxa that are presently considered as of infrasubspecific rank, and is in need of revision (see SEGERS & SHIEL, 2008). Considering this, it is noteworthy that the variety *socialis*, which was also observed in one of the present samples, had, to date not been recorded from the Oriental region.

Sinantherina sp. near *triglandularis* Arora, 1963 – (Figs 13-14). We found a single specimen of a *Sinantherina* that eluded identification. It most closely matches the general shape of *S. triglandularis* by the foot being relatively short, giving the impression as if the ovifer is situated basally, but we were unable to assess the presence of the three pairs of gastric glands that are purportedly diagnostic for the species (ARORA, 1963). In addition, the corona of the Cambodian specimen is much larger than reported for *S. triglandularis*. The basal position of the ovifer is further shared with *S. spinosa* (Thorpe, 1893), but that species has prominent ventral spines, which are absent in our material. We tentatively identify the species as an undescribed one, close to *S. triglandularis*.

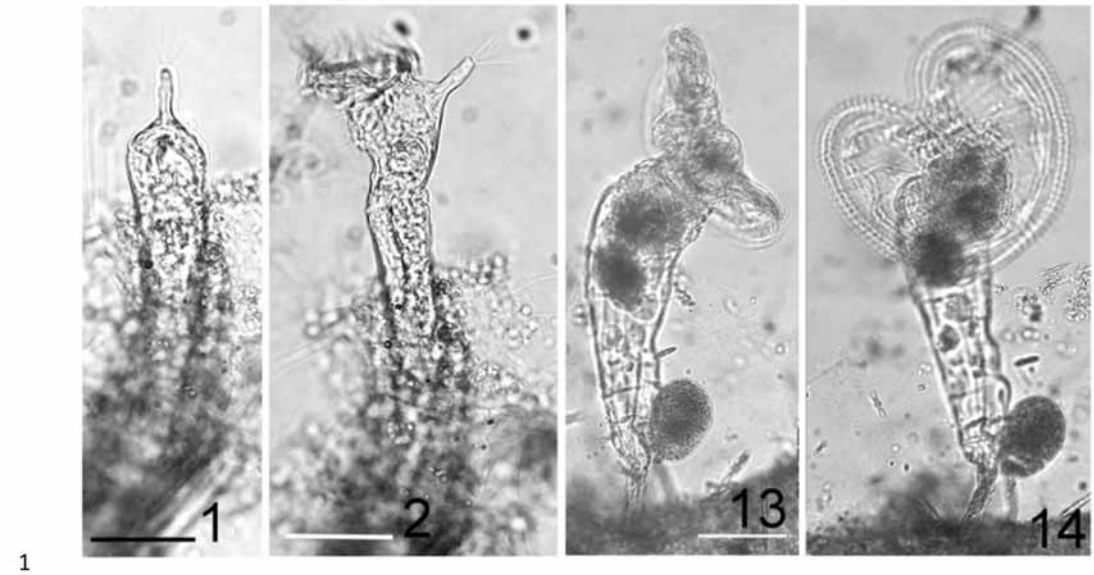
TABLE 2

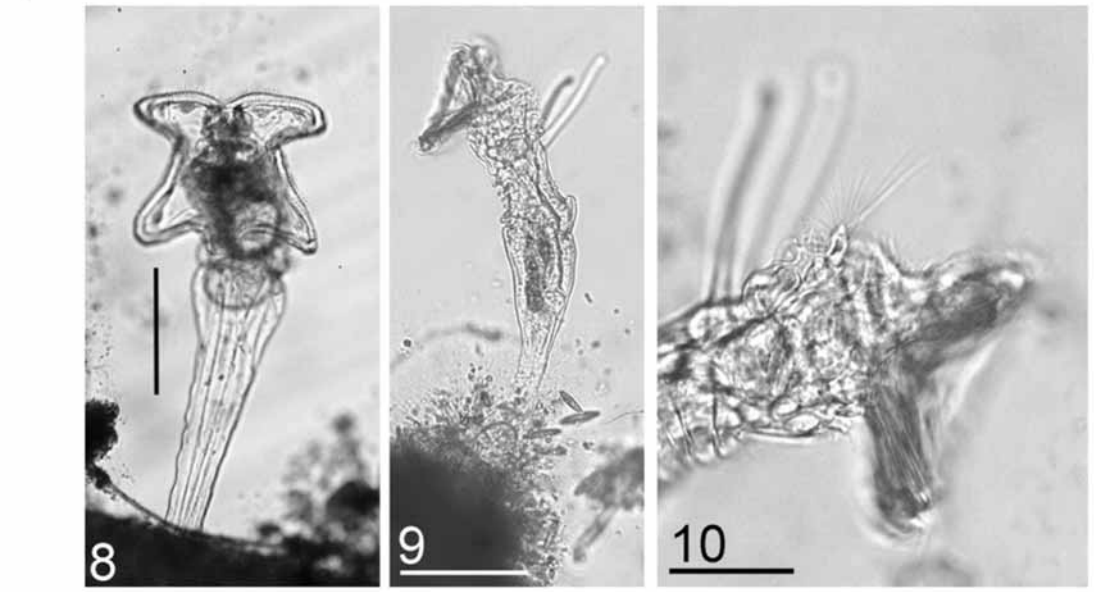
Comparison of *P. brachiata, P. linguata* and *P.* cf. *linguata*.

	P. brachiata*	***P. linguata*****	***P.* cf. *linguata***
Attachment stalk	Present, short	Present, elongated	Present, variable in length (age-dependent?)
Dorsal hooks	Two, hooked	Two, large, with opposed points, containing a pair-shaped gland	Two, sharp, with sizeable projection in between hooks
Corona	Bilobed, with ventral concavity; dorsal gap wider than the ventral; supported by strong rods	Bilobed, dorsal gap narrow, ventral notch deep, wide	Bilobed, dorsal gap narrow, ventral notch much deeper; corona supported by rods
Tube	Gelatinous, incorporating diatom cells (cultured animals!)	Gelatinous, laminated horizontally; dense inner sheath	Gelatinous, laminated horizontally; walls relatively thick
Additional diagnostic features	Lateral antennas with swollen bases	Lateral antennas extremely long; buccal area with cylindrical tongue-like process covered with short ciliae; pair of small conical projections in neck region	Buccal area with short, cylindrical process covered with elongate ciliae

* Based on the original description by HUDSON & GOSSE (1886); KOSTE's (1970, 1978) report does not correspond with HUDSON & GOSSE's (1886) in several aspects and is therefore not retained as representative for this species.

