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BIOLOGICAL DATA FROM POST MORTEM ANALYSIS OF OTTERS IN HUNGARY

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In this paper we examined the characteristics of reproduction parameters, stomach content and inner organ weights on carcasses (male, n = 67, female n = 57, unknown n = 3) of otters (*Lutra lutra*) collected in Hungary between 1999 and 2006. Most otter carcasses (90.6%) were collected as road casualties. In breeding females (n = 28), the mean (±SE) number of placental scars was 2.22±0.17. One female was pregnant (3 embryos). Suckling were detected at four females, the number of active teats (2–4) equalling the number of placental scars. Reproduction period, calculated from the age of juveniles, was long (from winter to summer). No seasonal difference was found in the gonado-somatic index values of adult males, and births were estimated to have occurred from winter to summer. The stomach was empty in 31% of the cases, while mean weight of the stomach content was 49 g the main food eaten by otters was small-size, non-commercial fish. In the adult age group, the organ weight index of the kidneys ($P < 0.01$) and adrenal glands ($P < 0.05$) was greater in females, while in case of the other organs: heart, liver, spleen, tyroid glands and lungs no significant difference was found.

Key words: *Lutra lutra,* uterine horn, placental scar, testis, organ index, Hungary

INTRODUCTION

One possibility to monitor the populations of rare and hiding species such as the case with wild otters (*Lutra lutra* L.) is the *post mortem* examination of individuals found dead (SIMPSON 1997, REUTHER *et al*. 2000, SIMPSON 2000). In numerous parts of Europe or the world researchers (MASON & MACDONALD 1986, KRUUK 1995, SMIT *et al*. 1998, SHORE *et al*. 2000, HAUER *et al*. 2002*a*, ELMEROS *et al*. 2006) have already performed detailed post mortem analysis or examined the accumulation of different pollutants in otters. Initially, these studies were motivated to reveal the effects of industrial and agricultural contamination or catastrophes (e.g. BOWYER *et al*. 2003, RIDOUX *et al*. 2004), causing major environmental problems. More recently, monitoring the accumulation of toxic substances in the food chain (ROOS *et al*. 2001), long-term population trends (ELMEROS *et al.* 2006), threatening factors (KRUUK & CONROY 1991, SIMPSON 1997, HAUER *et al*. 2002*a*), determination of age composition (HAUER *et al*. 2002*b*), study of reproduction characteristics (ELMEROS & MADSEN 1999, HAUER *et al*. 2002*b*) and temporal population changes (e.g. age structure: HAUER *et al*. 2000) have become also important.

While still distributed country-wide in Hungary (HELTAI 2002, KEMENES 2005), due to increasing road traffic and poaching, otters in fact remain threatened even nowadays (RAKONCZAY 1989). Its study and protection contributes to the conservation of other important species and their habitats e.g. to the maintanance of artificial fish-ponds supporting fish-eating (breeding or migrant) species.

From the point of otter conservation, it would be important to collect more detailed information (KRUUK 1995) about its little-known reproductive characteristics (e.g. how reproductive physiology is adapted to environmental conditions, litter size). Where food availability is fluctuating, its reproduction may be seasonal (ERLINGE 1967, KRUUK *et al.* 1991), but in the majority of its area, females are continuously polyoestrous (CHANIN 1985, MASON & MACDONALD 1986, SIDOROVICH & TUMANOV 1994). Reproduction characteristics of males are hardly known (ELMEROS & MADSEN 1999). Less information available about the characteristics of reproductive biology, health status and organ weight of the Hungarian otter population.

The examination of stomach content may bring new information useful in designing conservation policies primarily through studying the correlation between the quantity of stomach content and other factors (e.g. the cause of death, season, sex, body weight, age). The food composition of otters is most often studied by using the internationally accepted method of non-invasive spraint analysis, using larger samples sizes. The examination of stomach content composition may at best reinforce the general results gained through spraint analysis. Our post mortem examination of otters started in 2002, following the serious cyanide and heavy metal spills on the eastern rivers flowing to Hungary from Romania in 2000. Otter carcasses originated from areas, where general fish stock is abundant in line with the distribution of the otter in Hungary (KEMENES 2005).

