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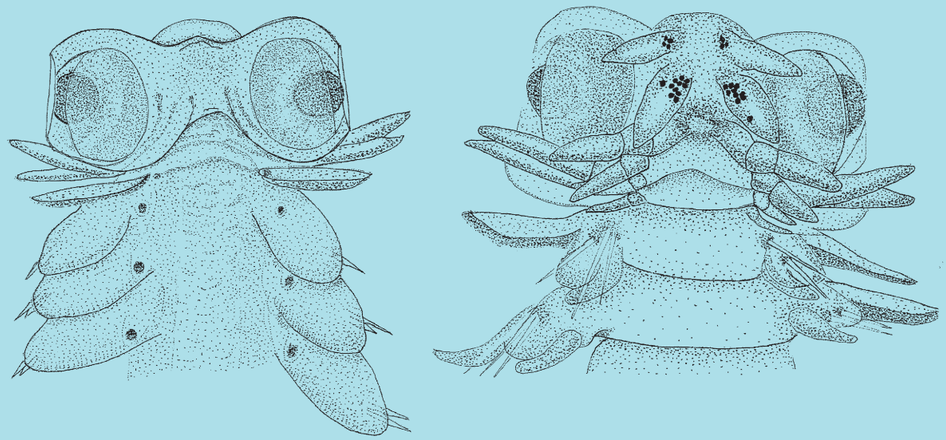

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Numbers and distribution of Red-footed Falcons (*Falco vespertinus*) breeding in Voivodina (northern Serbia): a comparison between 1990-1991 and 2000-2001

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ABSTRACT. The survey of population size and distribution of Red-footed Falcons proceeded in June-July 2000 and 2001 respectively, ten years after the first census (1990 and 1991). Data of only those nests were processed in which there was breeding. Breeding success was calculated from the number of offspring per reproductive female. During the survey in Voivodina in 1990-1991 there were 308 and 124 pairs, respectively, whereas ten years later, in the year 2000 there were 116, and in 2001 only 61 pairs of Red-footed Falcons. Even if the marked fluctuations observed are not considered, the Red-footed Falcon population breeding in Voivodina shows a declining tendency. The south-western margin of the distribution area has moved towards the northeast by about 50-70 kilometres. More than 90% of the nesting sites, including the larger nesting colonies, are found in the Banat region, i.e. east of the Tisa River. More than 90% of the Red-footed Falcons continue to nest in Rook colonies. No significant change has occurred in their breeding success.

KEY WORDS : Red-footed Falcon, *Falco vespertinus*, census, fluctuation, nesting strategies, breeding success, Voivodina, Serbia.

INTRODUCTION

The Palearctic breeding range of the Red-footed Falcon (*Falco vespertinus*) extends across a broad band of steppe, forest-steppe, and cultivated north temperate areas (CADE, 1982). It is estimated that there are 18,000-44,000 pairs nesting in Europe, with the majority (15,000-40,000) found in Russia's European areas (TOMIALOJC, 1994; BIJLSMA, 1997; HEATH et al., 2000). Breeding numbers in the stronghold of the Carpathian basin, Hungary, declined from about 2000-2200 pairs in the early 1990s (TOMIALOJC, 1994; BIJLSMA, 1997; HARASZTHY, 1998; MAGYAR et al., 1998; HEATH et al., 2000) to 1300-1500 pairs in 1997 (TÓTH & MARIK, 1999) and still further to 700-800 pairs in 2000 (BAGYURA et al., 2001). In recent decades, there has been only sporadic nesting west of the Danube (HARASZTHY, 1998). Numbers of Red-footed Falcon breeding in Voivodina, the northern province of Serbia, have been estimated several times over the last 30 years (HAM, 1977; VASIĆ et al., 1985; VASIĆ, 1996; BIJLSMA, 1997; HAM & RAŠAJSKI, 2000). A detailed assessment carried out in 1990-1991 (PURGER, 1996). HAM & RAŠAJSKI (2000) estimated as high as 17.2% increase of the Red-footed Falcon population in Voivodina and an extension of its distribution. Red-footed Falcons use the abandoned nests of various crow species (CRAMP & SIMMONS, 1980) as nest sites and may be solitary, semi-colonial, colonial when nesting. HARASZTHY & BAGYURA (1993) reported that Red-footed Falcons nesting in colonies have significantly higher breeding success. Thus, it is important to know the proportion of pairs that breed in colonies.

The aim of this study was to assess changes in population size, distribution of nesting sites, nesting strategies, and breeding success of Red-footed Falcons nesting in Voivodina, ten years after the first census (PURGER, 1996).

MATERIALS AND METHODS

Study area

The province of Voivodina (44°38'-46°10'N; 18°10'-21°15'E) is predominantly a flat region, occupying the south-eastern part of the Carpathian Basin. It is divided into three regions by the rivers Danube, Tisa and Sava (Fig. 1); Bachka (Bačka) is a wide plain bordered by the Danube, the Tisa and the Hungarian border (8956km^2); Banat is bordered by the Tisa, the Danube and the Romanian and Hungarian border (8886km^2); while Srem is the area between the Danube and the Sava and the Serbian and Croatian border (3838km^2). Within Voivodina there are four loess plateaus (Banatska-, Tamiška-, Titelska-lesna zaravan and Telečka), two sandy areas (Deliblatska peščara and Subotičko-Horgoška peščara), and two low mountain ranges (Fruška gora in Srem at 539m, and Vršačke planine in south-eastern Banat at 641m a.s.l). It is a forest-steppe region with a temperate-continental climate, in which central-European and Mediterranean influences are noticeable (STEVANOVIĆ & STEVANOVIĆ, 1995). The potential natural vegetation of Voivodina consists of climo-zonal vegetation, hydrologically conditioned vegetation, sandy and saline vegetation (PARABUĆSKI & JANKOVIĆ, 1978). According to STEVANOVIĆ et al. (1995) natural vegetation includes: steppes (*Festucion rupicolae*), forest-steppes (*Aceri tatarici-Quercion*), mezophylic Slavonian-oak hardwoods (*Quercion roboris*), mezophylic beech and oak-hornbeam forests (*Fagion moesiacae*, *Querco-Carpinion betuli*), and thermophylic Turkey oak-Italian oak forests (*Quercion frainetto*). More than 75% of the province is used agriculturally; only 6.6% is covered by forests (MARKOVIĆ, 1990). The remnants of steppe and forest-steppe areas are decreasing and being modified by agricultural expansion (MATVEJEV & PUNCER, 1989). The distribution

of natural woodlands and fast-growing plantations has also decreased recently.

Methods

Our survey of the population size and distribution of Red-footed Falcons in 2000 and 2001 followed the same routes taken a decade before (PURGER, 1996). With one or two observers the author drove all main roads and a lot of dirt roads, usually ca. 300-400km per day. However, the majority of trees and bushes along roads, railways and canals have been cut down recently (this is particularly striking in Bachka and in the northern and central parts of Banat). Therefore nesting opportunities for Rooks (*Corvus frugilegus*), Hooded Crows (*Corvus cornix*) and Magpies (*Pica pica*) and consequently for the Red-footed Falcons have decreased. Thus carrying out the survey (total counts) took less time than during the first study. The census was done between 21-24 June and 7-12 July 2000, and between 17-20 June and 13-18 July 2001. It took ten days in both years: one day was spent in Srem, where its breeding is probable in some years, two days in Bachka, where nesting sites are mostly isolated, and seven days in Banat, because the main breeding sites are in northern and central part of Banat (PURGER, 1996; HAM & RAŠAJSKI, 2000). The types of nests occupied by the Red-footed Falcons were recorded: birds nesting in Rook colonies were considered "colony nester" irrespective of the number of pairs, whereas those occupying Magpie or Hooded Crow nests were regarded as "solitary nesters". For raptors POSTUPALSKY (1974) proposed at least two checks of each occupied nest per breeding season. Due to the frequent rains, it was not possible to check all nests several times, thus breeding success was studied only in 2001 at the colony in Mokrin, which was easily accessible. The number of pin-feathered or fledging chicks (mean number ± sd) per reproductive female was used as basic unit for calculating breeding success, in order for the results to be comparable with the earlier survey (PURGER, 1995). Checking nests was done using mirrors, by climbing trees and by counting chicks sitting on nest.

In the statistical analysis for comparing proportions (numbers of colony nester and solitary pairs) we used 2×2 contingency tables with Yates' correction. For comparing the means of small samples we used two tailed t-test. The probability threshold for rejecting null hypothesis was 5% (FOWLER & COHEN, 1995).

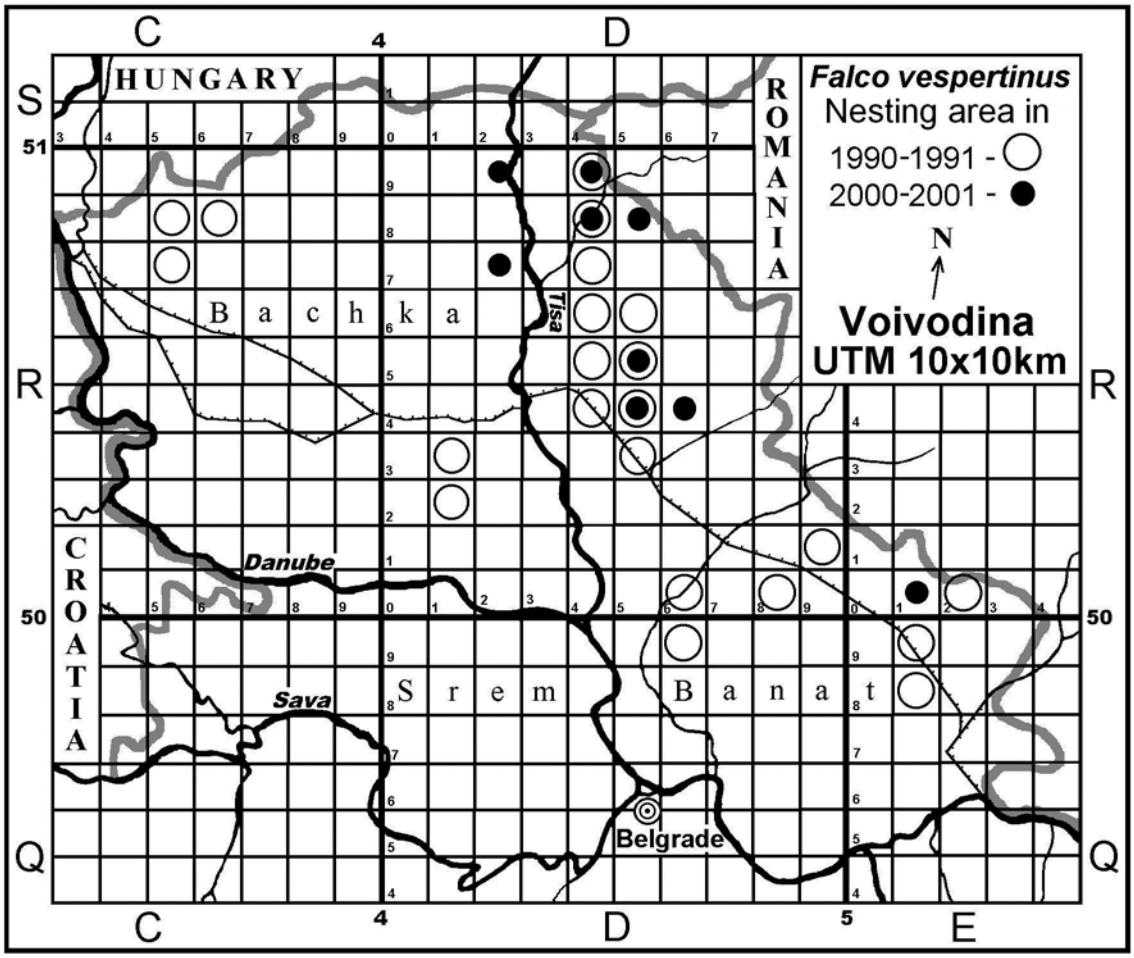

Fig. 1. – Breeding distribution of the Red-footed Falcon in Voivodina in 1990-1991 (according to PURGER, 1996, open circles) and 2000-2001 (original data, black points) based on the bird census data.

RESULTS AND DISCUSSION

A total of 116 and 61 pairs of Red-Footed Falcon nested in Voivodina in 2000 and in 2001, respectively (Table 1). Ten years before there were 308 in 1990 and 124 pairs in 1991 (PURGER, 1996). The survey in 1991 indicated a 60% population decrease since 1990, whereas the number recorded in 2001 was 47% less than in 2000. This suggests that the population size along the south-western margin of European area fluctuates greatly among years. Difference between the surveys in 1991 and 2000 was not big (124, 116 pairs), but whereas a total of 432 pairs of Red-Footed Falcon nested in the study area at the time of the first surveys (1990/91), there were only 177 pairs of them breeding during the second surveys (2000/01), which suggest population decline. Also, the number of nesting localities varied from year to year, being 19 in 1990 and 22 in 1991. Only 9 were used in both years (PURGER, 1996). Our survey revealed a further decline in the number of nesting localities used; 17 in 2000, 11 in 2001 and 6 were used in both years (Table 1). Only one nesting location used in the first survey was used again in 2000-2001 (2km south-east of Jazovo). In this colony 147 nesting pairs were counted in 1990 and 29 pairs in 1991 (PURGER, 1996), but only 12 pairs in 2000 and 6 pairs in 2001 (Table 1).

TABLE 1

Number of breeding pairs of Red-footed Falcon found in Voivodina during 2000 and 2001.