** After EDMONDSON (1939)





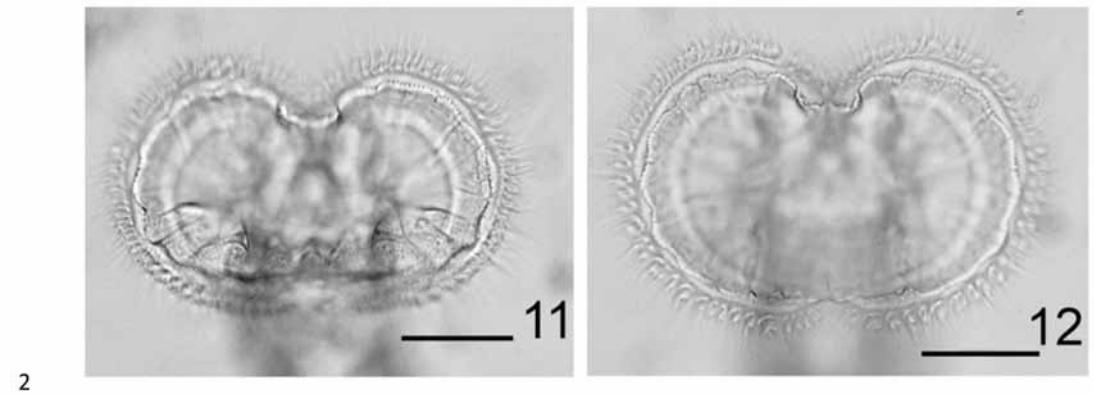


Fig. 1-2. – *Beauchampia* cf. *crucigera*. 1: ventral view, corona retracted; 2: lateral view, corona extended. Scale bars: 20µm.
Fig. 8. – *Pentatrocha gigantea*, habitus. Scale bar: 250µm.
Fig. 9-12. – *Ptygura* sp. near *linguata*. 9: habitus, lateral view; 10: head and corona, lateral view (note the ciliated projection in the mouth region); 11, 12: corona, frontal view. Scale bars: 50µm (Fig. 9), 20µm (Figs 10-12).
Fig. 13-14. – *Sinantherina* sp. near *triglandularis*, habitus. 13: lateral view, 14 dorsal view. Scale bars: 100µm.