In the present study, using data collected during this analysis, we aim at (1) examining the reproduction characteristics of otters to study reproduction seasonality, (2) analysing the stomach content and weight pattern of inner organs to study possible season, condition and/or age dependent differences. With these examinations, as part of a larger study, we aim to record basic data for further otter population analyses and to contribute to efficient otter conservation programs.

MATERIAL AND METHODS

Study area and studied material

The otter carsasses examined were collected by the staff of Hungarian national parks, with permission of the Ministry of Environment and Waters (837/6/2005). Altogether n = 127 otters were analysed. Year of origin and numbers, 1999: 2, 2000: 2, 2001: 3, 2002: 21, 2003: 20, 2004: 25, 2005: 21, 2006: 26 respectively, and unknown: 7 individuals (some individuals were collected before the study started). The bodies originated from near Lake Balaton and Kis-Balaton (n = 17), artificial fishing places, fish ponds and gravel pit ponds (n = 29), backwaters (n = 2), marshlands (n = 1), the Danube (n = 7), and Tisza river valley (n = 16) as well as other rivers (n = 9), streams, canals and ditches (n = 32), settlements (n = 1), unknown (n = 13). Regional distribution of otter carcasses collected are shown on Figure 1.

The examined bodies were categorised according to body weight, measurements and teeth characters (HEGGBERGET 1984, REUTHER 1999) into adult (>2 years), subadult (between 1 and 2 years) and juvenile (<1 year) age groups. No detailed year determination has been carried out on the basis of incremental cementum of teeth (HEGGBERGET 1984). The number of males were n = 54, 7 and 6, and that of the females n = 33, 18 and 5 in the three age groups, respecively. Furthermore, in case of one female, and 3 bodies of unknown sex, age was impossible to determine (in these cases only certain organs could be examined). The causes of death were the following: road kill: 112 cases (88.2%), road kill and dog bites together (judging after bite traces) 2 cases (1.6%), road kill and otter bites together 1 case (0.8%), shooting 2 cases (1.6%), beating to death 1 case (0.8%), dog attack and direct human intervention together 1 case (0.8%), dog attack (regardless whether caused stray or human-instructed animals) 4 cases (3.1%), poisoning 1 case (0.8%), drowning in fyke nets 1 case (0.8%), otter attack 1 case (0.8%), and organ disfunction 1 case (0.8%) (LANSZKI *et al.* 2007). The seasonal distribution of carcasses collected was the following: autumn (n = 32), winter (n = 34), spring (n = 25), summer (n = 23) and unknown (n = 13).

Sampling methods and evaluation

The bodies were stored frozen (–18°C) until analysis. The post mortem analysis was basically carried out using SIMPSON's (2000) description. During the examination we recorded the general data