UTM	Locality	2000	2001
	Bachka		
DR27	1 km south of Sterijino Selo	1	-
	2 km south of Sterijino Selo	2	-
DR29	2.5 km north-west of Zimonjić (Kapetanski rit)	6	2
	Banat		
DR48	2 km south-east of Jazovo	12	6
DR49	1 km north-west of Podlokanj	1	-
	1 km south-west of Vrbica	1	-
	1 km west of Banatski Monoštor	1	-
	1 km east of Banatski Monoštor	-	2
	2 km east of Crna Bara	1	-
	3 km east of Crna Bara	1	-
	3.5 km east of Crna Bara	-	1
	5 km south-east of Crna Bara	-	1
DR54	Torda	25	14
	2 km south-west of Torda	4	2
DR55	4 km north of Bašaid	1	-
DR58	Mokrin (Vašarište)	42	26
	1 km north-west of Mokrin	9	5
DR64	1 km south-west of Čestereg	2	-
	Banatsko Karađorđevo	-	1
	1 km north-east of Banatski Dvor	1	-
	1 km north of Banatski Dvor	-	1
ER10	7 km north-west of Vršac	6	-
	Total	**116**	**61**

Breeding pairs were found only in Bachka and Banat (Table 1, Fig. 1). In the north-western and south-eastern parts of Bachka there were 4 and 5 pairs nesting in 4 and 3 locations in 1990 and 1991, respectively (PURGER, 1996). By 2000 there were 9 pairs in 3 locations and in 2001 only 2 pairs at 1 location in the north-eastern part of the region (Table 1, Fig. 1). The shift of nesting locations towards the north-east coincided with a contraction of their range that was associated with declining numbers. However, the majority of Red-Footed Falcons in Voivodina (1990 – 99%, 1991 – 96%, 2000 – 92%, 2001 – 97%) bred in the northern, central and eastern parts of Banat (Table 1). After a period of ten years, not only the number of nesting locations, but also the number and size of larger colonies (with more than ten pairs of Red-footed Falcons) had declined. In 1990-1991, 88% and 72% of pairs nested in 5 and 4 large colonies respectively, and by 2000-2001 only 3 and 2 larger colonies (Jazovo, Torda, Mokrin) remained (Table 1). Thus, despite the population decline, 73% and 68% of Red-footed Falcons nesting in the areas beyond the Tisa River continued to nest in larger colonies (Table 1). The majority of nesting areas in Banat are about 10-20km from the Tisa, and are aligned parallel with the river (Fig. 1). No nesting Red-footed Falcons were found in the central parts of the region during the survey in 2000-2001. In 2000 there was still a smaller colony near Vršac (ER10), but it had disappeared by 2001 (Table 1). Overall, nesting areas in Voivodina have shifted to the north-east by 50-70 kilometres (Fig. 1).

The 1990-1991 results suggest that there were less birds nesting within Voivodina in 1991; only about a third of the 1990 total. Also, the number of solitary nesting pairs was significantly higher in 1991 (χ^2=17.77, df =1, P< 0.001). In contrast, there was no significant difference (χ^2= 0.16, df=1, ns) in nesting strategies between 2000 and 2001 (Table 2). Red-Footed Falcons nesting in Voivodina used Rook nests even if they were situated in human settlements. In 1991, 11.3% of nests were found in the villages Bašaid and Sakule (PURGER, 1996). This association has subsequently increased. In 2000, 59.5% of nests (Mokrin, Torda, Čestereg) and in 2001, 67.2% of nests (Mokrin, Torda, Banatsko Karađorđevo) were located within villages (Table 1).

TABLE 2

Numbers of Rook and Hooded Crow and Magpie nests occupied by Red-footed Falcons in 1990 and 1991 (according to Purger 1996), and in 2000 and 2001 (original data). No significant difference was found between the proportions of the two groups in the two study periods (χ^2 = 0.26, df = 1, ns).

Years	Rook nests	Hooded Crow and Magpie nests
1990	301 (98%)	7 (2%)
1991	108 (87%)	16 (13%)
Both years	409 (95%)	23 (5%)
2000	107 (92%)	9 (8%)
2001	58 (95%)	3 (5%)
Both years	165 (93%)	12 (7%)

In the Rook colony in Mokrin in 2001 (DR58, Table 1) the mean number of offspring per reproductive female was 2.46±0.76 (n=26) and was not significantly different in either 1990 (t=0.95, df=48, ns) or 1991 (t=-0.398, df=56, ns). There was no significant difference (t=1.31, df=54, ns) between breeding success in 1990 (2.66±0.76, n=24) and 1991 (2.34±0.87, n=32) (PURGER, 1995).

From our results in 2000 and 2001 (116 and 61 pairs, respectively) it appears that HAM & RAŠAJSKI (2000) overestimated the size of the Red-footed Falcon population (250-336 pairs between 1994-1996) breeding in Voivodina. Several authors have presented various distribution maps of nesting areas (PURGER, 1996; PURGER & MUŽINIĆ, 1997; RAŠAJSKI, 1997; HAM & RAŠAJSKI, 2000), but those can not be used for assessing population fluctuations. The spatial distribution of recent data reveals that Red-footed Falcons have traditional breeding locations with larger colonies in Banat where there are more extensive steppe grasslands, but the boundaries of the distribution area do fluctuate. Such fluctuations in range can best be determined if the survey is repeated every year. Our data indicate that the Red-footed Falcon population is declining and its range contracting towards the north-east. Thus, both trends are similar to what has been observed in Hungary (HARASZTHY, 1998; TÓTH & MARIK, 1999; BAGYURA et al., 2001).

Nesting strategy appears not to have changed during the past ten years: the majority of the Red-footed Falcons in Voivodina nest in Rook colonies, even when those are located inside villages. In Hungary only one third of the birds breed in colonies and the rest nest solitarily or in semi-colonies (TÓTH & MARIK, 1999). If solitary nesters have lower breeding success than birds in colonies (TÓTH, 1994; TÓTH & MARIK, 1999; HARASZTHY & BAGYURA, 1993), then the decline of the Hungarian population may be assisted by the lower breeding success. In Voivodina, south from Hungary, Red-footed Falcons continue to nest in colonies, but the population is still declining. Moreover the proportion of birds nesting in Rook colonies was even higher in 2000-2001. Maybe if population size falls under a critical limit, colonial nesting results in growth again through the effect of higher breeding success? This question can be answered only if population size and changes in nesting strategy are monitored for several years. It is likely that the disappearance of nesting sites and feeding grounds greatly influences both nesting strategies and population trends (TÓTH, 1994; TÓTH & MARIK, 1999). The Red-footed Falcon population in Voivodina is threatened by the increase of monoculture plantations and by the use of biotoxins (HAM & RAŠAJSKI, 2000). Changes in agricultural practice are the main factor implied in the population decrease of The Lesser Kestrel (*Falco naumanni*) and other steppary birds of prey (TELLA et al, 1998). This is supported by SNOW & PERRINS (1998), the major causes of Red-footed falcon population decrease being the decline of insect prey because of pesticide use, and habitat changes.

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Seasonal Variation in blood plasma sodium and potassium concentrations in the lizard *Agama nupta* (Agamidae)

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ABSTRACT. Seasonal changes in the concentrations of Na and K ions in the blood of *Agama nupta* in summer are reported from a population in Iran. These lizards do not have access to free water and acquire all their required water from their diet of insects. The hypernatraemia and hyperkalaemia reported here may be the result of chronic dehydration but could also result from the accumulation of ions in the extracellular fluid, as has been shown in other species of lizards. In contrast to herbivorous species of lizards, *A. nupta*, which is insectivorous, does not appear to have an active nasal salt gland for excreting sodium and potassium as salt-loading in the laboratory failed to evince any secretions.

KEY WORDS : *Agama nupta,* sodium, potassium, blood.

INTRODUCTION

There have been a number of published studies with lizards showing that many species, but not all, experience increased blood sodium (hypernatraemia) and potassium (hyperkalaemia) concentrations in summer when supplies of water are at a premium (BRADSHAW, 1986; BRADSHAW, 1997). Hypernatraemia has been shown to result from excessive dietary intake of sodium ions (BRADSHAW & SHOEMAKER, 1967). This study is the first to examine water and electrolyte balance in the Iranian agamid lizard *Agama nupta*, and to examine the maintenance of electrolyte homeostasis during the hot dry season near Khoramabad. Another desert agamid lizard, *Uromastix acanthinurus* has been reported as being able to maintain electrolyte constancy in its blood throughout the summer (BENTLEY, 1976; GRENOT, 1976), but subsequent careful analysis of changes in plasma sodium levels from separate populations near Beni-Abbes in Algeria showed that this species also experiences hypernatraemia in summer (LEMIRE et al., 1979; LEMIRE et al., 1982; BRADSHAW, 1986).

The study of changes of the amount of electrolytes in the blood of different lizard species and during various seasons has attracted a considerable deal of attention. BENTLEY (1959), working with the skink, *Tiliqua rugosa*, reported the existence of high concentrations of Na^+ in plasma during mid-summer. In addition, he found that sodium injections in the laboratory also resulted in hypernatraemia. BRADSHAW & SHOEMAKER (1967), carried out field studies on water and electrolytes balance in the agamid lizard *Amphibolurus* (now *Ctenophorus*) *ornatus* in Western Australia and found that high concentration of Na^+ in the plasma of this lizard during summer, were associated with a significant isosmotic expansion of the extracellular fluid. Plasma sodium levels were found to increase to as high as 300mmol.L^{-1} in extended droughts and were associated with significant mortality.

In lizards, an active nasal gland for discharging salt has attracted zoologists' attention, and in some species the role of this active nasal gland in excreting ions has been investigated. In some lizards species however, no research has been performed on nasal glands and their function remains unknown.

Since the discovery of nasal salt glands in many species of reptiles some zoologists have shown that in natural and laboratory conditions, a group of terrestrial reptiles can exude the salt in a highly-concentrated solution from the nasal gland (SCHMIDT-NIELSEN et al., 1963; DUNSON, 1974; 1976).

This subject has been reviewed by CLOUDSLEY-THOMPSON (1971), and LEMIRE & VERNET (1982), reported that some species of terrestrial reptiles usually exude relatively smaller amounts of salt relative to marine and estuarine species. A nasal gland in the form of a special gland specialized for salt secretion is common in herbivorous desert lizards, in particular those where their only water sources are the plants that they eat (GABE & SAINT GIRONS, 1971; 1976). These animals ingest significant amounts of electrolytes particularly potassium and they must adapt to quantitative changes in ion concentrations in their food. The importance of nasal gland secretions in regulating plasma electrolyte concentrations in the family Iguanidae, residing in deserts of Northern America, has been demonstrated in both *Sauromalus obesus* (NORRIS & DAWSON, 1964; TEMPLETON, 1964; SHUTTLEWORTH et al., 1987); and *Dipsosaurus dorsalis* (TEMPLETON, 1966; SHOEMAKER et al., 1972; HAZARD, 2001). GRENOT (1976) showed that these lizards are similar in their ecology to the north-Africa agamid lizard, *Uromastix. acanthinurus*. However despite salt being gathered on the nasal cavities in normal conditions (GRENOT, 1968), and having a salt gland in the nose which has specialized morphologically (LEMIRE et al., 1970; 1972; LEMIRE, 1975), the manner of adaptation of the nasal gland in Agamidae against increasing salt ions in comparison with Iguanidae of Northern America has remained unknown. Nasal gland structure in *U.acanthinurus* has been studied by LEMIRE & GRENOT (1974), LEMIRE et al. (1970), and in *Agama*

mutabilis by LEMIRE & GRENOT (1973), and its function has been investigated by GRENOT (1968), LEMIRE & VERNET (1981; 1982; 1983) and by BRADSHAW et al. (1984).

SAINT GIRONS & BRADSHAW (1987) have studied the histology and cytology of nasal glands among Australian lizards such as Varanidae, Scincidae, Gekkonidae, Agamidae and Pygopodidae families. They reported that nasal salt secreting of glands are most highly developed in the Varanidae and to a lesser extent in Scincidae. Salt- secreting elements have been reported only and then in Gekkonidae and Agamidae, and never to date in the Pygopodidae.

Agama nupta is an insectivorous lizard that never drinks water and obtains its required water for body activity through its food. Adaptations of *A. nupta* to its environment and the manner of its reaction against effective factors in critical conditions have not been studied previously. The aim of this study was to investigate whether *A. nupta* lizards around Khoramabad are able to regulate concentrations of Na^+ and K^+ in the blood during summer when free water is scarce and whether they possess an active salt-secreting gland that assists in this process.

MATERIALS AND METHODS

Lizards weighting between 103 to 170g were collected weekly or biweekly (some times more) over a two-year period from May to September. 90 *Agama nupta* were collected during the investigation as follow:

5 individuals in May, 17 in June, 17 in July, 29 in August and 22 individuals in September in Khoramabad (50° 3'E, 34º 22'N). The animals were captured by hand (FERGUSSEN & BRADSHAW, 1992) usually in the morning to about noon. The mean size/weight ratio of animals was 5.6. Animals were collected and bled several times per month, 2 times in May, 4 times in June, 5 times in July, 6 times in August and 7 times in September. Habitat, temperature, and humidity were all recorded. Animals were transferred to laboratory, and blood samples in triplicate were collected into heparinised microhaematocrit tubes, from the infra orbital sinus of each lizard (HALPERN & PACAUD, 1951). Blood was obtained 2-4 hours after animals captured, and then the animals were released. Blood was centrifuged at 1500g and plasma was separated and then stored frozen at –20°C until analyzed (SAINT GIRONS et al., 1992).

Subsequently Na^+ and K^+ concentrations were measured with a flame photometer (Cornic 405). In addition to the above 90 mentioned lizards, and in order to stimulate nasal gland functions of *A. nupta*, for becoming active and secreting salt, 15 adults weighing between 103 to 170g, were trapped and transferred to the laboratory of zoology, they were divided into two groups, each group housed in a special cage, then they placed in climatic chamber for experimental conditions (see LEMIRE et al., 1980). The lizards were not fed during the experiments. One group was given daily subcutaneous injections of 2mL of sodium chloride (2mmol NaCl per 100g body weight) and the other group received 2mL potassium chloride (2mmol KCl per 100g body weight) for 5 days at 24 hours (LEMIRE & VERNET, 1983). The encrustations around the nostrile were checked every day to find the nasal secretions (because in lizards possessing active nasal gland, salt secretions are crystallized spontaneously on those areas). But no secretions of Na or K ions were seen through the nasal gland, after injections, to measure the nasal salt secretions. ANOVA was applied to determine significant differences between mean electrolyte concentrations. The confidence limit in all analyses was $P<0.05$. To detect differences between the different months Sceffe test was used.

RESULTS

Results obtained from tests on *A. nupta* collected around Khoramabad, indicate that the concentration of sodium ions in the plasma rise during the warm season (differ significantly from May to September, $P<0.05$) and is associated with small changes in the concentration of potassium ions (see Fig. 1). Temperature rises from a typical 30°C in May to 37°C in June, then to 42ºC in July, finally reaching 46ºC in August. Plasma sodium concentrations show a progressive rise through this period, reaching a maximum of approximately 175mmol.L^{-1} in late August, before falling in September. Changes in potassium concentrations mirror sodium reaching a maximum of 7mmol.L^{-1} in late summer (see also Fig. 1). This increase continues until early September. As the temperature of the environmental decreases, ions concentrations reach their normal level, means 140-150mmol.L^{-1} for sodium and 5mmol.L^{-1} for potassium. There was significant difference for sodium among August and June, July, May, September, and also among May and June, July and September ($P<0.05$). Significant change was observed between June and July ($P<0.05$). No significant difference for potassium concentrations was observed in the blood plasma among May and June, September and also between June and September ($P>0.05$). There was no mean difference for potassium between July and August ($P>0.05$). But significant difference for potassium concentrations was observed in the blood plasma among May and July, August and also among June and July, August ($P<0.05$). There was significant difference for potassium between July and September and also between August and September ($P<0.05$).