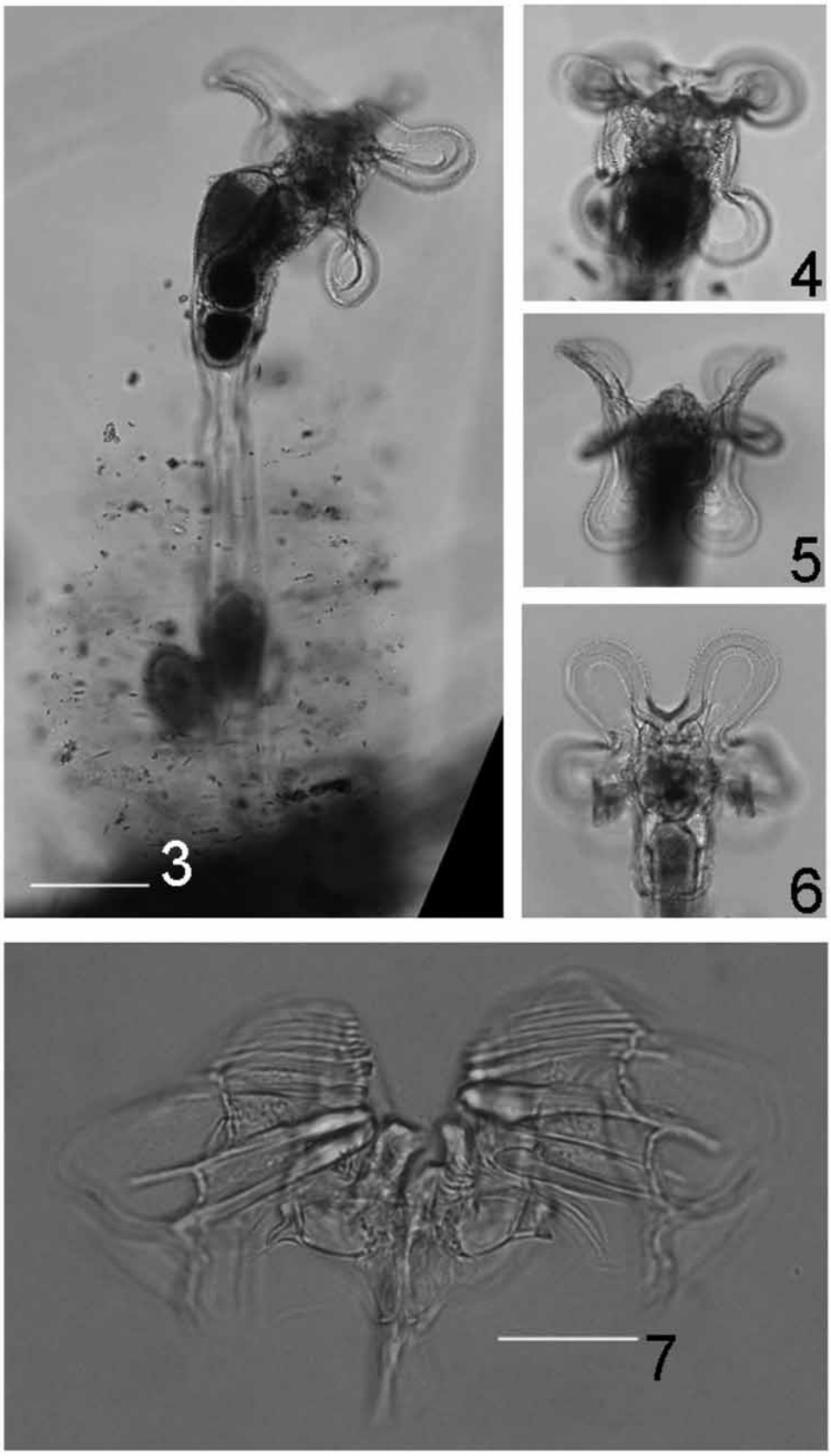


Fig. 3.7 – *Octotrocha speciosa.* 3: habitus, lateral view; 4-6: corona, different views; 7: trophi, frontal view (Thai specimens) Scale bars: 3: 250µm, 7: 20µm.

CONCLUSIONS

As mentioned before, all of the species recorded here are new to the records of Cambodian fauna, and several represent taxonomic novelties. The significance of this is questionable, as this fauna is notoriously understudied. A more meaningful comparison could be made with the much better known Thai fauna, but even so only seven of the species we report here have been recorded from that country (see Table 1). A comparison with the relatively well-known Chinese fauna (ZHUGE et al., 1998) reveals that 11, or nearly half of the taxa in our samples, have not been recorded from China. This once again illustrates the lack of information and comparative data on the diversity and biogeography of the rotifers, and in particular of the sessile ones, of Southeast Asia.

ACKNOWLEDGEMENTS

The authors wish to thank Dr R.L. Wallace (Ripon College, Ripon, WI, USA) and three anonymous reviewers for their comments and suggestions on this paper. The trip to Cambodia was made possible by the Center of Applied Taxonomy of Khon Kaen University, Khon Kaen, Thailand. The Vietnamese material mentioned was observed during a training workshop on rotifer taxonomy held in the Center for Coastal Management and Development Studies of Hue University, Hue, Vietnam, sponsored by the Belgian Development Cooperation through the Belgian Focal Point to the Global Taxonomy Initiative.

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Received: April 23, 2010
Accepted: October 16, 2010
Branch editor: Artois Tom

Belg. J. Zool., 140 (2) : 241-243 July 2010

SHORT NOTES

Derivation of variance estimators and statistical inference for indices of sexual size dimorphism: an example

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KEY WORDS: statistical inference, indices, Delta method

Null-hypothesis significance testing is the primary, but not only, tool for sexual dimorphism research (1). A large variety of different indices have been developed for quantifying sexual dimorphism. Statistical tests offer better technique and a mathematically more grounded decision-making algorithm to test differences in size between the two sexes, but indices have advantages as well. Often it is easier to have a single numerical value to express the phenomenon under study. Index outcomes are interpretable as scalar expressions, and similar values always indicate similar phenomena, facilitating comparisons between studies.

One of the most frequently used sexual dimorphism indices was introduced by LOVICH & GIBBONS (2). This ratio index is rather intuitive and easy to interpret

$$SDI = \frac{\theta_x}{\theta_y} - 1$$

where θ_x represents the size parameter for the larger sex (x) and θ_y is the size parameter for the smaller sex (y). Simply put, this is size of the larger sex divided by size of the smaller sex. In order to centre the ratio around zero, one is subtracted. Usually size is quantified by sample mean or by 80th percentile. The outcome of the index is arbitrarily expressed as positive if females are larger and negative if males are larger. This index is widely used although scientists concentrate only on its calculated value without paying attention to its variability. Regardless of the method of size quantification, this SDI is a product of two random variables and therefore should itself be regarded as a random variable.

The following rather simple example will illustrate the necessity of considering the index's variance. A hypothetical study aims to quantify sexual dimorphism of a bird species that inhabits two connected forests. In the first forest 100 birds were measured, 50 females averaging 22cm, and 50 males averaging 20cm. In the second forest, due to more extreme conditions and time constraints, the researcher managed to measure only 24 birds: 20 females averaging 21cm, and only 4 males also averaging 21cm. Analyzing the collected data in the first forest the researchers observe a sexual dimorphism (SDI) of 0.1 while in the second forest the sexes exhibit no dimorphism at all, SDI_2=0. As the two populations intermix, the researcher would expect the same degree of dimorphism. As the sample sizes are not equal and the sample size for the second forest is quite low, intuition leads the scientist to disregard the second forest data and only trust the index from the first. A keener look at the data set shows that the male measurements (27, 22, 19 and 14cm) from the second forest are not just too few but also have high variability and consequently lower estimation precision. This example shows the problem of incorporating not only sample sizes but also the variability in the index, and the need to consider not only the magnitude and sign of the index but also the level of confidence that the estimate has. One way to address this would be to develop Lovich & Gibbons's index to incorporate these parameters. This has serious disadvantages as it would be very difficult to incorporate sample size and variability but still preserve the intuitive simplicity and ease of interpretation. The alternative approach set forth here is to present the index with a statistic of dispersion e.g. $SDI \pm 1SD$. In the following, the mathematical formula is derived for the SDI variance and the performance of the estimator is evaluated. Through a concrete example the advantages of the improved index are demonstrated.

Calculating exact variance for the Lovich & Gibbons index is difficult, although approximation techniques can be applied. An approximation commonly used is the Delta method (3), which is a procedure to find approximate mean and variance of a nonlinear combination of random variables. The delta method takes a function too complex for analytical derivation of the variance, creates a linear approximation of that function, then estimates the variance of this simpler linear function that can be used for statistical inference. The Delta method expands a function of a random variable about its mean, usually with a one-step Taylor approximation. If a function $f(x)$ has derivatives of order k, then for a constant a the Taylor series of order k about a is

$$T_n(x) = \sum_{j=0}^{k} \frac{f^{(j)}(a)}{j!}(x-a)^j$$

Statistical applications of a Taylor series are concerned with first order terms, $f(x) \approx f(\mu) + f'(\mu)(t - \mu)$. Taking expectation on both sides leads to $f(\mu)$. We can also approximate the variance of $f(x)$ by $Var[f(x_i)] \approx E[f(x_i) - f(\mu)]^2$.