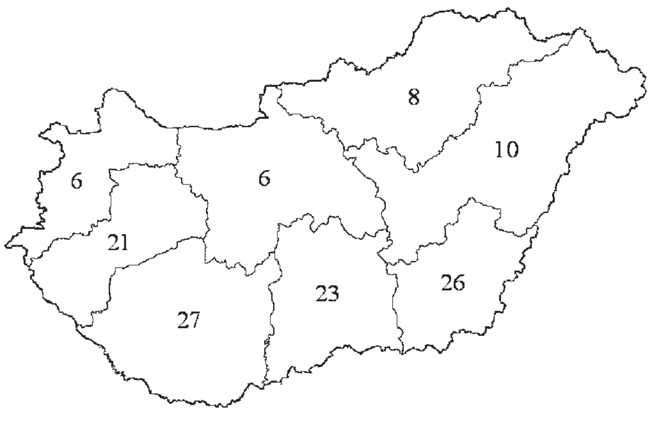

Fig. 1. Location of otter carcasses collected in different regions of Hungary

(date of collection, location, circumstances), measured the body dimensions (weight, body and tail length) and registered casualties (wounds on food pads and claws, eyes, ears, skin). We examined the condition of the mammary glands, measured (with 0.01 g accuracy) the weight of the internal organs and examined the health status of organs, then tissue samples were taken. The reproduction characteristics of otters were investigated in both sexes. Reproductive activity of adult females (earlier pregnancy) on the basis of occurrence and the number of embryos, counted by placental scars in the uterine horns (SIDOROVICH & TUMANOV 1994, ELMEROS & MADSEN 1999, HAUER *et al*. 2002*b*), furthermore the relationship between active teats and placental scar (or embryo number) was also examined. Seasonality of reproduction was examined in adult males on the basis of the gonado-somatic index, calculated from average testicle (without epididymes) weight and body weight (g/kg). Similarly, indices of inner organ weights meaning the ratio of organ weight (g) and body weight (kg) were calculated. Mean (±SE) body weight of adult males was 8.68±0.29 kg, and that of females was 6.02±0.13 kg (for more details see LANSZKI *et al*. 2007).

The percentage composition of food was determined on the basis of both relative frequency of occurrences, and weight (measured at the 0.01 g accuracy) of individual food remains found in the stomachs, identified using atlases and reference collections (for more details see e.g. LANSZKI *et al.* 2001, KEMENES 2005).

The differences in sample size were due to loss of tissue (e.g. by road accident) or insufficient data (e.g. location, date, skinned body).

In case of paired organs, we used the average weight in the calculations. The difference between the sexes in inner organ indices in the adult age group, and in stomach content weight was evaluated using independent samples t-test. Stomach content weight among the age groups as well as gonado-somatic index and stomach content weight among seasons were determined with single-factor variance analysis (LSD post-hoc test). The distribution of empty and non-empty stomachs were determined with Chi-square test. To analyse organ weight differences between the age groups – pooling the juvenile and subadult groups together – independent samples t-test was used. Spearman rank correlation was performed on the number of occurrences of each food item (Table 1) in stomaches and summarized weight of each food item measured, all using the statistical program SPSS (1999).

RESULTS

Reproduction characteristics

In the adult age group of female carcasses, taking all examined bodies (n = 28 from 33 adults, 5 cases were indeterminable due to missing uterus) into account, on average 1.43±0.23 placental scars (±SE) were counted in the uterine horns. In almost one-third of these (30.8%), no placental scars or embryos were found (non-breeding females). Among breeding females, one placental scar (and thus probably one cub) was found in 11.1%, two in 61.1%, three in 22.2% and four (at the same time 4 sucked teats) in 5.6%. One female was pregnant (carcass collected in April), in one uterine horn we found two embryos and in the other uterine horn one, but this horn was damaged. Mean number of placental scars was 2.22±0.17 in the females with earlier pregnancies (n = 18). Milk secretion was detected at four females (carcasses collected in May, September, December and in one case in unknown month). The number of active teats (2, 2, 3 and 4) was equal to the number of placental scars, i.e., two (0+2 and 1+1 placental scars in the left and right uterine horns, respectively), three (2+1) and four (1+3). The birth season, estimated on the basis of the age of examined juveniles was winter in 1 case, spring in 4 cases and summer in 4 cases.

In the adult age group, the highest gonado-somatic index value was measured in males in summer, however, the difference among seasons was not significant (ANOVA, $F_3 = 2.190$, $P = 0.107$). The mean index value (±SE) was 0.21±0.01 g/kg in autumn, 0.23±0.03 g/kg in winter, 0.194±0.03 g/kg in spring, 0.28±0.03 g/kg in summer, and 0.21±0.03 g/kg in the case of individuals from unknown months.

Stomach content

The mean (±SE) weight of food in the examined stomaches was 49±7 g (n = 113). In nearly one third of the cases, the stomach was empty (31%) (Fig. 2). The greatest weight of stomach food content was 400.2 g. There was no significant difference in stomach content weight across seasons (ANOVA, $F_3 = 0.323$, $P = 0.808$), sexes (independent samples t-test, $t_1 = 0.641$, P = 0.523) and age groups (ANOVA,

Table 1. Stomach content of otters in Hungary. N – number of food items, RFO – relative frequency of occurrence, W – weight of food, n = 113 stomaches.