The 15 *A. nupta* collected to check whether or not they have an active salt secreting gland were exposed to sodium chloride or potassium chloride. During their keeping period in laboratory, no food was given to them. 2mL sodium chloride or 2mL potassium chloride (see Materials and Methods) were injected over 5 days through subcutaneous injections. Before injecting the salt solutions, no a signs of secreted salt were observed around the nostrils. Also, after injecting the salt solutions in a number of days to the lizards, no secretions of Na^+ or K^+ from the nasal gland were identified in *A. nupta*. This means that, in contrast of other species of herbivorous lizards, such as *U. acanthinurus* and members of the Iguanidae, *A. nupta* do not appear to possess an active nasal salt gland.

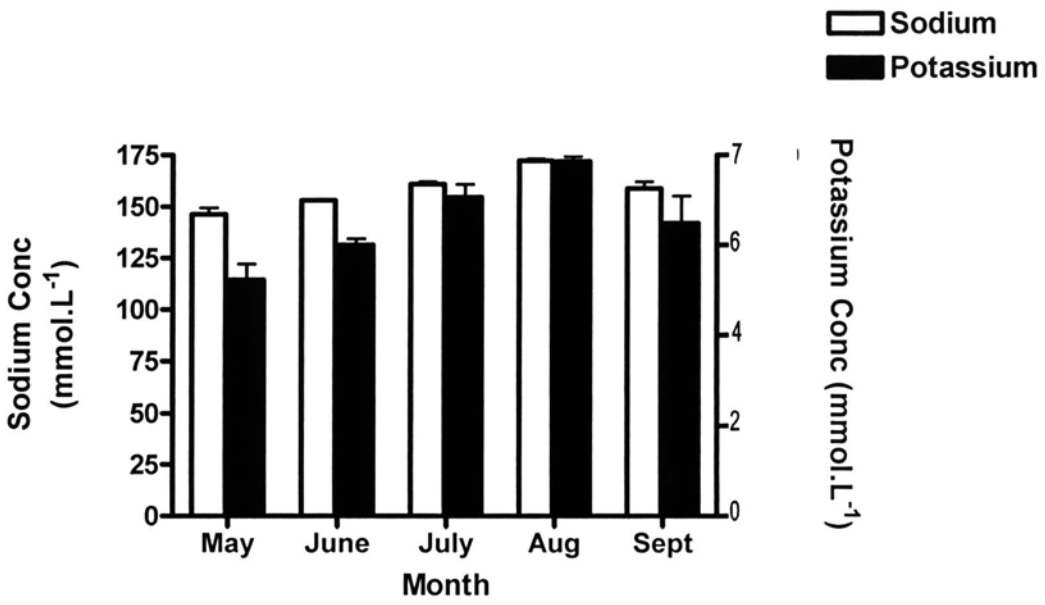

Fig. 1. – Bar chart of seasonal variation in Na^+ and K^+ concentration in the blood plasma of *Agama nupta* in Khoramabad.

DISCUSSION

Our investigation which carried out with *A. nupta* around Khoramabad has indicated that lizards fall into hibernation during the cold season, emerging to become active at the beginning of spring. The lizards are typical heliotherms, during the warm season. They use sunlight to regulate their body temperature. Typical thermoregulatory behaviour is indicated by *A. nupta* including the changes of dermal colour, which may assist in thermoregulation (RICE & BRADSHAW, 1980). *A. nupta* do not drink water and their required water is supplied solely through their insect diet.

The results obtained from the present investigation on *A. nupta* in Khoramabad indicate that plasma electrolyte concentrations increase significantly during the summer months with clear evidence of both hypernatraemia and hyperkalaemia. As in desert populations of lizards studied by LEMIRE & VERNET (1981) the plasma salt level changed according to season, increasing markedly during the warm season. In spring when environmental heat is lower than in summer, the concentration of sodium and potassium is lower in these lizards, and in summer through increasing environment temperature, the concentration of the ions particularly sodium goes up, and reaches a relatively high level in August and early September. Then, as September progresses, and temperatures fall, sodium levels decrease to reach essentially normal values. It should be considered that the percentage of humidity in spring is higher than in summer, and this factor may increase the amount of water present in the lizards' food. In summer when the humidity is low, the rate of evaporative water loss from the lizards' body is high and there is a potential for plasma Na^+ and K^+ concentration to increase through dehydration. BENTLEY (1959) reported high levels of sodium in the Australian skink *Tiliqua rugosa* in the middle of summer. BRADSHAW & SHOEMAKER (1967) studied *Amphibilurus ornatus* in Western Australia and reported a similar case of hypernatraemia, but one induced by sodium loading from the diet, rather than from dehydration. Increasing sodium concentration in some of the species depends on environment conditions and type of feeding, and may reach to 300mmol/L^{-1} and for potassium 12mmol.L^{-1} (BRADSHAW, 1997).

The secretory capacity of nasal salt gland has been examined in some species of lizards (MINNICH, 1981; LEMIRE & VERNET, 1982) with experimental loads, but due to differences in morphology and in the doses used, it is not always easy to obtain a clear idea of the potentialities of the nasal gland.

The first investigation on salt secretion on reptiles indicated that many of terrestrial lizards have a special gland in their nose, which has the property of producing a hyperosmotic secretion (SCHMIDT-NIELSEN & FANGE, 1958; SCHMIDT-NIELSEN, 1964). In the desert lizard *Uromastix acanthinurus*, which feeds on plants, electrolytes excreted from the nasal gland assist in maintaining the water and electrolyte balance in the animal (LEMIRE, 1976). There is a nasal gland with high electrolyte excretion to adapt to environment in desert reptiles. The importance of the function of nasal gland in excreting salt and regulating electrolytes concentration in some species of North America Iguanidae has been highlighted by NORRIS & DAWSON (1964), TEMPELTON (1964; 1966), SHOEMAKER et al. (1972).

Other investigations indicated that in intertidal lizard, *Uta tumidarostra*, as in other lizards, the nasal glands are able to eliminate of ions (HAZARD et al., 1998), and in desert Iguana, *Dipsosaurus dorsalis*, (HAZARD, 2001) nasal glands are capable to secret ions sodium, potassium, chloride and bicarbonate in different rates. The rate of secretions may be related to diet of lizards.

The structure of nasal gland has been studied in some groups of lizards with regard to the function of the secretory cells. In a group of lizards, like herbivorous species, such as *Dipsosaurus dorsalis*, *Sauromalus obesus* and *U. acanthinurus*, the nasal gland is obvious and cells with electrolytes-active transport features (the so called principle cells or cellules striées) can be recognized easily in histology sections (LEMIRE, 1976). In another group, consisting of desert's insectivorous Agamids like *Agama mutabilis,* the nasal gland is small and has no special cells for active transport of ions (LEMIRE, 1976). Therefore, the nasal gland is unable to act as a salt-secreting gland (SAINT GIRONS et al., 1977) to produce hyperosmotic solution. Interesting, the skink *Tiliqua rugosa* has a mixed cell population in its nasal gland but is capable of producing a mildly hyperosmotic solution (BRADSHAW, TOM & BUNN, 1984; SAINT GIRONS et al., 1977).The present study suggests that *A. nupta*, possesses a nasal gland with a structure similar to other insectivorous lizards such as *A. ornatus* and *A. mutabilis* (LEMIRE & GRENOT, 1974) that can not excrete sodium and potassium ions in an hyperosmotic solution, but this needs histological confirmation.

During the period of experiment, when *A. nupta* were exposed to sodium chloride or potassium chloride, no secretions from the nasal gland were seen. This data suggests strongly that *A. nupta* does not possess an active salt-secreting gland. Plasma sodium levels were not measured in these salt injected lizards, however, and confirmation would be needed that these were elevated by the treatment before confirming this conclusion. Histological examination of the gland is also needed to finally confirm that the gland lacks any salt-secreting ability. At this stage, it is not clear whether the hypernatraemia and hyperkalaemia evidenced by *Agama nupta* is the result of dehydration or of salt loading from the diet. To determine this, a body-mass length regression would be needed to examine whether field-caught lizards in summer were dehydrated (BRADSHAW & DEATH, 1991). If the changes on the other hand are diet induced, one would need to analyses the sodium content of the insects eaten by *Agama nupta* to ascertain whether this is hyperosmotic with respect to lizard plasma (BRADSHAW, 1986).

What is clear from the data is that *A. nupta* survives the very hot summer period in the region of Khoramabad with relatively modest increases in blood sodium and potassium concentrations. Plasma sodium concentrations of 175mmol.L^{-1} are quite low compared with other reported in literature and suggests that this species is well-adapted to its desert environment. Future research should focus on measuring a number of aspects of the physiology of this species, including its rate of evaporative water loss, the nature of its insect diet and also kidney function as all these factors interact to facilitate survival.

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Shell utilization by the land hermit crab *Coenobita scaevola* (Anomura, Coenobitidae) from Wadi El-Gemal, Red Sea

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ABSTRACT. We conducted a study in order to determine the shell utilization pattern of the land hermit crab *Coenobita scaevola* (Forskäl, 1775), the only species representing the family Coenobitidae in the Red Sea. Hermit crabs were collected during July 2003 and January 2004 along the sandy shores of protected area of Wadi-Elgemal, south Red Sea. Animals were fixed in 10% formalin and transported to the laboratory where they were weighed and measured for cephalothoracic shield length (CSL) and width, left propodus length and height. Gastropod shells species were identified, weighed and measured for shell aperture width and length and shell internal volume. A total of 391 individuals were collected (219 females, 172 males) and were found occupying ten shell species, with clear significant occupation of *Nerita undata*. A positive relationship was obtained between the size of the shells occupied and the hermit crabs. Analysis of shell internal volume and crab dimensions demonstrated that this shell dimension constitutes mainly the determinant for *C. scaevola* shell utilization. With respect to the size of the animals and the occupied shell type, *Nerita undata* was occupied by a wide range of CSL (2.5–8.5mm). Small sized crabs (2.5–3.5mm CSL) occupied *Planaxis sulcatus* and *Nassarius arcularius plicatus* while larger specimens (8.5–9.5mm CSL) occupied *Turbo radiatus*, *Polinices milanostomus* and *Monodonta canilifera*. Variations in the shell occupation were also recognized among male and females. Comparisons among populational and shell use features led us to suggest the use of this land hermit crab as key-species in the preserving program of shores and protected areas, since this species is the first organism to disappear from any shore when a new tourist establishment is implemented.

KEY WORDS : Crustacea, Decapoda, population, hermit crab.

INTRODUCTION

Among Decapod Crustacea, terrestrial and semi-terrestrial members are unusual and only small numbers of species from various taxa, including here the hermit crabs, have been more or less successful in occupying the land (WILDE, 1973). On the contrary, hermit crabs represent an important portion of the many intertidal and moderately deep benthic marine communities worldwide, where they play an important role in the food chain (FRANSOZO & MANTELATTO, 1998). Although one family, the Coenobitidae, composed of two phylogenetic closer genera, i.e. the monotypic coconut *Birgus* and *Coenobita* (MORRISON et al., 2002), both well adapted for semi-terrestrial conditions, other families and genera are almost exclusively marine, and the majority have gastropod-shell-inhabiting species with soft and asymmetrical abdomens (FOREST et al., 2000).

The genus *Coenobita* currently contains 16 species (TUDGE & LEMAITRE, 2006), and is a wide-ranging, largely tropical and subtropical genus of typical land hermit crabs inhabiting insular and coastal regions (KURTA, 1982; HARTNOLL, 1988). This semi-terrestrial hermit crabs show ubiquitous pattern of movement and migrations tendencies that are influenced by many factors as food, water, tide rhythms, rainfall, including shell exchange and availability (see KURTA, 1982; BARNES, 2003 for review).

The subject of this study is *Coenobita scaevola* (Forskäl, 1775), the only coenobitid species in Red Sea waters from a total 31 hermit crab species previously recorded (LEWINSOHN, 1969; VINE, 1986). This semi-terrestrial species is very abundant above the sea levels on the beaches of the Red Sea and the highly arid shores of Sinai Peninsula, but is totally dependent on the sea for water and consequently limited to the nearshore area (ACHITUV & ZISKIND, 1985). It lives in burrows or rest in shaded areas among coastal vegetation during daytime and then emerge at night to scavenge close to high water (VINE, 1986).

Although shell utilization by hermit crabs has been examined in other areas of the world (see MANTELATTO & GARCIA, 2000 for review), little information is available to our knowledge about the pattern of shell utilization by hermit crabs of the Red Sea. EL-DAMHOUGY (1995) investigated the mouthparts structure and mechanisms of feeding of *Calcinus ornatus* and *Calcinus nitidus* from Hurgada while EL DAMHOUGY & SADIQ (2003) studied the factors affecting the age of glaucothöe stage of *Clibanarius signatus* at initial shell entry. On the other hand, six species has been reported further north the Red Sea from the Gulf of Suez and studied for their substrate preference (EL-DAMHOUGY & HAEBA, 2003). Specifically on *Coenobita scaevola* the only work that we found was by VÖLKER (1967) who reported this species to inhabit 29 shell species in Hurgada, based on a punctual sample.

In contrast, the gastropod fauna of the Red Sea has been well documented (SHARABATI, 1984; VINE, 1986). Nevertheless, no information is available on that of the protected area of Wadi El-Gemal.

Gastropod shells are clearly important in all aspects of hermit life cycle as the main source utilized by these crustaceans to protect their soft and vulnerable abdomen. The crabs prefer to occupy shells of certain gastropod species to others, a preference not necessarily based on previous experience with these shell species but on certain shell properties that vary among gastropod species (CONOVER, 1978). Shells may function as a limiting resource to these crabs when in low abundance or adequacy (KELOGG, 1976). They may restrict crab growth (VANCE, 1972; BERTNESS, 1981), enhance their predation risk (VANCE, 1972), reduce fecundity (BERTNESS, 1981; ELWOOD et al., 1995), and modulate reproductive activity (BERTNESS, 1981; LITULO, 2004) and success (HAZLETT, 1989; HAZLETT & BARON, 1989). The patterns of shell utilization vary between hermit crab populations and are influenced by the type and size of shells available in the survey, the locality and the hermit crabs' shell preference (MANTELATTO & GARCIA, 2000; MEIRELES et al., 2003; MANTELATTO & MEIRELES, 2004).