Solving this equation leads to the Delta approximation for the variance

$$\sigma_{\varsigma}^2 = \sum_{i=1}^{n} \left(\frac{\partial g}{\partial x_i} |_{\mu_i} \right)^2 \sigma_i^2$$

A particularly useful characteristic of the Delta method is the convergence in distribution. For a given function $f(x)$ with existing first order derivative

$$\sqrt{n}[f(x) - f(\mu)] \to N(0, \sigma^2[f'(\mu)]^2) ,$$

that is a normal distribution with mean zero and variance $\sigma^2[f'(\mu)]^2$ (4).

The Delta method for the Lovich & Gibbons index leads to the following variance equation

$$\sigma_{SDI}^2 = \frac{\theta_y^2 \sigma_x^2 + \theta_x^2 \sigma_y^2}{\theta_y^4}$$

Here θ represents the size parameter, σ_x^2 the variance for the larger sex and σ_y^2 variance for the smaller sex. The variance of both sexes can be estimated from the data

$$\sigma_x^2 = \frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2$$

Since size is quantified as the population average, the variance will be

$$\sigma_{\bar{x}}^2 = \frac{\sigma_x^2}{n}$$

The variance equation derived by the delta method is just one of all theoretically possible estimators. Most likely estimators derived by approximate methods will not be unbiased ones. Bootstrap simulations demonstrate that the bias of the derived indices is negligible (Table 1) and genuinely small. The sign of the bias has no specific pattern, thus there are no systematic errors, and no corrective applications are recommended.

TABLE 1

Bias estimation of the proposed variance index by Monte-Carlo simulation. The two samples are normally distributed with noted mean and standard deviation and different sample sizes (n and m).

Sample 1	**Sample 2**	**Bias**			
$\bar{X}$**+1*SD***	$\bar{X}$**+1*SD***	**n=5, m=75**	**n=25, m=75**	**n=50, m=75**	**n=75, m=75**
25±15	20±10	-0.0037	-0.0022	-0.0011	0.0011
25±2.5	20±1.2	0.0019	-0.0014	-0.0021	-0.0014
21±15	20±10	-0.0018	-0.0020	0.0016	0.0012
21±1.8	20±1.6	-0.0012	-0.0013	-0.0005	0.0016

Pelobates fuscus and *Bobina variageta* are two relatively widespread European toad species with pronounced sexual size dimorphism in the former and less pronounced dimorphism in the latter. Here we analyse and test their sexual dimorphism in snout-vent length using Lovich & Gibbons` SDI based on measurements from two central European populations.

Males of *B. variegata* averaged 40.81mm (±1SD:4.14) (n=44) while females averaged 40.75mm (±1SD:4.92) (n=78). The estimated dimorphism index SDI=-0.0012, while its standard deviation is 0.0209. The bias is very small 0.0001, being 26 times smaller than the estimated variance. Although it is obvious that in this case there is no sexual size dimorphism, we will use the calculated SDI and its standard deviation to demonstrate the construction of confidence intervals (CI hereafter) and null hypothesis test of the index. As a standard method we follow the normal distribution approximation

$$P\left(\widehat{SDI} - z_{\alpha/2} \sqrt{Var(\widehat{SDI})} \le SDI \le \widehat{SDI} + z_{\alpha/2} \sqrt{Var(\widehat{SDI})} \right) = 1 - \alpha$$

where z is the normal distribution quantile, while α is the desired significance level. $\widehat{SDI}$ is the estimated sexual dimorphism and *SDI* is the true population value. Generally α=0.05 significance level is used, leading to z=1.96. Using the above described formula, a 95% CI from -0.039 to 0.042 is obtained. As the CI contains zero, one can be confident that the SDI for *B. variegata* does not differ significantly from zero.

A Monte Carlo simulation based on the *B. variegata* data was run to validate the build confidence interval against the generally applied computer intensive methods. A total of 1000 simulations were run. We tested the coverage probability of the build confidence interval.

Given a nominal coverage of 95% and 1000 simulations we expected that the estimated coverage level would fall in the interval 93.64 to 96.35 (5).Any value below or above the given bounds is an indication of systematic under- or over-coverage. The observed coverage probability (94.6%) of the built approximate confidence interval was slightly lower than the nominal 95% but well within the acceptance limits. As a comparison a 95% percentile bootstrap confidence interval (6) based on 1000 re-samplings resulted in a coverage probability of 94.3%. The width of the bootstrap confidence interval was slightly lower, 0.0793, than the width of the confidence interval based on our approximation, 0.0802. When comparing confidence intervals based on different methods (e.g. approximate against bootstrap intervals) we wish to choose the one with coverage probability closest to the nominal value. Between two confidence intervals, given an optimal or close to optimal coverage probability, we choose the narrowest. In our case, the proposed confidence interval and the one based on bootstrapping show no genuine difference either in coverage probability or width. Consequently, we postulate that the derived approximate confidence interval offers a valid tool as good as bootstrapping for researchers aiming to draw inference about the observed and quantified sexual dimorphism.

The probability equation for the CI easily can be transformed to a formal significance test formula

$$z = \frac{\widehat{SDI} - SDI_{H_0}}{\sqrt{Var(\widehat{SDI})}}$$

where z is the standard normal quantile and $\widehat{SDI}$ the estimated sexual dimorphism while *SDI* is the assumed null hypothesis value. The P-value associated with the observed z-score can be obtained from standard normal

distribution tables, or even better, from statistical software (e.g. R). In the case of *B. variegata* there is no significant difference in SDI from zero (z=0.061, P=0.950). As a comparison, a classical Student t-test for equality of means would give t=0.059 with the associated P-value of 0.953.