Food items	N	RFO (%)	W (%)
Carassius spp.	11	11.2	24.4
Common carp (*Cyprinus carpio*)	4	4.1	10.4
Small-sized cyprinids	7	7.1	6.4
Perch (*Perca fluviatilis*)	1	1.0	0.6
Brown bullhead (*Ictalurus nebulosus*)	1	1.0	0.5
Undetermined small-sized fish	38	38.8	36.1
Water vole (*Arvicola terrestris*)	2	2.0	0.6
Rat (*Rattus* spp.)	1	1.0	2.2
Undetermined small mammals	6	6.1	3.1
Small song birds (Passeriformes)	2	2.0	0.5
Medium-sized birds	3	3.1	6.4
Grass snake (*Natrix natrix*)	1	1.0	0.1
Frog (*Rana* spp.)	13	13.3	8.5
Insects	7	7.1	0.1
Plant matter	1	1.0	< 0.1
Number of items	98		

$F_2 = 0.828$, $P = 0.439$). The distribution of empty and non-empty stomaches did not differ significantly across the seasons ($\chi^2 = 2.50$, df = 3, $P = 0.476$).

In the otter stomaches containing food remains, 98 different taxa were found (Table 1). Weight-wise, the main food eaten by otters was fish of which *Carassius* spp. (mainly Prussian carp *Carassius auratus gibelio*), and other small-sized (less than 100 g) fish were the most frequent. The proportion of common carp in the total food was 10.4%. Besides, considerable amounts of frogs (*Rana* spp.), small mammals (e.g. *Rattus* spp., *Arvicola terrestris*), and medium-sized birds (6.4%) were consumed by the otters examined. In lower proportions, also small passerine birds, reptiles, insects and leaves of *Carex* spp. could also be detected in the stomaches. Close relationship (n = 14, $r_S = 0.701$, $P < 0.01$) was found between the number of occurrences of each food item in the stomaches and summarized weight of each food item measured.

Organ weight indices

The mean weight and organ weight index patterns of otters as a function of age and sex are shown in Table 2. Typically, organ weight indexes were higher in the combined juvenile and subadult (immature) age group than in the adult (or mature) group, however, these differences were significant in males in liver (independent samples t-test, $t_{43} = 5.140$, $P < 0.0001$), kidney ($t_{53} = 2.143$, $P < 0.05$), thyroid gland ($t_{56} = 3.921$, $P < 0.001$) and lung ($t_{48} = 2.419$, $P < 0.05$), while no significant differences were found in females. In the adult age groups, females tended to have higher organ weight indices, however, differences were not significant in the case of heart (independent samples t-test, $t_{69} = 0.043$, $P = 0.966$), liver ($t_{52} = 1.925$, $P = 0.059$), spleen ($t_{58} = 0.715$, $P = 0.477$), lung ($t_{58} = 0.09$, $P = 0.931$) and thyroid gland (males: n = 45, 0.183±0.011 g/kg, females: n = 22, 0.207±0.013 g/kg, $t_{65} = 1.305$, $P = 0.196$). Significant difference was only found in case of the kidneys ($t_{68} = 3.063$, $P < 0.01$) and the adrenal glands ($t_{67} = 2.117$, $P < 0.05$). Kidney stones were only found in one case, in one adult female with a bad conditon index (0.84) collected from a fish pond.