On the other hand, research investigating aspects available worldwide on coenobitid hermits has focused on reproductive aspects (see TUDGE & LEMAITRE, 2006) and the mechanisms of migration (see BARNES, 2003 and NIEVES-RIVERA & WILLIAMS, 2003 for review). Nevertheless, the shell utilization approach for these land crustaceans has received little attention in recent years and is poorly known even though its significance. Since movement is particularly costly in the terrestrial environment, the crabs must carry their shells all the time and sometimes inadequate ones in function of their size and weight.

As an initial step to evaluate the ecological parameters affecting population of land hermit crabs in south Red Sea, we characterizes the shell utilization pattern of *C. scaevola* in a sandy beach in the protected area of Wadi El-Gemal at Marsa Alam, Red Sea.

MATERIALS AND METHODS

Hermit crabs were obtained from the protected area of Wadi El-Gemal in Marsa Alam, Red sea on July 2003 and January 2004. The animals were collected by hand at low tide from a sandy shore in the early morning by one person during a 20min walk over an area of 300m long. They were fixed in 10% formalin in seawater and transported to the laboratory. Processing started by careful removal of crabs from their shells in an anticlockwise fashion. Crab specimens were sexed by observing the position of the genital opening (gonopores). They were weighed (WW) and measured for cephalothoracic shield length (CSL=measured from the tip of the rostrum to the V-shaped groove at the posterior edge) and width (CSW), left chelar propodus length (LPL) and height (LPH). Shells were weighed (SWW) and measured for shell aperture width (SAW) and length (SAL) and shell internal volume (SIV=determined by the amount of water required to fill the empty shell by means of a measuring pipette). Measurements were carried out using a 0.1mm venier caliper. Gastropod shell species were identified according to SHARABATI (1984). The normality of hermit crab size (shield length) data was checked by Kolmogorov-Smirnoff test (ZAR, 1996). The chi-square test (χ^2) was used to compare the absolute frequency of occupation of shell species between sexes. To determine correlations between the dimensions of hermit crabs and occupied shells, regression analyses were performed (Spearman test) and by correlation coefficients using the power function equation ($Y=a.X^b$).

RESULTS

A total of 391 individuals of *C. scaevola* were collected (172 males and 219 females). No ovigerous females were observed in the samples. There was a unimodal size distribution for each sex and the size frequency distribution showed a prevalence of specimens measuring 3.5 to 8.0mm in shield length (Fig. 1). The size ranged from 2.5mm (males and females) to 9.0 and 9.5mm in shield length for females and males respectively. Overall sex ratio was 1: 1.2 in favour of females and was significantly different from the expected 1: 1 (χ^2=5.64, d.f.=1, P<0.05).

The hermit crabs were found occupying ten species of gastropod shells in different percentages (Table 1). *Nerita undata* was clearly the most occupied (86%), with no difference in shell use between sexes (χ^2=1.06, d.f.=1, P<ns), followed by *Turbo radiatus* (7.2%). On the other hand, there were significant differences in gastropod shell species occupation between sexes. *Littorina scabra, Monodonta canilifera* and *Nassarius arcularius plicatus* were occupied only by males (χ^2=16.9, d.f.=9, P<0.05). Regarding to the shell *versus* hermit correlations, shell utilization in *C. scaevola* was strongly associated with shell internal volume.

Shell species occupation as a function of hermit crab size is illustrated in Fig. 2. The diversity of shells utilized increased with the increasing of individual size. Among the occupied shells, *N. undata* dominated and was occupied by a wide range of size classes (2.5–8.5mm CSL). Small sized crabs (2.5–3.5mm CSL) occupied *Planaxis sulcatus* and *Nassarius arcularius plicatus* while larger specimens (8.5–9.5mm CSL) occupied *Turbo radiatus, Polinices milanostomus* and *Monodonta canilifera.*

Since almost all individuals (86%) were found occupying *N. undata*, we presented the regression equations showing the relations between *C. scaevola* dimensions and *N. undata* (Table 2). The equations ranked the relationships between the crab dimensions and internal volume of the occupied shell as recognized by the high correlations (r>0.80).

DISCUSSION AND CONCLUSION

The population of *C. scaevola* showed unimodality in the size-frequency distribution for the total individual analyzed. This life trait is the most common pattern among the hermit crabs (see MANTELATTO & SOUZA, 2000 for review) and reflects continuous recruitment and mortality of the species (DÍAZ & CONDE, 1989). Sexual size dimorphism was observed in *C. scaevola*, where males attained larger sizes than females. This dimorphism is a common one reported for marine hermit crabs (i.e, MANTELATTO & GARCIA, 2000; BIAGI et al., 2006), associated

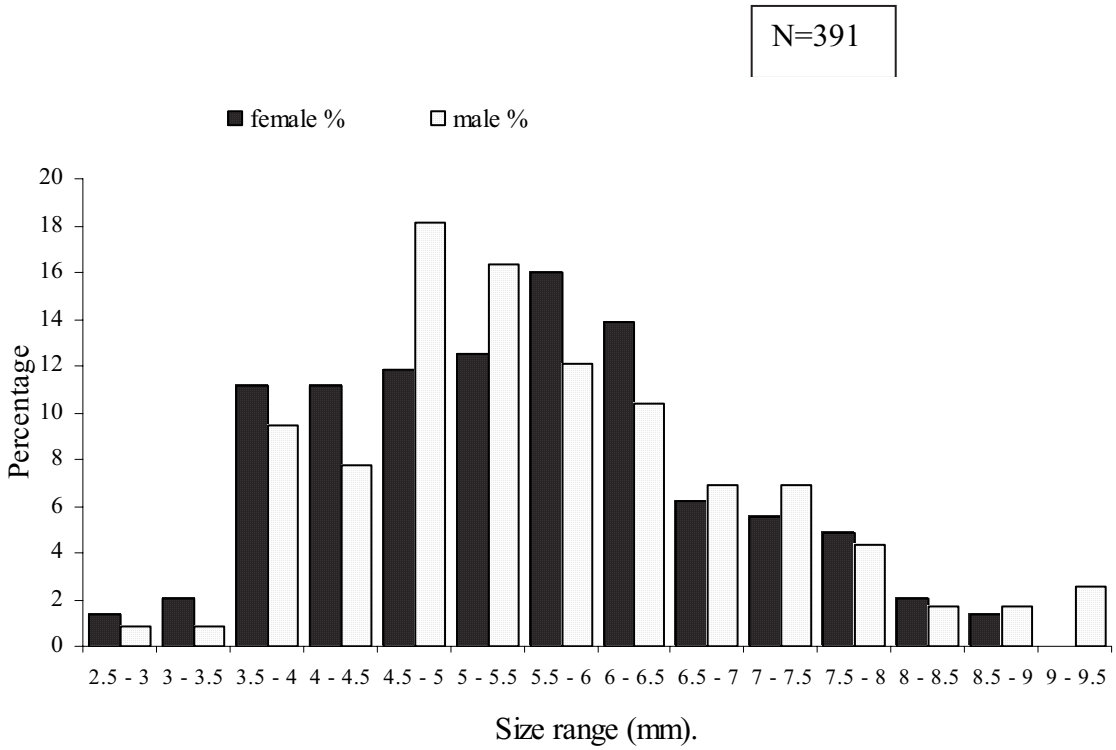

Fig. 1. – *C. scaevola.* Size frequency distribution (CSL) for the total number of individuals obtained from the protected area of Wadi El-Gemal in Marsa Alam, Red sea on July 2003 and January 2004.

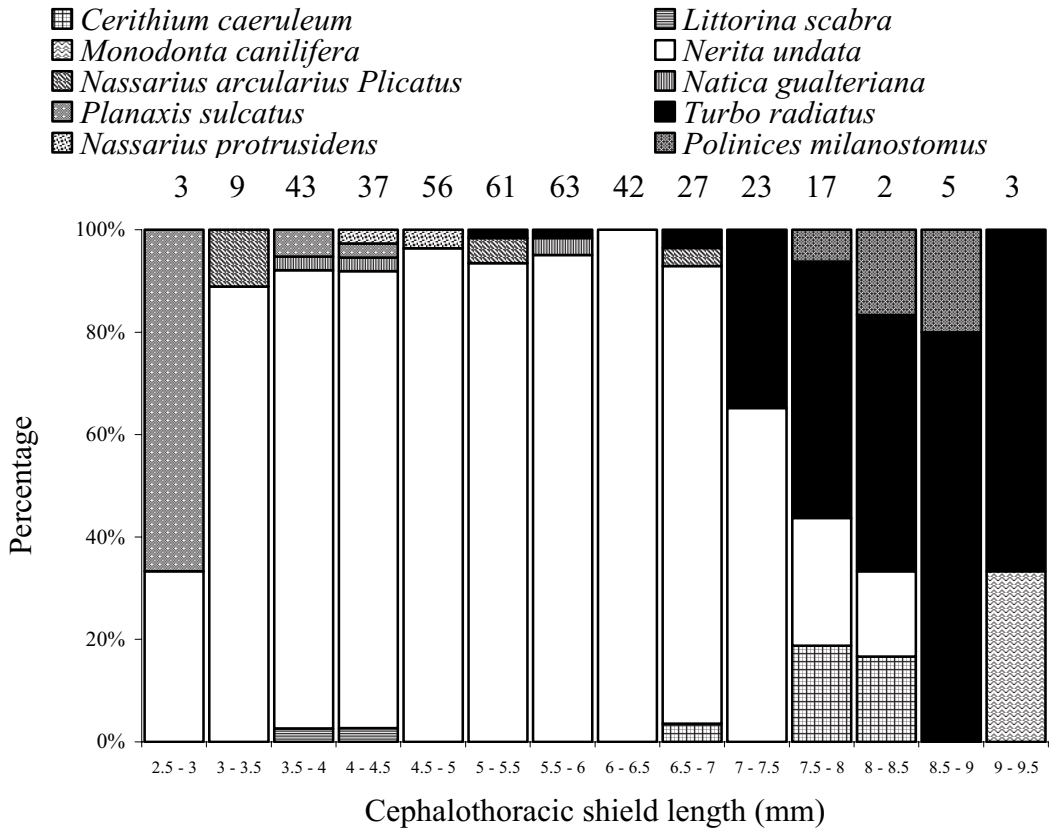

Fig. 2. – *C. scaevola*. Gastropod shell species occupation as a function of hermit crab size. The numbers above the bars indicate the total sample size for each size class.

TABLE 1

Total number and percentage of gastropod shells occupied by *Coenobita scaevola* in Wadi El-Gemal, Red Sea. (N=number of specimens).

Gastropod Shell Species	Males N (%)	Females N (%)	Total N (%)
Cerithium caeruleum (Sowerby, 1855)	3 (1.74)	2 (0.91)	5 (1.3)
Littorina scabra (Linnaeus, 1758)	2 (1.2)	–	2 (0.5)
Monodonta canilifera (Lamarck, 1816)	1 (0.6)	–	1 (0.3)
Nerita undata (Linnaeus, 1758)	138 (80.2)	197 (90.0)	335 (86.0)
Nassarius arcularius plicatus (Roding, 1798)	5 (3.0)	–	5 (1.3)
Natica gualteriana (Recluz, 1844)	2 (1.2)	2 (0.9)	4 (1.0)
Planaxis sulcatus (Born, 1778)	3 (1.7)	2 (0.9)	5 (1.3)
Turbo radiatus (Gmelin, 1791)	14 (8.1)	14 (6.4)	28 (7.2)
Polinices milanostomus (Gmelin, 1971)	2 (1.2)	1 (0.5)	3 (0.8)
Nassarius protrusidens (Melvill, 1888)	2 (1.2)	1 (0.5)	3 (0.8)
Total	**172 (43.99)**	**219 (56.01)**	**391**

TABLE 2

Regression equations for the relations between hermit crab *Coenobita scaevola* dimensions and the most occupied shell *Nerita undata* (**R^2**=determination coefficient; CSL=cephalothoracic shield length; CSW=cephalothoracic shield width; LPL=left propodus length; LPH=left propodus height; WW=hermit crab wet weight; SAW=shell aperture width; SAL=shell aperture length; SWW=shell wet weight; SIV=shell internal volume; N=391).

Relations	Y= a.X^b	R^2
SAW x CSL	$SAW=0.6401CSL^{0.9586}$	0.49
SAW x CSW	$SAW=0.4293CSW^{0.9681}$	0.49
SAW x LPL	$SAW=0.7574LPL^{0.9469}$	0.53
SAW x LPH	$SAW=0.4910LPH^{1.0645}$	0.50
SAW x WW	$SAW=0.0018WW^{2.5603}$	0.48
SAL x CSL	$SAL=1.3051CSL^{0.7578}$	0.67
SAL x CSW	$SAL=0.8851CSW^{0.7631}$	0.67
SAL x LPL	$SAL=1.7377LPL^{0.6804}$	0.62
SAL x LPH	$SAL=1.1556LPH^{0.8064}$	0.66
SAL x WW	$SAL=0.0124WW^{2.016}$	0.66
SWW x CSL	$SWW=4.3139CSL^{0.3454}$	0.53
SWW x CSW	$SWW=2.9382CSW^{0.3542}$	0.56
SWW x LPL	$SWW=5.0633LPL^{0.3175}$	0.52
SWW x LPH	$SWW=4.1154LPH^{0.3723}$	0.55
SWW x WW	$SWW=0.2992WW^{0.9441}$	0.53
SIV x CSL	$SIV=5.8882CSL^{0.4691}$	0.79
SIV x CSW	$SIV=4.0399CSW^{0.4765}$	0.79
SIV x LPL	$SIV=6.747LPL^{0.4384}$	0.77
SIV x LPH	$SIV=5.7532LPH^{0.5065}$	0.79
SIV x WW	$SIV=0.6855WW^{1.2696}$	0.79

principally to differences in energetic repartition between sexes (Abrams, 1988) and is important in selective pressure as intra- and interspecific fights for food, copulation, territory and shell (Mantelatto et al., 2005). This dimorphism can be attributed to such factors as differential mortality and growth rates between sexes (Abrams, 1978) with males reaching larger sizes within a shorter time than females, but being influenced by shell limitation, a fact that may imply reduced survival (Fransozo & Mantelatto, 1998; Mantelatto & Garcia, 2000).