The measured specimens of female *P. fuscus* (n=104) averaged 63.14±4.76mm while males averaged 55.22±3.54mm (n=185). The estimated sexual dimorphism is 0.143±0.01 with a narrow 95% CI (-0.163, -0.123). The variance's bias is -0.00002, 36 times smaller than the estimated variance. The estimated SDI differs significantly from zero (z=14.28, P<0.0001). A Student t-test for equality of means also evidenced a similar result (t=16.05, P<0.0001).

It is possible to compare the difference in sexual dimorphism between the two species using the following equation

$$z = \frac{\widehat{SDI}_i - \widehat{SDI}_j}{\sqrt{\widehat{Var}_i(SDI) + \widehat{Var}_j(SDI)}}$$

The test statistic z has a standard normal distribution.

Comparisons made between *B. variegata* and *P. fuscus* evidenced that the magnitude of SDI is significantly larger for the latter species (z=-4.66, P<0.0001).

Since its introduction, the SDI index has consistently proved its value. With the improvements presented in this note SDI can function as a more nuanced and flexible tool. Flexibility is gained by facilitating comparison not only with a hypothetical value, but also between studies or even organisms, if needed. It also needs to be emphasized that the variability of all biological indices should be considered. The methodology used here can be applied successfully to find and assess variance estimators of biological indexes. Computer intensive methods such as bootstrapping could be employed easily both for variance estimation and statistical inference. However the Delta method offers an easy tool that can be applied to collected data directly and also data gathered from the literature. Here we chose to use the Lovich & Gibbons index, however the methodology outlined here can be used for any index of sexual size dimorphism.

ACKNOWLEDGEMENTS

We would like to thank Robin Biddulph, Kinga Öllerer and two anonymous reviewers for advice and comments on the manuscript.

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Received: January 2, 2008
Accepted: July 15, 2010
Branch editor: Adriaens Dominique

Belg. J. Zool., 140 (2) : 244-245 July 2010

First record of *Synurella ambulans* (Müller 1846) (Amphipoda: Crangonictidae) in Belgium

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KEY WORDS: exotic species, Ponto-Caspian, Vloethemveld

Macroinvertebrates are one of the groups that are monitored to assess biological water quality. In Flanders (Belgium), they have been sampled during the past 20 years by the Flemish Environment Agency. As a consequence, a large collection of more than 20,000 samples is currently available. More than 2,500 samples containing crustaceans, from the period 1989-2008 and distributed all over Flanders, were investigated at the Laboratory of Environmental Toxicology and Aquatic Ecology (Ghent University) in the scope of an ongoing PhD related to the spread and impact of exotic macro-crustaceans in Flanders. Analysis of these samples revealed that *Synurella ambulans* (Müller 1846) was sampled for the first time in Belgium in 2003 and again in 2004. However, because identifications were previously limited to the family level, the species remained unnoticed until 2009. These samples with *S. ambulans* from 2003 and 2004 were taken by handnet, as described by Gabriels et al. (1) by the Flemish Environment Agency in three places in Snellegem (province Western Flanders). Two of the sampling locations were situated in a small stream (Walbeek) in Vloethemveld, which is in a former military domain that is currently classified as a nature reserve. The third location was in a stream (Kastanjebeek) directly connected to the first one. Both streams run dry during summer and contain dense vegetation in the riverbed. General characteristics of the water are a circumneutral pH (7.0-7.5), a low conductivity (310-395µS/cm) and a high oxygen concentration (7.3-9.1mg/L). The ecological water quality of both streams is moderate, with a MMIF of 0.55 (Multimetric Macroinvertebrate Index Flanders, (1)). Some taxa sensitive to pollution, such as Trichoptera and Plecoptera, were also present in the samples. In total, the three samples contained 96 individuals (adults and juveniles) of *S. ambulans*. Two of the samples were collected at the beginning of May and one sample at the end of April. Several females of *S. ambulans* carried eggs and 18 juveniles were sampled, indicating that *S. ambulans* reproduces in these streams. It is unclear how *S. ambulans* reached this location. One of the possible avenues is anthropogenic introduction via fish ponds, which are situated nearby.

S. ambulans can be found in freshwater habitats in lowland as well as highland rivers, in small ponds, swamps, temporary pools and small streams (2). Moreover, subterranean populations of *S. ambulans* have been recorded (3). Common characteristics of all colonized habitats are a low flow velocity and a stable water temperature, which is often influenced by the inflow of groundwater. A typical biotic habitat characteristic is the presence of dead plant material. As observed and confirmed by others, this species can be found in surface waters that periodically run dry (4; 5). Although Arbačiauskas (5) hypothesized that *S. ambulans* has some type of diapause stage, enabling the species to survive in seasonal water bodies, a diapauses stage may not be necessary, since many streams with dry periods have an understream all year long that may act as a refuge.

S. ambulans is an amphipod species which is native to the Ponto-Caspian region and Central and Eastern-Europe (4-8). Until now, this species has only been discovered at a few isolated places in Germany, Italy and Switzerland outside its natural range (3; 9). Despite the wide distribution of *S. ambulans*, the species rarely occurs in large numbers and usually only a few individuals are encountered (6).

The holarctic genus *Synurella* belongs to the family Crangonictidae. The genus *Synurella* consists of 18 species, 14 of which occur in Europe and Asia Minor, three in the southeast of the USA, two in northeastern Siberia and one species in Alaska (10; 11). *S. ambulans* is characterized by spot-shaped eyes, consisting of different ommatidia, a yellow spot at the apex of the head, fused urosome segments and a distinguishable third pair of uropods (12) (Fig. 1). The sex can be determined based on the presence of sternal papillae on segment seven of the pereonite in males and the presence of oostegites in females (6). The maximum size of females and males was found to be 7.0mm and 4.7mm, respectively (6). In the Belgian samples, the maximum size was 8.8mm for females and 5.3mm for males, with an average of 6.7mm (females) and 4.5mm (males), respectively, which is comparable to the published size variation (6). In Belgium, the species can only be confused with *Crangonyx pseudogracilis* Bousfield 1958, which originates from North America but has recently invaded Europe (13). However, the urosome segments of *C. pseudogracilis* are not fused. Research carried out by Konopacka & Blazewicz-Paszkowycz (6) about the life history of *S. ambulans* in Poland indicated that females were more abundant than males with seasonal changes in sex ratio throughout the year. In our samples, males were more abundant than females with a sex ratio of 1:0.7. This difference could be due to the relatively limited number of individuals that were encountered in Belgium. Moreover, seasonal changes in sex ratio could not be investigated since all samples were taken in spring. It has been reported that *S. ambulans* has an iteroparous, univoltine life cycle with a reproductive peak from May until July and a sexual maturation of the new generation in March of the consecutive year (6). The two samples collected in May contained juveniles and

gravid females. As a consequence, it can be concluded that the reproductive cycle of the Belgian specimens is similar to the one found in their native area.