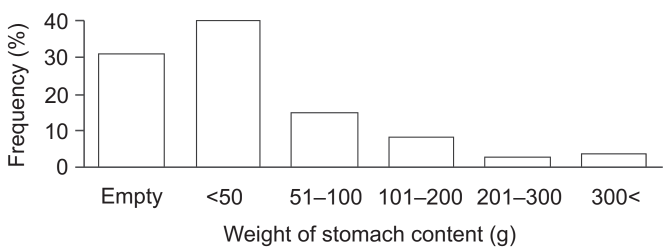

Fig. 2. Distribution of otter stomach contents according to weight categories in Hungary

Table 2. The weight and the organ weight index of otter internal organs, in Hungary. Age classes: 1 – juvenile, 2 – subadult (1–2 years), 3 – adult (older than 2 years)

Organ	Age class	Weight of organ (g)				Index (g/kg)			
		Males		Females		Males		Females	
		N	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE
Heart	1	50	69.3±1.95	28	48.6±1.34	46	8.1±0.14	26	8.0±0.19
	2	7	52.7±4.39	16	37.0±1.11	7	8.5±0.52	15	8.0±0.29
	3	6	21.8±4.21	5	21.6±4.84	6	7.7±0.84	5	8.3±1.10
Liver	1	36	285.0±10.22	22	229.7±11.64	34	34.3±1.10	21	37.6±1.87
	2	7	285.1±35.79	14	180.8±15.17	7	45.2±4.07	14	38.7±2.09
	3	4	157.0±17.07	5	109.3±20.95	4	53.1±5.38	5	42.7±2.85
Spleen	1	41	39.7±2.04	24	29.5±1.89	38	4.5±0.22	23	4.7±0.27
	2	7	29.4±4.71	12	24.2±2.73	7	4.7±0.67	12	5.0±0.47
	3	4	10.7±4.51	4	8.6±1.75	4	3.2±0.78	4	3.0±0.16
Lung	1	40	125.7±5.77	25	90.9±5.57	37	15.2±0.90	24	15.0±1.04
	2	7	123.6±18.64	15	81.2±4.63	7	20.1±2.87	15	17.7±0.98
	3	6	51.5±6.97	5	45.9±11.26	6	19.1±2.20	5	18.1±2.64
Kidney	1	47	31.3±0.73	29	25.5±0.61	43	3.7±0.13	28	4.3±0.11
	2	7	25.7±2.57	14	19.2±0.73	7	4.2±0.39	14	4.3±0.22
	3	5	12.6±1.46	5	12.6±2.38	5	4.5±0.28	5	5.1±0.45
Adrenal gland	1	47	0.51±0.020	28	0.430.027	44	0.06±0.003	26	0.07±0.006
	2	7	0.40±0.064	15	0.28±0.018	7	0.07±0.011	15	0.06±0.007
	3	5	0.22±0.029	5	0.23±0.053	5	0.08±0.009	5	0.09±0.015
Thymus	1	40	5.38±0.623	30	4.71±0.653	38	0.61±0.069	26	0.79±0.125
	2	7	5.58±1.910	15	5.17±0.962	7	0.88±0.304	14	1.12±0.227
	3	6	1.80±0.532	5	1.93±0.984	6	0.76±0.273	5	0.92±0.538

DISCUSSION

One of the important characteristics of reproduction is litter size, which varies between 1 and 4 (6) in wild otters (KRUUK 1995). Under the conditions of lush waterside vegetation in Central Europe, direct observation of otters reproduction is problematic (occcasional and biased), in contrast with diurnally active otter living in marine habitats (KRUUK *et al*. 1987) and with studies conducted in Mediterranean areas (RUIZ-OLMO *et al.* 2002). Expedient post mortem analysis (based on wild living otters) provide more reliable data about wild otter populations, while data e.g. from captive ones could easily be biased. The number of placental scars found in the examined carcasses indicate that the litter size of otters in Hungary is most often probably 2 or 3. Nevertheless, these indirect data don't necessarily reflect true litter size, but there seems to be a strong agreement between the estimated numbers and the actual number of offsprings counted. In a study conducted in Eastern Germany (HAUER *et al.* 2002*b*) similar, under European conditions moderate (KRUUK 1995, ELMEROS & MADSEN 1999, RUIZ-OLMO *et al.* 2002) litter sizes were found. Mean litter size was 2.77 and 2.55 based on thriving and regressive corpora lutea counts (respectively), 2.27 on embryo counts, 2.39 on placental scar counts and only 2.02 according to the number of cubs (HAUER *et al*. 2002*b*). It is not known why nearly third of the adult (older than two years) females never produced litter. Otters (both sexes) usually reach sexual maturity in their second year of their life (STUBBE 1969), but a part of the females may not have yet been ready to breed (HAUER *et al*. 2002*b*), or their older placental scars were not visible, and maybe also other reasons, e.g. early pre-natal mortality, insufficient habitat (resource) conditions (ERLINGE 1967) may have contributed to the high proportion of adult non-breeding females.