Coenobita scaevola has the greatest number of zoeal stages (7 stages) and the longest zoeal life span (54-80 days) among the members of genus *Coenobita* (see Al-Aidoroos & Williamson, 1989 and Wang et al., 2007). Comparatively, the larval development of *Coenobita clypeatus* and *C. rugosus* require about 22-30 days to attain the glaucothöe respectively, and at least an additional month to the first crab, the presumed settling stage (Provenzano, 1962; Shokita & Yamashiro, 1986). Since in the studied period we have not found ovigerous females we may expect that juveniles are not being recruited to the population all over the year. This condition was corroborated by a low number of small sized crabs (2.5–3.5mm CSL) found in the Wadi El-Gemal. Thus we infer that settlement could take place in a different habitat other than the usual one for the adults, due to the fragility of these organisms and necessity of small shells supply. The presented pattern has been reported for other marine hermit crabs (Mantelatto & Sousa, 2000; Garcia & Mantelatto, 2001a; Macpherson & Raventos, 2004). According to Ball (1972), it appears that small sized individuals of *C. compressus* are much more sensitive to desiccation than larger animals and great aggregations can be found under ledges, in small rocky caves where is slightly more moisture.

Differences in gastropod shells utilization can occur as a function of the area of occurrence of the hermit crabs (Garcia & Mantelatto, 2000). *Coenobita* species are frequently found in areas, such as sandy beaches, where shells are extremely scarce (Ball, 1972). Specimens of *C. scaevola* of Wadi El-Gemal were found occupying ten species of shells with relative differences between sexes. *Littorina scabra, Monodonta canilifera* and *Nassarius arcularius plicatus* were occupied only by males. This fact probably indicates the resource competition/partition occurring mainly to guarantee a good adequacy of individual size to shells available in the survey. Differences in shell utilization between sexes were also observed by Imazu & Asakura (1994) and Bertini & Fransozo (1999). These differences may be due to differences in body size, competitive ability or reproductive behaviour,

respectively (BERTNESS, 1981; BLACKSTONE, 1985; IMAZU & ASAKURA, 1994). Also, in semi-terrestrial hermit crabs a well-fitting shell is essential for maintaining low evaporation rates and carrying ample water. An appropriately sized shell in good condition allows invasion of inland environments offering more shade, food, and fresh water for *C. clypeatus* studied on Curaçao (WILDE, 1973). According to this author, the hermit crabs with broken, ill-fitting shells are restricted to the coast, must rely on drinking seawater, and appear to be in relatively poor conditions.

A population of *C. scaevola* studied almost 40 years late by VÖLKER (1967) in the close region of Hurgada were found occupying a greater diversity of gastropod shells species (n=29) than the population studied here on the sandy shore of Wadi El-Gemal (n=10). Several hypotheses are possible to explain this apparent discrepancy in gastropod shell occupancy rates found between the two populations: 1) Gastropod life cycle – availability of different shell types (species) in nature is determined by the relative abundance of different live gastropods and their mortality rates (MEIRELES et al., 2003); 2) Environmental conditions – differences in abiotic characteristics of these two areas in terms of water dynamics (wave activity, intensity of currents, food supply) are determinant of installation of some invertebrate species (FRANSOZO & MANTELATTO, 1998); 3) Predation pressure – several combined actions from natural (crabs) and artificial (human tourism) predators can act in different ways to reduce the diversity of gastropod shells in the region.

Similarly as other marine hermit crabs, *C. scaevola* shows highlight occupation of one species of gastropod shells, *N. undata*, over other ones. This shell was occupied by a wide range of individuals of both sexes with CSL varying from 2.5 to 8.5mm, while large sized crabs (8.5–9.5mm CSL) occupied a variety of shells. Although that shell availability was not evaluated, this intense occupancy would indicate active selection behaviour in *C. scaevola* in the field. Interestingly, in the past study VÖLKER (1967) found that shells generally used by *C. scaevola* were found in the same frequency in the gastropod fauna, revealing a close relationship between shell use and availability of the resources. According to the energy savings hypothesis, proposed by OSORNO et al. (1998), *C. scaevola* of Wadi El-Gemal preferred *N. undata* because it's the lightest of the shells available thus reducing the cost of bearing and carrying a shell. Large sized crabs might have modified their shell preference and occupied other species when large shells of *N. undata* became no longer available. The energy saved by carrying a light shell may be used to increase growth rate and egg production of intertidal hermit crabs, which ultimately improve fitness (GUILLÉN & OSORNO, 1993; BERTNESS, 1981). Also, crabs occupying shells large enough that they can withdraw completely and block the shell aperture with the chelipeds are much harder to extract from their shells than crabs which are too large to withdraw completely, and for this reason they would presumably be less vulnerable to predators (BALL, 1972). In conclusion, this pattern should be associated with the availability of resources and/or to the better suitability of these shells species to the condition of the individuals in the natural habitat.

Competition for shells may not be as intense among land hermit crabs as among their aquatic relatives. ABRAMS (1978) reported no fights for shells among *Coenobita compressus* in the field in Panama, whereas marine hermit crabs frequently fight. In opposite way when compared with marine hermit crabs, larger individuals of land hermit crabs tend to inhabit shells nearer their preferred size, i.e., shells that are modified by previous hermit crab use. The new shells have too little interior volume and are enlarged by hermit use (WOLCOTT, 1988). In this connection it is interesting to note that the majority of the shells occupied by *Coenobita* seem to be missing the columella (BALL, 1972). A similar observation has been made by KINOSITA & OKAJIMA (1968) on shells of *Nerita striata* occupied by *Coenobita rugosus* from Japan. ABRAMS (1978) suggested that rather than competition, terrestrial hermits show "shell facilitation"; that is, larger populations of crab generate, through wear, larger numbers of shells suitable for adult crabs. He accounted for limited adult populations by limited "recruitment" of entry-level shells, or possibly by predation on or food competition between adult hermits.

Interestingly and on the contrary to the proposed by VÖLKER (1967), that affirm "land hermit crabs seem to have no relation to the shells of a given snail species", we found that shell dimension constitutes mainly the determinant for *C. scaevola* shell utilization, adopting in the Red Sea similar strategy developed by some other tropical and subtropical marine hermit crabs (GARCIA & MANTELATTO, 2001b).

The absence of ovigerous females during the two sampled periods was also an interesting and important point in the present study indicating the need for further frequent and systematized studies on this population since this is a rare pattern for these crustaceans. The presence of ovigerous females in some populations studied around the world have been previously documented (BALL, 1972; WILDE, 1973; VANNINI, 1976; SHOKITA & YAMASHIRO, 1986). So, despite the fact that reproduction in coenobitids has covered a range of topics in recent years (TUDGE & LEMAITRE, 2006), the factors responsible for the absence of egg-bearing females are not clear and may involve methodology design (only two sampled periods), shell resource availability, interspecific competition for food and shelters, and others. The data obtained from two dates by daylight collection provided limited information about the reason for this absence. However, some hypotheses can be raised taking in account the limited range of the collection: 1) peaks of presence of ovigerous females probably occurs in different periods; 2) ovigerous females may be taking shelter at other points along this beach showing a cryptic habit during daylight and active at night as *C. compressus* (BALL, 1972), a common occurrence observed among ovigerous females of crustaceans; Particularly for *C. scaevola*, and other coenobitids, VANNINI (1975) showed either daily or seasonal migrations depending upon rainfall. Also, spawning females of *C. clypeatus* presented unusual pattern since they did not enter the water, but move toward the sea at low tide to drop or fling their eggs onto the wet rocks (WILDE, 1973).

As a result of the intensive unregulated tourist development in the Red Sea area, a shore-monitoring program

was established for the protected area of Wadi-El-Gemal. Continuous surveying of shores and beaches by rangers has led to the observation that *C. scaevola* is the first organism to disappear from any shore when a new tourist establishment is constructed (W. SALLAM, pers. comm.). Accordingly, this study suggests the possibility to this species to be used as a key-species in the preserving program of this protected area. Its rarity or abundance on any shore could reflect the degree of healthiness of that shore and might assist in assessing the amount of deterioration of the shoreline which has a considerable impact on the different biota.

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A review
Historical evolution of preformistic versus neoformistic (epigenetic) thinking in embryology

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To the memory of Professor Doctor Lucien Vakaet sr

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ABSTRACT. In the classical embryology there exist two main concepts to explain the rising of a living being. At one hand there exists the theory of preformation i.e. all the parts of the future embryo would already exist preformed during the preembryonic or early embryonic period. On the other hand there is the epigenetic view in which it is propounded that all parts arise by neoformation from interacting (induction) previously existing, apparently simpler structures. We found evidence that initially a kind of preformation (or pre-existence), disposed in concentric circular layers, exists in the full grown oocyte and early germ. After normal development these circular layers will settle successively, from the centrum to the periphery, into the central nervous system, the notochord, somites, lateral plates (coelom) definitive endoderm. Under influence of mechanical and gravitational forces an early epigenetic development starts by unequal oblique uptake (unidirectional chaos) and segregation of ooplasmic determinants in the germ. So an epigenetic cascade of early general body plan formation occurs in the vertebrate embryo. Recently, we demonstrated by hemi-sectioning of avian unincubated blastoderms that both a kind of preformational (mosaicism) as epigenetic (regulation) behaviour depends on the spatial, ooplasmic distribution of Rauber's sickle material, homologous to the also sickle-shaped Wnt expressing gene region in ascidians. This clearly brings preformation and epigenesis much closer together since there is a common influencing ooplasmic factor. An ooplasmic continuity bridging the premature oocyte ooplasm to the embryonic primordial germ cells of the following generation is demonstrated. Both nuclear and ooplasmic continuity in the oocytes, present in the ovary of birds and mammals, is shown by radioactive premeiotic DNA labelling.

KEY WORDS : preformation, mosaicism, epigenesis, predispositions, ooplasmic determinants.

INTRODUCTION

Among all biological topics, embryology (developmental biology) presents most uniformity in the animal and even in the vegetal kingdom. So, not with standing the pronounced structural differences between species, it forms a common link among all living organisms. All embryological processes are based on cell biological, genetical and biochemical principles since every organism is formed by isolated or associated cells. In embryology one studies the very complex processes by which these cells differentiate and associate from one single initial more or less voluminous cell: the egg. The egg is the link between two generations and already by its volume it gives the impression to contain more then an ordinary cell. Our mind needs, already from its earliest manifestation, to know how a young animal, a child or a plant makes its appearance. It is still one of the first questions that children ask for when they begin to think and realize their own existence. Moreover their own origin, as is the case for most mammals is most difficult to observe since the mammalian embryo is completely hidden from direct observation. By contrast, avian embryos are more accessible to direct observation. For instance chickens lay eggs, incubate them by their own body heat and after approximately 20-21 days, as touched by a magician, hatching of a young bird takes place. It is easy to open the eggs at the successive stages of development, sometimes even without killing the embryo. This has been and is still one of the bases of modern embryology.

EARLY HISTORY OF IDEAS IN RELATION WITH THE ORIGIN AND NATURE OF THE GERM

Ancient Arabs and old Germans thought that only mothers have the possibilities to give origin to the development of a child, whilst people in Greece considered that the father was only responsible (SHORT, 1977). Greek natural philosophers generally interpreted female seminal fluids as menstrual blood. The female usually was considered inferior to the male: due to a lack of internal heat, this menstrual blood did not form true seed. This did happen in males, leading to male seed or sperm, which also was thought to form out of blood (generally conceived as a life giving principle). Hippocrates (460-380 B.C.) "the father of medicine" was the first to control theories with real experiments. He made from embryology a separate science and made comparative embryological studies between the chicken and the human embryo. He accepted that both the female as the male contributed equally to the formation of the germ. ARISTOTELES in the 4th century before J.C., also called "father of the natural history", was the first to describe the "punctum saliens", the early beating heart of the avian embryo and followed its evolution, day by day during further incubation. While he observed

the evolution of the chicken embryo and it's appearing organs, he concluded that the organs appear successively and not at the same moment. Therefore he proposed the so-called epigenetic development in contrast to the preformation hypothesis. Aristoteles did not consider uterine blood but the male seed as the essential factor for germ formation. Aristoteles saw it as an unmaterial principle of movement "triggering" the menstrual blood to further selforganize. The female "seed" is to be considered as the material cause of any embryological process. Ancient people must have observed the existence of egg sacs with large eggs in the ovaries from birds. The yellow aspect of the large follicles in the ovary and a similar aspect in the laid egg were the first indication that eggs are formed in the ovary. So the link between ovarian oocytes and the avian germ in the laid egg (oviparity) was established. But the principle of viviparity (development of an embryo in the female organism) could originally not been explained. ARISTOTELES in the 4th century before J.C. has made embryological observations on much different kinds of animals. He followed not only methodically the development of the chicken embryo but has also observed the embryos of dolphins and larval stages of insects (CAULLERY, 1957). The role of the male and female respectively in the generation process could be explained according to Aristoteles by the example of the chicken. Indeed, the latter taken apart from the rooster still continues to lay eggs in which however no embryos develop. Therefore he concluded that generally speaking the female furnished the constituting material of the embryo whilst this material was vitalized by the male. The place where this occurred in the chicken egg would be the cicatricular region where the germ disc, forming the base of the future embryo develops. Departing from Aristoteles's natural philosophy, VAN SPEYBROECK et al. (2002) have historically shown that epigenesis gained alternating attention from the 17th century onwards. It was considered to be the opposite of the preformationist tradition. Where preformationism stated that the germ cells of each organism contain preformed miniature adults that unfold during development, epigenesis held that the embryo forms by successive gradual exchanges in an amorphous zygote. CLAUDIUS GALENUS (131-201) one of the founders of anatomy and physiology was born in Pergamon. He made dissections of animals, mainly apes, because it was not allowed to study human corpses. His influence has been very important until in the 16th century. Galenus described the female ovaries as testes and propounded that the male sperm was secreted by the testes. So the notion of gonads (genital glands) male or female was established. Galenus holds a very naturalistic view on the embryological processes in terms of growth, nourishment and genesis (change and shaping). After Galenus during 13 centuries, science and particularly embryology were completely influenced by religious ideas and no new discoveries were made.