Little is known about the feeding habits and the impact of this species on other macroinvertebrates. Therefore, additional research is needed to assess the impact of this new Ponto-Caspian invader on indigenous macroinvertebrate communities.

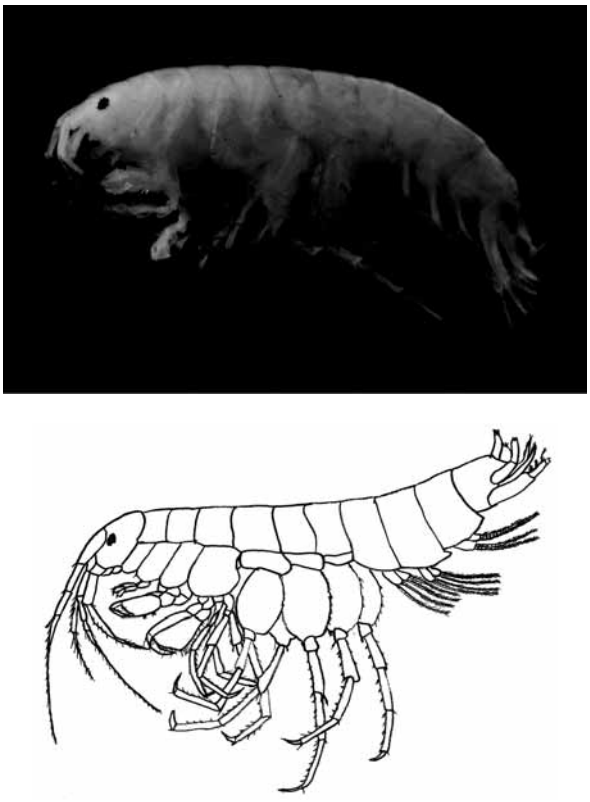

Fig. 1. – Photograph (above) and drawing (below) of *Synurella ambulans*.

ACKNOWLEDGEMENT

We would like to thank the Flemish Environment Agency for the opportunity to study their samples. Koen Lock is currently supported by a post-doctoral fellowship from the Fund for Scientific Research (FWO-Vlaanderen, Belgium).

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Received: October 9, 2009
Accepted: March 10, 2010
Branch editor: Schön Isa

Belg. J. Zool., 140 (2) : 246-248 July 2010

On the mechanical stability of the air volume trapped within the diving bell of the water spider *Argyroneta aquatica* (Araneae;Cybaeidae); a thermodynamic analysis based on a model

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KEY WORDS: *Argyroneta aquatica*, diving bell

In this contribution it is shown that the effective surface property of silk threads used by the spider *Argyroneta aquatica* to produce a diving bell in which air is trapped in a mechanically stable state has to be hydrophilic. The arguments leading to this conclusion are based on a model. This approach is similar to the one followed by Crisp and Thrope who analysed the water-protecting property of insect hairs (1).

The water spider *Argyroneta aquatica* has several adaptations to its aquatic life. Its systematic status is still uncertain (2). In Germany, *A. aquatica* is an endangered species. The results reported here can contribute to conservation efforts for this species because the stability of the air trapped in the diving bell can be influenced by the absorption of surfactant molecules on the surface of the silk threads forming the diving bell. Such surfactants are most likely present in polluted water of the aquatic habitat of this spider.

In a recent publication DE BAKKER et al. reported on the structure of silk threads produced by the water spider *A. aquatica* (2). It uses silk threads to build its diving bell, which functions as an air reservoire. DE BAKKER et al. noticed "that a kind of film was drapped over and woven between the strands. It is not certain whether this film is produced by the spider (serving probably as a water repellent layer) or deposited by other aquatic organisms." It is the primary aim of this study here to charaterize the effective surface property of the silk using a theoretical approach on which future experiments can be based. Futhermore, the results of this study contribute to the knowledge of surface functions in biology, a subject that is currently attracting considerable interest in botany, zoology, and biophysics (see the recent review (3)).

The analysis of the mechanical stability of an air volume trapped within the diving bell of *A. aquatica* is based on the law of Laplace in the form of equation(1),(4). It is assumed that the meshes of the diving bell are circular with the same radius.

$$P_e^w(x) = \rho_w \cdot g \cdot x = \left[P^w(x) - P^a(x)\right]_{\cdot e} = -\frac{2 \cdot \gamma_{a/w} \cdot \cos(\theta_e)}{r_m} \quad (1)$$

Whereby P is the hydrostatic pressure, ρ_w the density of water, g the gravitational constant, x a space coordinate (see Fig. 1), r_m the radius of a circular mesh and θ_e the effective contact angle at the line of contact between air, water and silk. By definition the contact angle θ_e is measured across the liquid. The subscript e refers to mechanical equilibrium state. The term "effective contact angle" takes into account the possibility that the silk threads carry an adsorbed layer that can give a surface a property different from that of pure silk. The parameter $\gamma_{a/w}$ is the air/water surface tension.