In seasonally breeding mammals the reproductive system undergoes profound changes throughout the year (CHANIN 1985). For example in males, after the breeding season gonads become much smaller and sperm production terminates. In American river otters, which are more seasonal breeders than Eurasian ones, testis size reaches a maximum in spring (LIERS 1951). In our study, the gonado-somatic index of males didn't show any tpyical seasonality, concurring with other, not too numerous researches in this field (HEGGBERGET & CHRISTENSEN 1994, SIDOROVICH & TUMANOV 1994, ELMEROS & MADSEN 1999). This indicates that in the Carpathian Basin, similar to other temperate European regions, the fertility of males remains constant throughout the year. In females, the time of breeding cannot exactly be determined in post mortem examinations (from the uterine horns). Furthermore, the collection period of suckling females with active teats cannot be used in determining the seasonality of reproduction, as otters suckle their cubs for a long time, i.e. 8–12 month (summarized KRUUK 1995).

The long period of juvenile dependence may block the next oestrus cycle of the female (SIDOROVICH & TUMANOV 1994), and food availability is also a limiting factor which could cause a seasonality in reproduction. This is also reinforced by the fact that on the basis of the age of young otter carcasses collected throughout the year, most of them were born in spring and summer, but winter birth also occured. The experienced non-seasonal breeding of otters draws attention to the importance of winter fish food availability on wetlands in Hungary.

In the fish ponds the harvesting takes place in the autumn (LANSZKI *et al.* 2001, 2006): in this period food deficinecy develops very quickly and its abundance increases in spring again. The carcasses were collected mainly during autumn (28.1%) and winter (29.8%). However, most examined bodies were not collected from these habitats, and the fish stock of large lakes, rivers and oxbows is much more stable (LANSZKI & SALLAI 2006). The correspondance between the number of active teats and placental scars may be important from the physiological point of view (especially if also proven in larger sample sizes), meaning that cubs always have the possibility to suck simultaneously. Results of stomach content examination are – in spite of considerably lower sample size – in agreement with spraint analyses (LANSZKI *et al.* 2001), both regarding relative proportion of food elements and weight ranges.

The high proportion of empty stomaches and the ones containing very little (<50 g) food – almost three-quarter of the cases – indicates that mostly hungry individuals, in search of food became victims of car traffic. Analysis of stomach contents corroborate the studies performed in the Pannonian ecoregion using spraint analyses (e.g. KEMENES & NECHAY 1990, LANSZKI *et al.* 2001), namely that otters do not consume commercially important fish in high proportions. This information should be also applied in the design of conservation programs of the otter e.g. in Hungary.

The organ weight data and the indexes calculated as a function of body weight play a role in the description of the basic patterns of Hungarian otter population and in collecting better and more detailed knowledge about the species. Unfortunately less information is available in this field of Eurasian otter biology, data published about American river otters are more abundant (HARDING & ELLIOTT 1996). The use of organ index data may also be necessary in future comparative studies among populations or indviduals in different health status, sexes and/or seasons. In our case, decreasing tendency in organ weight, while increasing tendency was found in case of liver, lung and kidney indices depending on age classes.

In summary, through the detailed *post mortem* examination of otter carcasses collected, we gained biological data about the wild populations which couldn't be studied otherwise or only at high costs. Thus, the study serves the efforts of conservation of otter.

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