PERIOD OF THE EARLY ANATOMISTS

Fabricius Ab Aquapendente (teacher of William Harvey) had a chair of anatomy at Padua and made an enormous contribution to embryology (ADELMANN, 1942). He

Fig. 1. – Preformation concept during the 17th century: drawings of the early developmental stages of the chicken embryo in which, according to Fabricius Ab Aquapendente, already the adult form could be seen.

was probably the first investigator to give an exact account of the role of the ovary in the formation of the hen's egg, for he observed that besides that the yolk was formed in the ovary that the egg white, the shell membrane and the shell were all formed during the transit of the egg down the oviduct. This key observation was the first indication that the egg might be produced directly by the female rather than as the result of the union of male seed "and female soil" as supposed by Aristoteles. He also thinks that the avian egg is fecundated by the so-called "aura seminalis" (an emanation from the semen of the rooster). FABRICIUS was also a preformationist since he pretended that a "little birdie" was visible in the egg during the earliest developmental stage (1637) (Fig. 1). However the initial developmental stages of most of the embryos have a size which makes them invisible to the naked eye. The first microscopes appear in the 17th century, but only in the 19th century a higher degree of perfection permitted their real use for embryological observations. Embryos in advanced developmental stages are more or less visible to the naked eye and already have more or less the form and structure of the adult animal. This suggested the attractiveness of the theory of preformation i.e. that development was only the result of the progressive enlargement of the germ, having from the beginning its final constitution and complexity. The preformation theory seemed to be first propounded by the arab scientist, AVERROES (also called Ibn-Roschd) who was a teacher at Cordoba (1115-1198). Contrary to this theory, the theory of epigenesis was formulated: the complex structure of the definitive adult forms and even the embryo is only realized progressively by transformation of more simple structures. In the course of the history of embryology, as a research discipline, it appeared that a kind of progressively build up preformation (pre-existence) exists in the egg (already before fertilization), which is indispensable for early embryonic development. Initially, epigenesists defended the view that the embryo progressively forms out from a homogeneous matter.

Aristoteles, but also HARVEY (1653) claimed that it is most certain that in the egg there is no prepared material at all. It takes until the studies of Albrecht Von Haller (1708-1777) first adhering to epigenesis, later becoming a strong ovist-preformationist, to conclude that the egg does contain differences in viscosity.

EARLY EXPERIMENTATORS AND USE OF MICROSCOPES

WILLIAM HARVEY (1578-1657), medical doctor from the king (Charles I) of England, discovered the blood circulation (1628) and made dissections from female deer's after coitus and states that the young germs of the mammals are present in the form of eggs. In 1653 appears his book: "Exercitationes de generatione animalium".

He did point out a place where fecundation would occur, but at the same time, his research on deer did not allow him to conclude that the male semen made any material contact with the egg (or left any visible trace) on the female egg. According to him the little white cicatricula on the top of avian eggs is the place where fecundation occurs. After incubation this region extends and forms a semi-liquid mass (colliquamentum) in which soon the first traces of the embryo and particularly the heart appear. From him comes the aphorism "ex ovo omnia": every living being develops from an egg (1651). REGNIER DE GRAAF (1672) who was an excellent experimentator (LINDEBOOM, 1973) observed that after killing, female rabbits at different moments after coitus present a progressive evolution in the vesicles bulging at the surface of the ovary, followed by scars after ovulation. These "eggs" (sic) were found afterwards in the uterus. He thinks erroneously that the vesicles bulging at the surface of the ovary before the coitus were really the eggs themselves (SAWN, 1997). He observed that once they were found in the rabbit uterus they are first small but later larger, adhere to and become fixed into the wall of the uterus (implantation). Now we know that the ovarian vesicles described by Regnier De Graaf are not the eggs but are follicles surrounding the real, much smaller eggs. These follicles are now still called Graafian follicles in modern literature. At nearly the same moment, thanks to the use of the first microscopes, spermatozoa were seen in semen (perhaps by HAMM, or HARTSOEKER, 1694; fig. 2) but they were surely seen, drawn and described by the Dutch researcher ANTONIE VAN LEEUWENHOEK (1678; 1683). The main discovery of Van Leeuwenhoek was his finding in 1677 of "little animals" (animalcules) in semen (LA BERGE, 1999). He found these animalcules in semen from men but also from roosters and in the semen of different mammals. So Van Leeuwenhoek became an "animalculist" or "spermist" who thought that later the embryo was formed from one of these little animals (Fig. 2) or spermatozoa (Von Baer used this word). According to Van Leeuwenhoek, De Graaf's egg, if it did anything, provided no more than nourishment to the embryo. During the next century little further research was done on the topic, although there were much theoretical discussions between the animalculists and the ovists (who considered the egg as the location in which the preformed embryo resides). De Graaf's ideas on the egg as being the precursor of the embryo fell in some disfavour because the evidence for the animalculists was easy to obtain by simple observation of semen under the microscope, which seemed to demonstrate the animalculist preformation theory (LINDEBOOM, 1973). The ovistic hypothesis of De Graaf was on the contrary more difficult to control. It was in fact only much later, 100 years after De Graaf's death, that CRUIKSHANK repeated and confirmed his studies on embryonic development in rabbits. Marcello Malpighi (1628-1694) described the capillary circulation in the lungs of frogs and also studied the development of the chicken embryo. His study "De formatione pulli in ovo" appeared in 1672. This biologist used systematically a microscope (fabricated by JANSSEN in 1590). He described the cicatricula as the most important part of the avian egg for the development of the future embryo after incubation. With Malpighi the microscope became an indispensable tool for biological research (LAMS, 1935; LÜTHY, 1996) and he adhered to ovism. In 1740, at the age of 20, CHARLES BONNET (Switzerland) describes for the first time the phenomena of natural parthenogenetic development (from a virgin egg) in a plant-louse. These observations of BONNET on virginal reproduction were confirmed by RÉAUMUR and BEGUELIN, TRIMBLEY and ALBERT VON HALLER. This discovery of parthenogenesis was strongly in favour of the ovist theory of preformation. However, the eventual "emboîtement" of the successive generations could hardly been explained. We must also mention the theory of organic molecules developed in 1745 by MAUPERTIUS (DOLLANDER & FENART, 1973). Seminal liquids both from the mother as from the father, coming from all parts of the body, should mix after mating in the uterus. So, Maupertius tries to explain that some characteristics of the father or mother are recognized in the children. By contrast both ovists and spermatists (or animalculist) believed in the theory of preformation i.e. that the egg or the spermatozoon contains a miniature individual with all the parts of the adult. FABRICIUS AB AQUAPENDENTE (1637) and MALPIGHI (1669) were convinced preformists and according to them the avian germ disc contains a miniature organism already in its definitive form (Fig. 1) (ADELMANN, 1942; 1966). By growing, its different parts unfold progressively into an adult form. Albertus Von Haller (1753) had a more evoluated idea (with epigenetic background) about preformism. He wrote: "nulla est epigenesis, nulla in corpore animali pars ante aliam facta est et omnes simul creatae existunt". He found no recognizable organs in the chicken germ but considered that only their Anlagen were present and that these will progressively develop into their adult form. Von Haller was an authority in embryology and studied the chicken embryo at early stages after incubation. He wrote a book in French and described the development of the heart, the eye and respiratory phenomena (see the title page of his book: Fig. 3). He uses the chicken embryo to explain the formation of some anatomical structures in the human.

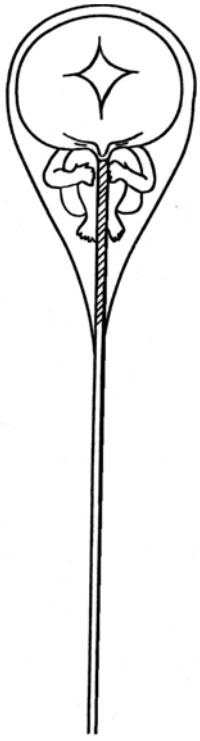
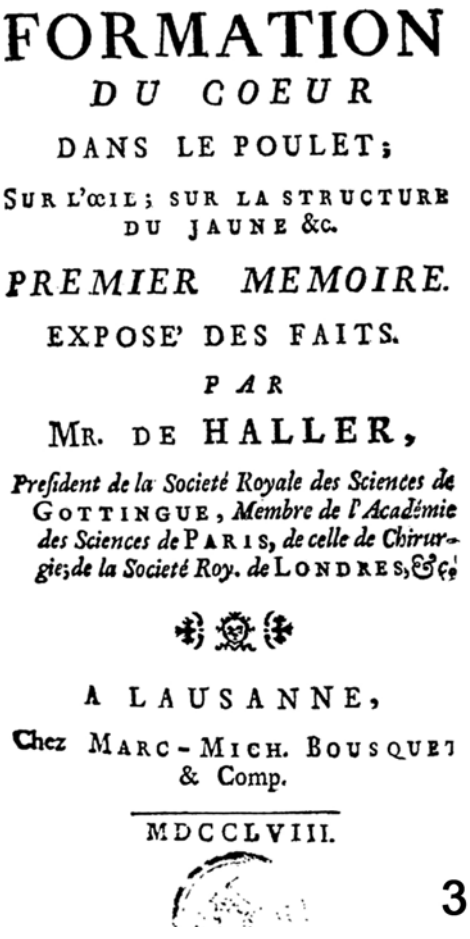


Fig. 2. – Drawing of the microscopic aspect of a human spermatozoon of animalculist Hartsoeker, in which he seems to see a miniature baby as was also accepted by Van Leeuwenhoek.



FORMATION
DU COEUR
DANS LE POULET;
SUR L'ŒIL; SUR LA STRUCTURE DU JAUNE &c.
PREMIER MEMOIRE.
EXPOSE' DES FAITS.
PAR
MR. DE HALLER,
Preſident de la Societé Royale des Sciences de GOTTINGUE, Membre de l'Académie des Sciences de PARIS, de celle de Chirurgie; de la Societé Roy. de LONDRES, &c.
A LAUSANNE,
Chez MARC-MICH. BOUSQUET & Comp.
MDCCLVIII.

Fig. 3. – Tittle page of the book of Von Haller (1758) in which he still accepts original preformation concepts.

EVIDENCE FOR EPIGENETIC DEVELOPMENT

The old preformistic view dominated in embryology up to the middle of the 18th century. During that period a new view on embryonic development was introduced by C. F. Wolff (1734-1794). He was born in Germany where he started his research in anatomy and embryology. This resulted in the publication of a dissertation entitled "Theoria Generationis", published in Germany in 1759. In this study, C. F. Wolff developed and defended a so-called epigenetic view based on careful microscopical observations of early embryonic development. According to Wolff the chicken embryo is not formed by the accumulation of juxtaposed parts but the organs arise progressively the one after the other from more simple structures. Thus the gut is composed first from a flat membrane which later forms folds and finally has the general cylindrical aspect of a digestive tract. In the same way he describes the development of the neural groove followed by the medullary cord. So in the early germ nothing exists "in facto" but all "in potentio". Thus from already pre-existing structures new and more complicated structures and organs were formed.

Wolff did not so much stress the existence of pre-existing structures. He did argue in favour of "an active nature". This is however contrary to the preformistic concept of nature as a dead mass unto which blind mechanical forces work. It is in this regard that Wolff talks about "inorganic matter", as the heterogeneous, but still unorganized, matter out of which an embryo self-organizes. At that time however this theory of epigenesis was not accepted in German scientific circles. So Wolff could not find a place in the German universities and immigrated to Russia. At the end of 1766, the St. Petersburg Academy of Sciences invited him to work as academician in the anatomical department. Finally he settled in St. Petersburg where he worked in the field of embryology, teratology and anatomy. The discovery of organic transformation and neoformation by C. F. Wolff was misunderstood by his contempories. According to Blumenbach's unordered matter does not have the power to order itself, thus life cannot spring from non-live. The organization one sees in life, is due to a physiological impecunious principle of internal correspondence (Bildungstrieb), ungraspable to the human ratio. This principle is not equal to the mechanical formative power or Bildungskraft that inorganic matter also possesses. Blumenbach's *nisus formativus* or "Bildungstrieb" is not entirely the same as Wolff's *vis essentialis*. Whereas Wolff's "wesentliche Kraft" is single in nature, producing but one effect, varying only through the influence of the surrounding context, Blumenbach's *nisus formativus* was a multiple active force which could produce many different things by itself, making it by itself sufficient to generate a new organism. This makes Blumenbach much more into a vitalist than Wolff.

For Wolff the *vis essentialis* produces the different parts of the organic body no longer merely through itself and according to its nature, but rather with the help of countless other concurring causes, "and what it does through itself alone, becomes a completely simple effect, as attraction or repulsion, and is worlds apart from the

building of organic bodies" (WOLFF, 1789 in ROE, 1981: 117). This point is very important, since it was and still is often thought that Wolff deduced the total formation of matter from the *vis essentialis*. Even his opponent, Von Haller, did not grasp this point fully: "why does this *vis essentialis*, which is one only, forms always and in the same places the parts of an animal which are so different, and always upon the same model, if inorganic matter is susceptible of changes and is capable of taking all sorts of forms? Why should the material coming from a hen always give rise to a chicken and that from a peacock give rise to a peacock? To these questions no answer is given" (VON HALLER in NEEDHAM, 1959: 202). Wolff asserted several times that people paid too much attention to his *vis essentialis* and that his theory of attraction and solidification would have been the same without it. A follower of Wolff's epigenetic theory was TREDERN DE LÉZÉREC, a usually forgotten pioneer of chick embryology. He submitted his thesis in Jena (1808). The importance of the male semen, next to the female egg for reproduction was shown during the same period by Lazarro Spallanzani (1729-1799). Using amphibian eggs, SPALLANZANI applicated the artificial insemination method used by JACOBI (published in the Hannover magazine in 1763) for fecundation of trouts or salmon. This method of Jacobi is still used at present to populate waters. Spallanzani did not believed in the "aura seminalis" theory of Harvey, as he was convinced of the necessity of material contact between egg and semen (more specifically spermal fluid). He demonstrated that the fecundation capacities of the semen of an amphibian (*Rana temporaria*) disappears after filtration or heating. That fertilization is the result of the effect of spermatozoa on an egg was concluded by PREVOST and DUMAS in 1824. They repeated the experiments of Spallanzani and demonstrated unequivocally that the spermatozoa are the real fecunding elements in the semen. The idea that the spermatozoa play a major role in fertilization by penetration, as was propounded by Prevost and Dumas, was not accepted by all biologists in that time. TH. W. BISCHOFF (1807-1882), in 1842 and J.P. MULLER (1801-1858) in 1844 doubted about this. Bischoff claims in "Entwicklung des Hunde-Eies (1845): "ich habe nie im Inneren eines Eies einen Spermatozoide auffinden können. Die Wirkung des Saamens auf das Ei halte ich dann zunachst fur eine chemische". By contrast, L'ALLEMAND believed strongly that fecundation consist of the union of two living parts: "un fluide ne peut évidemment transmettre la forme de la vie qu'il ne possède pas". The fusion of spermatozoa with eggs has been really observed only for the first time clearly in Ficus by THURET (1854). The development of the embryology has also been influenced by evolutionistic concepts during the 19th century by LAMARCK (1802) and DARWIN (1859). In succession of F. MÜLLER (1844), HAECKEL formulated the so called biogenetic fundamental law, according to which ontogenesis summarizes phylogenesis. This means that the development of an individual being is a partial recapitulation of the evolution of his ancestors. The checking of this hypothesis has been at the origin of an important boom in descriptive embryology during the second period of the 19th century. Although Wolff's epigenetic ideas in embryology were not accepted in Germany, his influence was great on the founders of the Russian embryological school, C. H. Pander and K.E. Von Baer (SANDER, 1996; MIKHAILOV, 1997). Pander, a Russian zoologist born in Riga (12/7/1794) made a thorough description of the developing chick embryo with the three layers forming the body. His schemes of chick embryonic, development at different stages, surprisingly resemble modern classic descriptions. Pander demonstrates for the first time in history that a bird embryo develops from three germ layers (Fig. 4). PANDER (1817) distinguishes clearly in the cicatricula (germ disc region) of the unincubated avian egg the superficial "blastoderma" (now called blastoderm) and the central underlying "nucleus cicatriculae" (now called nucleus of Pander). Pander observed that the tinny membranes which will form the germ also contain a great generative power (*nisus formativus*) already described by BLUMENBACH (1789). Karl Ernst Von Baer (1792-1876) was born in Piep (Estonia) and, after graduating from the university, he started his career as zoologist and embryologist. He worked as professor of Anatomy at the University of Königsberg. His microscope was already much better then from his predecessors. Later he worked in the St. Petersburg academy of Sciences, where he worked for nearly 30 years and published more than 400 manuscripts. His scientific work was as he described himself influenced by two persons: "So dürfen die vorliegenden Untersuchungen sich rühmen, eine Folge jener für die Naturwissenschaft ewig denkwürdigen Verbinding zu sein, in welcher ein in physiologischen Forschungen ergrauter veteran (Döllinger), ein von Eifer für die Wissenschaft glühender Jüngling (Pander) sich verbanden um durch vereinte Kräfte eine feste Grundlage für die Entwicklungsgeschichte des thierischen Organismus zu gewinnen" (cited by VAKAET, 1965; page 137).