The surface tension can have positive values only (e.g. $\gamma_{a/w} > 0$). This is due the fact that a differentially small amount of mechanical work δW (> 0) has to be done on a constant mass of water to increase its air/water surface A by a differentially small amount δA (> 0): at constant temperature: $\delta W = \gamma_{a/w}\, \delta A$. Mechanical work has to be done to bring a water molecule from the interior of the liquid to its surface to increase the surface area between air and water. This requires a certain amount of energy (mechanical work). Therefore a molecule located in the surface of a liquid in contact a gas is in a state of higher energy compared to a molecules in the bulk. (Mechanical work is the amount of energy transferred by a force through a distance.)

In the present context, the term "mechanical equilibrium" means that the air trapped in the diving bell remains trapped even when the (hydrostatic) pressure $P^w(x)$ and the pressure difference $[P^w\text{-}P^a]$ change slightly, caused by external disturbances (fluctuations). In mechanical equilibrium, the value of the term $[P^w(x)\text{-}P^a(x)]_e$ on the left hand side of equation (1) is equal to the value of the term

$$\left[-\frac{2 \cdot \gamma_{a/w} \cdot \cos(\theta_e)}{r_m} \right]$$

on the right hand side.

Figure 1 shows a schematic drawing of a vertical cut across a few cubic centimetres of air enclosed in a net-like structure submerged in a container filled with water. The net enclosing the air is shown as a curved, dashed line in this figure. The distances between the dashes indicate the positions of the curved air/water surfaces bordered by the silk threads that form the diving bell. The net carries at its bottom a large opening where the spider can enter or leave the interior of the diving bell. The diameter of this opening is so large that the air/water surface there can be considered as flat. The position of the entire net is fixed by additional threads. It is not taken into account here that the threads forming the diving bell could consist of more than one type of thread as suggested by DE BAKKER et al. (2).

It can be concluded from equation (1), that the sign of the pressure difference $[P^w(x)\text{-}P^a(x)]_e$ across the bulged air/water surface bordered by silk threads is determined by the sign of the term $[\cos \theta_e]$. If the value

of the contact angle θ_e along the line of contact of air, water and silk is found to be smaller than 90° i.e. ($\cos(\theta_e) > 0$), the effective surface property of the silk is, by definition, called hydrophilic. Consequently (see equation (1)), the pressure of the trapped air in the diving bell has to be larger than the pressure in the water surrounding the diving bell at the same space coordinate x (i.e. $P^a{}_e(x) > P^w{}_e(x)$). If the value of the contact angle θ_e along the line of contact between air, water and silk is larger than 90° (i.e. $\cos(\theta_e < 0)$, the effective surface property of the silk is, by definition, called hydrophobic. Consequently (see equation (1)), the pressure in the water surrounding the diving bell has to be higher than the pressure of the air trapped in the diving bell at the same space coordinate x (i.e. $P^w{}_e(x) > P^a{}_e(x)$).

It will turn out (see below) that along the path $x_1 \rightarrow x_2$ (see Fig. 1) the pressure of the air trapped in the diving bell is higher than that in the water surrounding the diving bell (i.e. $P^a{}_e x) > P^w{}_e(x)$). If this fact is taken into account in equation (1), it must be concluded that the effective surface property of the silk threads forming the diving bell of *A. aquatica* must be hydrophilic to keep the air trapped within the diving bell mechanically stable.

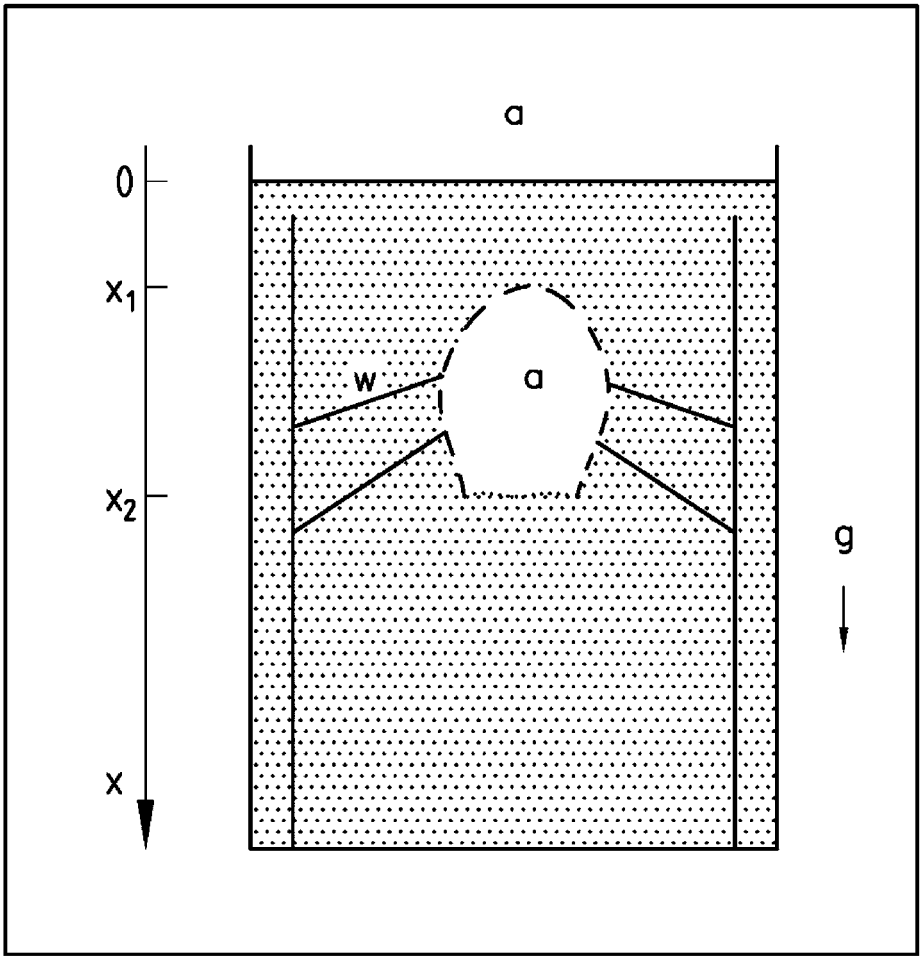

Fig. 1. – Schematic drawing of a vertical cut across a diving bell of the water spider *Argyroneta aquatica* (not drawn to scale). The contour of the bell is marked by a curved and dashed line. The unmarked distances between the dashes refer to the locations of the air/water surfaces bordered by silk threads. The bell is filled with a small, macroscopic volume of air. The diving bell is submerged in water. The position of the bell is stabilized against the force of buoyancy by threads. Abbreviations: *a*, air; *w,* water; *g*, standard gravity; *x*, space coordinate. The coordinate x_1 is the location of the highest point of the diving bell. x_2 is the location of a flat, macroscopic opening of the diving bell. It is the location at which the spider can enter or leave the interior of the diving bell. Figure 1 refers to equation (1).