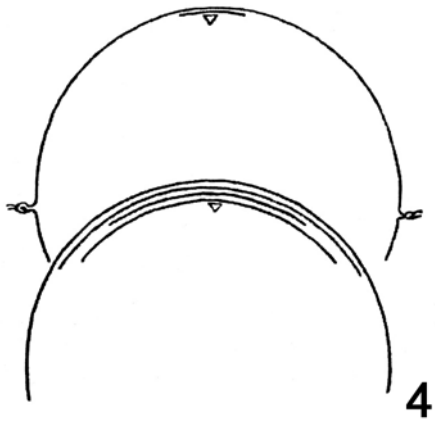


Fig. 4. – Early composition and disposition of the avian blastoderm and cicatricular region on the avian egg yolk ball, represented by PANDER in 1817: the so-called nucleus of Pander is seen as a triangular mass below the unincubated blastoderm (upper figure) and after incubation below the three germ layers (lower figure), clearly indicating epigenetic phenomena (neoformation).

In 1821, for the first time, VON BAER detected mature unfertilized eggs (oocytes) in the ovary of the female dog. In his paper "De ovi mammaliae et hominis genesis" (1827) he notes that they were identical to the ova found in the oviducts. Later, Von Baer started to analyze ovaries in rabbits, pigs and humans and described the human egg

and the structure of the Graafian follicle. Von Baer developed and enlarged Pander's ideas about germ layers and studied their fates during early embryonic development. He was able to demonstrate that the so-called "cutaneous" layer (external layer) transformed into superficial epidermis and central nervous system, that the so-called "muscular" layer (now merely somites) formed muscles, skeleton and connective tissues; that the internal "covering" layer of the digestive tract developed from the so-called "mucous" layer. From these three germ layers all body structures are formed according to an orderly process. By the study of chick embryos he detected a new embryonic organ for the first time i.e. the backbone "cord" (chorda dorsalis or notocord). Based on detailed comparative studies of embryonic development in different vertebrate embryos he formulated some conclusions: during embryonic development more specified characteristics appear later than the more general features and the general features of a large family of animals appear earlier than the features of a species. Von Baer recognizes a certain similarity only between the early embryos of different animal groups.

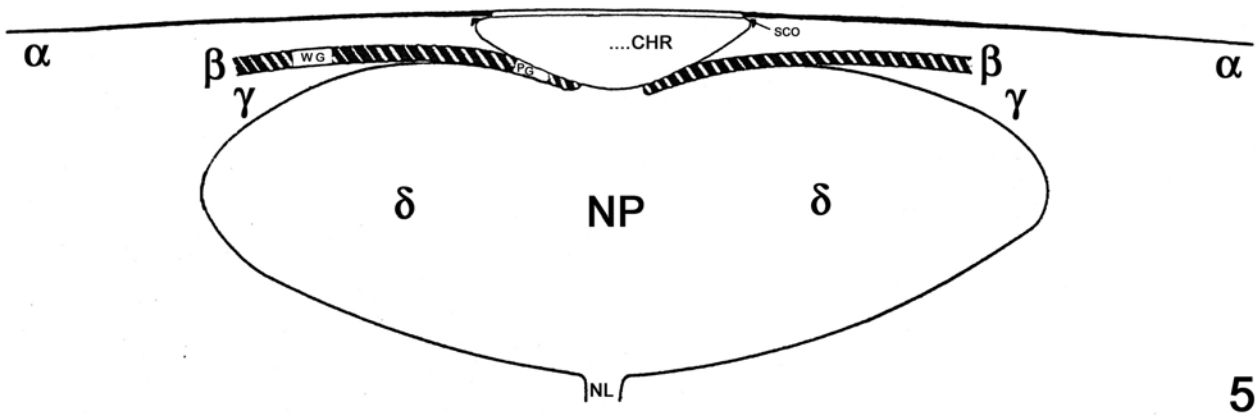


Fig. 5. – Schematic drawing representing the onion peel-like localization of the four ooplasms (α, β, γ, δ) at the end of oogenesis (modified after CALLEBAUT, 1975): the ooplasms will play a fundamental role, during the early development of the blastoderm; from peripherally and superficially to more centrally and deeply we see: the α ooplasm which after a centripetal movement functions during the cleavage stage (see Fig. 6); the β ooplasm is mainly incorporated in the primitive streak and in the embryo proper; a part of the γ ooplasm, surrounding the nucleus of Pander (NP), is spatially obliquely taken up caudolaterally in the blastoderm and forms Rauber's sickle (see also Fig. 8); part of the superficial part of the δ ooplasm (from the nucleus of Pander) is also taken up in a spatially oblique manner by the more central part of the blastoderm and forms the sickle shaped endophyll and primordial germ cells (see also Fig. 8); CHR: spherical postlampbrush chromosomes seen in the centre of the flattened germinal vesicle; SCO: perinuclear subcortical cytoplasmic organelles or ticos; WG: wedge granules layer; PG: polar granules; NL: latebra neck.

The germinal vesicle (the large nucleus of the oocyte) in the large ovarian oocytes of birds is transparent (Fig.5) in contrast to the white opaque structure of the surrounding cicatricula. PURKINJE (1830) describes for the first time the avian germinal vesicle and gives it the name of "vesicula germinativa": Habet itaque cicatricula ovi ovarii partem specialem et sibi propriam, vesiculam sphaericam subcompressam, membranula tenerrima constantem, lympha propria, fors generatrice repletam (inde vesiculam germinativam appellaverim), in fossam cumulo albo mammaeformi e globules composito. (Eggs in the avian ovary present in their cicatricular region a special characteristic structure with a spherical, partly compressed aspect. It contains its own liquid which is always surrounded by a tinny membrane and it is localized in a mammiform mass of globules, it has the power to generate a future germ (sic), therefore I give it the name of germinal vesicle). Thus for Purkinje the germinal vesicle was the germ-generating-structure. By contrast for Von Baer (1828) it was not yet clear if the avian embryo was only formed from the disintegrated germinal vesicle together with the spermatozoa, or that also material from the surrounding cicatricula (ooplasm) plays a role in germ formation. He speaks from "Umbildung" i.e. a series of transformations from simple towards more complex. It was COSTE (1850) who described for the first time the cleavage furrows in the avian blastoderm, formed in the cicatricula shortly after fertilization. So he demonstrated that the oocytal ooplasm of the cicatricula also gives rise to the avian germ. The germinal vesicle in birds, reptiles and cephalopods is according to COSTE (1850) always included in a fine granular layer, which forms the substrate for the later blastoderm from which the embryo will develop. SCHWANN (1839), who developed the general cell theory, was the first to understand that the oocyte (unfertilized egg) of a mammal must be considered as one cell. Owing to their enormous volume he still considered the large oocytes in the ovary of birds as multicellular. HOYER in 1858 clearly observed that the yolk of large avian oocytes contained no cells. Thus large intraovarian avian oocytes are single giant cells.

In 1865 JOHAN MENDEL discovered the fundamental laws of genetics. However his study has been negated and ignored for more then half a century (KRUMBIEGEL, 1957). The reason seems to be that in that period; Darwin described and propounded the variability of species whilst the work of Mendel just demonstrated a constant previsible evolution (DE VEER, 1969). Chronologically we can distinguish three steps in the pairing mechanisms of animals, resulting in the formation of a germ: pairing of the chromosomes during meiosis in the germ cells of the gonads of the parents, copulation of the parents, pairing of the gametes (unfertilized eggs with spermatozoa) at the moment of fertilization. By progression of the cytological

techniques, at the end of the 19th century, chromosomes in meiocytes (during oogenesis or spermatogenesis) were seen as most prominent structures. So VAN BENEDEN (1883) in the eggs of Ascaris megalocephala and VAN BAMBEKE (1885) found, that at the end of the meiotic division, only half of the number of chromosomes was found in the gametes (mature oocytes and spermatozoa). At the moment of pairing of the gametes (fertilization) the specific number of chromosomes is restored in the zygote. This demonstrated for the first time that both the father and the mother afford the same quantity of chromosomal material during the formation of the zygote, which gives rise to all the cells of the embryo. According to Van Beneden (University of Liège) and Van Bambeke (University of Ghent) this suggested that embryonic development is bound to chromosomal material charged with hereditary characteristics. However it is only in 1902 that SUTTON makes the link between the meiotic dissociation of chromosomes and the segregation of hereditary characteristics described by Mendel. The localization of genes on the chromosomes of Drosophila by MORGAN (1910) permitted further development of genetical knowledge. In recent decades the study of expression of genes during early developmental processes has become a powerful tool. Evolutionary and developmental biologists have joined forces to create a new field, unravelling the mysteries of evolution by studying the genes that control how an embryo develops (DEPEW & WEBER, 1995).

EXPERIMENTS ON EGGS

The theory of epigenesis, based on neoformation, as has been proposed by C.F. WOLFF since 1759, considers the egg not as a mosaic of territories with an achieved fate but as a progressive realization of stages which are conditioned by an earlier more simple stage (VAN SPEYBROECK et al; 2002). According to this epigenetic hypothesis the half of a germ can still give rise to a complete normal embryo of a smaller size. The egg is thus capable to regulate development of its parts. However in some species there is evidence for preformistic behaviour as the result of factors or territories present in the ooplasm. The link between ooplasmic structures (before fertilization) and structures in the germ (after fertilization) is not always obvious and differs from species to species. So the first experimental investigations in embryology by CHABRY (1887) and ROUX (1888) and CONKLIN (1905) suggested prelocalization – preformation mechanisms by mosaicism. Indeed in some species (ascidian or amphibian) an isolated hemisected egg (containing one of the two first blastomeres) will develop only in the corresponding half (left or right) of the embryo. The term mosaic development is used as originally defined by CONKLIN (1905) in ascidian species: each region of the whole fertilized egg would be able to form more or less independently on its own. The development of the entire embryo was regarded as being the sum of the development and interaction of the individual parts. Here the concept of "preformation" more particulary mosaicism no longer refers to the strong preformationistic theory described earlier in this review (pre-existence of parts), but as a soft preformation or pre-existence, inclining to a more sophisticated version of preformation, due to ooplasmic determinants. OSCAR HERTWIG introduced in 1916 the concept of "preformed epigenesis": the development of multicellular organisms from a fertilized egg is an epigenetical process whose species-specific course is firmly determined by the preformed hereditary substance which serves as its basis. In other species (echinoderms) an isolated half of an egg will produce a complete miniature embryo (DRIESCH, 1891) as the result of so-called regulation phenomena. This can however been explained by the all or not existence in the isolated halves or parts of all the different kinds of ooplasm in the blastomeres. Indeed during a study of the early embryonic avian development (CALLEBAUT, 1987), I found preformation evidence for the role of the four ooplasms (α, β, γ, δ) which present an onion-peel distribution in the oocytal germ disc region (Fig. 5). I demonstrated that α ooplasm plays a fundamental role during the cleavage stage by penetrating along with the cleavage furrows into the underlying ooplasms (Fig. 6). The β ooplasm originally mainly localized in the peripheral region of the area centralis becomes concentrated in the primitive streak by converging phenomena. The γ-ooplasm finally gives rise to Rauber's sickle. So for instance in the large blastomeres of the early avian germ disc the four fundamental ooplasms can still be observed as is the case in the whole younger germ (CALLEBAUT, 1987) (Figs 5; 6). That the caudocephalic axis of a vertebrate embryo is not preformed in the egg but can develop under influence of gravity was probably first observed by WINTREBERT (1922) in Selachians. Indeed, Wintrebert described the relationship between the spatial orientation of the early Selachian blastodisc on its egg yolk with respect to the vertical and the ensuing development of its caudo-cephalic axis. Also in the Selachian egg any part of the periphery of the blastodisc can give rise to the embryonic caudal edge: the only condition is that it should correspond temporally to its highest point. Still in the 20th century preformistic views concerning the craniocaudal axis of the future vertebrate embryo, persisted. So BARTELMEZ (1912; 1918) claimed that the craniocaudal axis of the future pigeon embryo was already established in the ovarian primordial follicle, visible as the long axis of the oocyte. According to VAKAET (1953; 1955) and FAUTREZ & VAKAET (1954) the plane of bilateral symmetry of the ovoviviparous teleostean fish, *Lebistes reticulatus*, is already predetermined in the oocyte during previtellogenesis. This view, is contested, however by CLAVERT & FILOGAMO (1957 a; b). Indeed, after fertilization the *Lebistes reticulatus* egg becomes movable within its follicle and undergoes an orienting rotation by gravity whereby the future germinal disc is turned to the upper pole of the egg. By contrast to vertebrates the cranio-caudal axis of the Drosophila embryo seems to be preformed by the localisation of ooplasmic determinants within the oocyte (ST. JOHNSTON & NÜSSLEIN-VOLHARD, 1992). Bicoid mRNA localised in the cranial pole is translated after fertilisation to give rise to a morphogen gradient of Bicoid protein that patterns the head and thorax (BERLETH et al., 1988). Similarly, oskar mRNA is localised into the caudal pole of the oocyte where it directs the assembly of the pole plasm, which contains the caudal and germ line determinants (EPHRUSSI & LEHMANN, 1992). J. BRACHET (1933; 1941; 1961) and CASPERSON (1941) discovered

the role of RNAs in the synthesis of proteins. In 1953, Watson & Crick found the basis of our knowledge about the replication mechanism of DNA synthesis, giving rise to the development of molecular biology. Particularly in oocytes, intense synthesis of RNA's and proteins takes place (BRACHET, 1941). These molecules (messenger RNAs) will in part pass into the ooplasm of the zygote after fertilization and will play a fundamental role during early embryonic development. According to the hypothesis of DALCQ & PASTEELS (1937) the original localization of the ooplasmic constituents in the egg, even not genetically determined, can influence early stages of embryonic development. Thus a kind of molecular preformism, present in the ooplasm, must be accepted. The term epigenetics was introduced by C.H. WADDINGTON (1940; 1952) over 60 years ago as the study of those processes involved in the unfolding of development. The discovery of the role of DNA in inheritance has cast a shadow over this discipline for decades (CAVALI, 2006). It was found that molecular machines act on the chromatin to regulate gene expression. These epigenetic regulators play a crucial role in the global shaping and maintenance of developmental patterning. Histone modifications seem to play a major regulatory role. Epigenetics has then been redefined as the study of heritable traits that are not dependent on the primary sequence of DNA (CAVALI, 2006). Ooplasm remains indispensable for embryonic development, since until now in the absence of ooplasm no cloning of individuals can be obtained. The transplantation of somatic nuclei into maturing amphibian oocytes by GURDON (1968; 1969) demonstrated the fundamental effect exerted by the ooplasm and eventually the nucleoplasm of the germinal vesicle on an implanted nucleus for further embryonic development.