What are the arguments leading to the statement that the pressure difference $[P^w(x)-P^a(x)]$ along the path $x_1 \rightarrow x_2$ has negative values? The values of the pressure P^w in the water surrounding the diving bell as a function of the space coordinate x increase linearly along this path: $P^w(x) = \rho_w g x$ (see Fig. 1). The value of the pressure P^a in the volume of air enclosed by the diving bell remains constant along this path: $P^a = P^w(x_2)$. This leads to the equation: $(P^w(x)-P^a(x_2)) = -\rho_w g (x_2 - x)$ (see Fig. 1). The location $x=x_2$ marks the point at which the spider can enter or leave the interior of the diving bell through a comparatively large opening in which the air/water surface is flat. The statement $P^a(x_2)=P^w(x_2)$ expresses the condition of mechanical equilibrium of water and air across a flat surface at constant temperature.

What conclusions can be drawn from this analysis on the mechanical stability of the air trapped within the diving bell of the water spider *A. aquatica* using the proposed model?

(1) A stable trapping of air volume in a diving bell formed by silk threads requires an effectively hydrophilic surface of the threads. The speculation of DE BAKKER et al. that a kind of film on silk forming the diving bell serves as a water repellent (hydrophobic) layer is not confirmed (2).

(2) At first sight, this finding may be unexpected. *A. aquatica* carries outside its diving bell a volume of air trapped on its abdomen under a layer of comparatively long hairs oriented parallel to each other. This air volume is completely covered by a layer of hairs without a (comparably) large opening. This (mobile) air volume acts as physical gill of the spider in a similar manner to the air volume enclosed in the diving bell. It is known that the surface of the hairs covering the trapped air on the abdomen is effectively hydrophobic (1),(5). It is also known that – outside the diving bell – the pressure of the trapped air on the abdomen has a value that is lower than that in the water surrounding the spider (1).

This suggests that the adaptation of *A. aquatica* to a life under water has led to the ability to produce materials in its body with different wetting properties depending on the function of the surfaces of the materials. The function of a diving bell requires an opening at its bottom. Its diameter has to be large enough to give the spider access to the interior of the bell. From a physical point of view, this has the consequence that the effective surface of the silk threads has to be effectively hydrophilic to trap air within the diving bell in a stable way. The pressure of the trapped air on the abdomen of the spider outside its diving bell is smaller than the pressure of the water surrounding the spider. From a physical point of view, this means that the surface of the hairs trapping the air on the abdomen of the spider has to be effectively hydrophobic. Thus, the chemical composition of the silk threads forming the diving bell can be expected to be different from the composition of the hairs on the abdomen (silk consisting of polypeptides while hairs consist of chitin/protein complexes).

(3) Equation (1) can be used to estimate the upper limit of the water pressure P_e^w at which the air volume, trapped within the diving bell of *A. aquatica* immersed in water, can be expected to be mechanically stable. The values of the parameters θ_e and r_m are necessary for this estimation. The choice of values of the contact angle can only be based on an "educated guess" because no experimental data for θ_e could be found: $\theta_e<90^o$, $\theta_e=60^o$ $\theta_e=80^o$. For the mesh radius r_m of the diving bell values between $1\mu m \leq r_m \leq 5\mu m$ are used. They are taken from published scanning electron microscope pictures (2). For the air/water surface tension $\gamma_{a/w}$ a value $\gamma_{a/w}=72.5\ 10^{-7}$ Jcm^2 (room temperature) is used, taken from the literature (6). The results of this estimation are compiled in Table 1. The range of the estimated values of P_e^w is in relative agreement with results of field studies (5).

(4) Scanning electron microscope pictures of sections of the diving bell show that the area of the air/water surfaces bordered by silk threads varies strongly, especially for the parameter r_m (2). On the basis of equation (1) it can be expected that the mesh with the largest diameter is the weakest point in the overall stability of the entire air volume trapped in the diving bell. The pressure difference $[P^a(x)- P^w(x)]$ has its largest value at the top of the diving bell ($x=x_1$). It vanishes at its bottom ($x=x_2$; $P^a(x_2)=P^w(x_2)$).

TABLE 1

Estimate of the upper limits of hydrostatic pressure P^w_e up to which a volume of air is trapped in a stable state within the diving bell of *A. aquatica.* The estimation is based on equation (1). It is assumed that the threads of the diving bell are made of material with hydrophilic surface properties. The parameters used for the calculation are: diameter of the mesh, $2\ r_m$; contact angle, θ_e 10^{-7} J=10^{-2}bar, corresponding to the height of a water column of 10cm.

$2\ r_m$ [μm]	P_e^w [10^{-3} Jcm^{-3}]	P_e^w [10^{-3} Jcm^{-3}]
	$\theta_e=80^o$	$\theta_e=60^o$
2	12.6	72.5
4	6.3	36.3
6	4.2	24.2
8	3.1	18.1
10	2.5	14.5

ACKNOWLEDGEMENTS

D. Neumann (Zoological Institute, University of Cologne/ Germany) drew the attention of D.W. to the water spider *Argyroneta aquatica.* D.W. thanks him for his encouragement and helpful discussions.

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Received: November 3, 2009
Accepted: May 12, 2010
Branch editor: Schön Isa