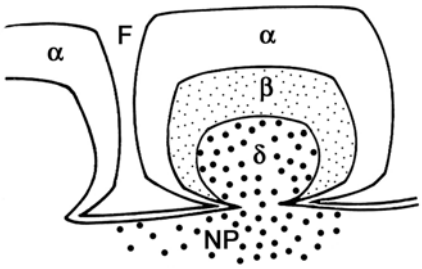


Fig. 6. – Schematic representation of the localization of the ooplasms in the first large "closing" blastomeres; the forming subgerminal space is lined by a narrow α layer; F: cleavage furrow; NP: nucleus of Pander containing δ ooplasm (after CALLEBAUT, 1987).

PERSISTENCE OF FEMALE GERM LINE CELLS FROM EMBRYO TO ADULT

Until the years '60 of the previous century numerous investigators claimed that the primitive sex cells in the ovary sooner or later all degenerate and completely disappear (FIRKET, 1914). Indeed a great part of the germ cells in the ovary degenerate and their investing follicular cells have a hormonal influence. Therefore it was thought that the definitive germ cells of the adult ovary develop secondarily by neoformation from somatic cells in the so-called germinal epithelium covering the ovary and develop epigenetically through transformation of the common coelomic epithelial cells (BLOOM & FAWCETT, 1962). This seems not to be the case. Indeed in the case of birds, by radioactive labelling with ^{3}H-thymidine of the chromosomes of embryonic oocytes during the ultimate premeiotic DNA synthesis period (CALLEBAUT, 1967), I could demonstrate that the labelling persisted into the chromosomes of the adult oocytes. The adult oocytes are thus derived from the embryonic oocytes, present already long before hatching (CALLEBAUT, 1973). In mice similar results were obtained by PETERS et al. (1962), LIMA DE FARIA & BORUM (1962) and CRONE et al. (1965). This demonstrates that birds and mammals contain a final stock of oocytes, at the end of their embryonic development, which will be exhausted progressively during further life [part of the hypothesis of WALDEYER (1870)].

DISCOVERY OF EMBRYONIC INDUCTION

In 1924, SPEMANN & MANGOLD describe the induction phenomena i.e. an embryonic structure functions as an organizing inductor on another structure (reactor) in its neighbourhood and modifies this reactor into a new structure without itself affording a cellular contribution to this new structure. SPEMANN & MANGOLD (1924) discovered the so-called "Spemann organizer" in amphibian embryos. They observed that after transplantation of a piece of dorsal blastopore lip of the early gastrula to the ventral side of another embryo, a secondary embryonic axis developed on this side. So the existence of induction phenomena between different associated parts of embryonic tissues demonstrated epigenetic development. The Spemann's organizer redeterminates the fate of a part of the host cells and induced them to form axial structures, more particularly a central nervous system. The formation of a neural plate which gives rise to the central nervous system was considered to be the result of an inductive influence from the underlying mesoderm with organizer property.

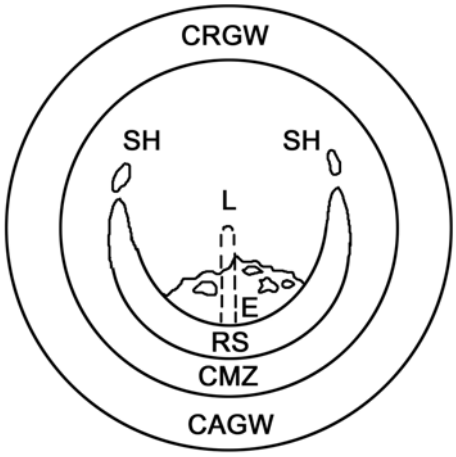


Fig. 7. – Schematic representation of the components of an unincubated avian blastoderm seen from its deep side after removal of the subgerminal ooplasm, ready for culture; CRGW: cranial germ wall; CAGW: caudal germ wall; CMZ: caudal marginal zone; E: incomplete endophyll sheet; L: lacune in the deep layer; RS: Rauber's sickle with its fragmentary sickle horns (SH) enclosing the area centralis. The vertical interrupted double line represents the future localization of the PS after incubation.

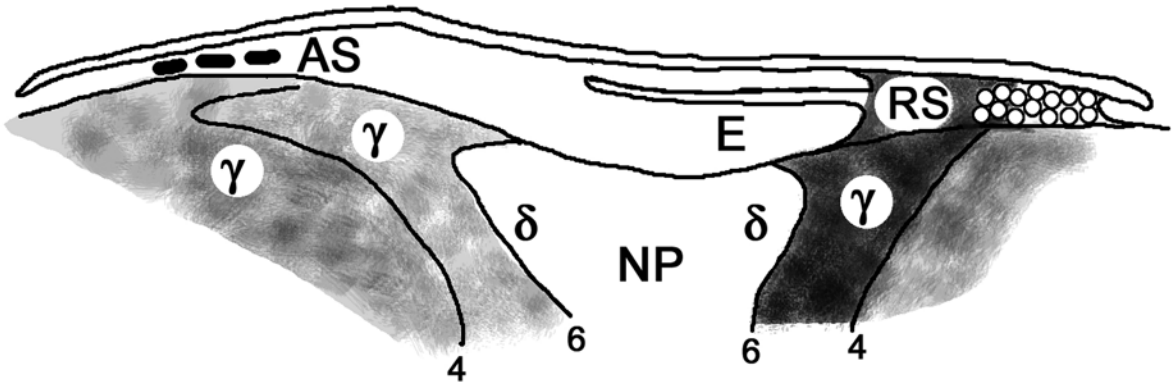


Fig. 8. – Schematic representation (simplified after CALLEBAUT, 1993b) of the localization of two intraoocytally radioactively labelled layers (respectively 4 or 6 days after a maternal radioactive injection) in the γ and δ ooplasms on a midsagittal section through an uninucubated avian blastoderm (presenting a sickle-shaped bilateral symmetrization). Note the permanent boot-shaped deformation of the ooplasmic layers around the nucleus of Pander (NP) composed of δ ooplasm (white) and in the surrounding γ ooplasm (grey). The toe-shaped part of both γ and δ layers are expanded and horizontally flattened. They remain in the underlying ooplasm below the anti-sickle region (AS) and they have lost contact with the blastoderm. Caudally, in the heel-shaped part, the γ ooplasm is more condensed and adheres vertically to the upper layer, forming Rauber's sickle (RS); the heel and middle part of the layers containing δ ooplasm of the nucleus of Pander (NP) are taken up in the caudal part of the area centralis of the blastoderm and later segregate progressively as endophyll (E) which also contains δ ooplasm (white) (CALLEBAUT, 1987; 1993a); the superficial upper layer is mainly composed of β ooplasm (after CALLEBAUT, 2005).

OOPLASMIC DETERMINANTS IN SOMATIC TISSUES

During the last decades it became clear that induction phenomena not only occur as the result of interaction between embryonic structures (in the sense of SPEMANN & MANGOLD, 1924) but also extraembryonic ooplasmic structures, all or not nucleized, play a fundamental role in early embryonic induction phenomena. Indeed in 1969 NIEUWKOOP discovered that during early blastulation in amphibians, signals are required from a region which is localized vegetal to the prospective dorsal blastopore lip, to initiate development of the mesoderm (Spemann's organizer). This vegetal region is now designated as Nieuwkoop's centre. In amphibians, Wnts seem to be the primary axis (formation of head and trunk-tail regions) inducing substances. Indeed in Xenopus, microinjection of several Wnts into the ventral cells of the early embryos leads to complete duplication of the body axis (CADIGAN & NUSSE, 1997; DEARDORFF et al., 1998). This duplication is believed to arise from the formation of a second Nieuwkoop's centre (dorsal vegetal cells) of the early blastula which then induces overlying tissue in order to it might become the Spemann's organizer (homologous to the avian primitive streak and nodus, WADDINGTON, 1932). Indeed in birds and mammals the properties of the Spemann's organizer are performed by the node (Hensen's node in birds) which is the rostral end of the full grown primitive streak. We found that in birds, RAUBER's sickle (1876) (Figs 7; 8) has a homologous function as Nieuwkoop's centre in amphibia. It functions indeed as the primary major organizer, initiating gastrulation phenomena and primitive streak formation (CALLEBAUT & VAN NUETEN, 1994; CALLEBAUT et al., 2003a). The localization and function of Rauber's sickle present a strong similarity with the localization and function of the ascidian Wnt gene, Hr Wnt-5 from *Halocynthia roretzi* (SASAKURA et al., 1998). Indeed HrWnt-5mRNA is present in the vegetal cortex of unfertilized eggs. After fertilization, HrWnt-5 moves to the equatorial region to form a sickle-shaped structure after which this mRNA is concentrated in the most caudal region of the embryo. That Vg1 (the axis inducer) present in the avian Rauber's sickle material (inclusive in the sickle horns) and not in the caudal marginal zone has recently been demonstrated (BERTOCCHINI & STERN, 2007). Hensen's node must be considered as a secondary major organizer linked to Rauber's sickle via a rostral outgrowth of the latter i.e. sickle endoblast. At the moment of the sickle-shaped bilateral symmetrization (characterized by the appearance of Rauber's sickle), there oocurs a spatially oblique, sickle-shaped uptake of γ and δ ooplasms by oblique position in utero which become incorporated into the deeper part of the avian blastoderm (Fig. 8). This provokes an unidirectional chaos (radial symmetry breaking) in the ooplasm according to the principle of PRIGOGINE (PRIGOGINE & LEFEVER, 1968) which is indispensable for further development. These ooplasms seem to contain ooplasmic determinants (CALLEBAUT, 2005) which initiate (perhaps by Wnt signalling) either early gastrulation or neurulation phenomena by positional information (CALLEBAUT et al., 2003a). First we found (cytological) evidence for a radial predisposition (preformation) in the premature avian oocyte i.e. radially symmetric and concentric distribution of groups of mitochondria (CALLEBAUT, 1972) (Fig. 9). After the eccentric sickle shaped tilting of the yolk and ooplasmic layers in the fertilized egg by oblique positioning (CALLEBAUT et al., 2000), we observed the presence of predisposed sickle-shaped anlage fields in the upper layer (CALLEBAUT et al., 1996). There is a strong similarity with the gastrulation and neurulation phenomena described by VANDEBROEK (1969) in selachian germs (also developing on very large eggs). Indeed, in the latter vertebrate group there exist also analogous sickle-shaped anlage fields, localized in the upper layer in the same succession order as in birds. These fields are localized in the

concavity of Rauber's sickle in unincubated diblastic avian blastoderms which thus present a sickle-shaped bilateral symmetry (Fig. 10). Finally from this sickle-shaped bilateral symmetric disposition a primitive streak and neural plate will develop by convergent extension movements under influence of signalling molecules secreted by Rauber's sickle (CALLEBAUT et al., 2003a) (Fig. 11) so a triblastic one-axis-containing embryo is formed. The early neural plate inducing structure which forms a deep part of the blastoderm is the δ ooplasm-containing endophyll (primary hypoblast) (Fig. 8). Together with the primordial germ cells it is derived from the superficial centro- caudal part of the nucleus of Pander which also contains δ ooplasm. It is indeed known that the prelaid nucleus of Pander (as is also the case for the endophyll) can induce the upper layer to form an early neural plate (CALLEBAUT et al., 2004b). The other structure (γ ooplasm) which is incorporated into the caudolateral deep part of the blastoderm forms Rauber's sickle (Figs. 7; 8). It induces first gastrulation (intramuros) and later blood island and coelom formation (extramuros). Rauber's sickle develops by ingrowth of blastodermal cells into the γ ooplasm (CALLEBAUT, 1994), which surrounds the nucleus of Pander (Fig. 8). The Rauber's sickle constitutes the primary major organizer of the avian blastoderm. Rauber's sickle generates only junctional and sickle endoblast and by positional information, organizes and dominates the whole blastoderm (first gastrulation, neurulation, and later coelom and cardiovascular system formation) (CALLEBAUT et al., 2003b). Fragments of the horns of Rauber's sickle extend far cranially into the lateral quadrants of the unincubated blastoderm, so that often Rauber's sickle material forms three quarters of a circle (Fig. 7). This explains the so called regulative capacities of isolated blastoderm parts with the exception of the anti-sickle region and/or the central blastoderm region, where no γ ooplasm and no Rauber's sickle material is present (which again demonstrates the influence of the γ ooplasm) (CALLEBAUT, 2005). Recently we demonstrated that in the one and the same species (*Gallus domesticus*) and the same unincubated blastoderm both mosaic development or regulation phenomena can be obtained (CALLEBAUT et al., 2007). Indeed after hemi-sectioning of unincubated chicken blastoderms and culturing both halves formatted *in vitro*, two kinds of development can be discerned: 1. When the unincubated blastoderms were hemi-sectioned according to the plane of bilateral symmetry, going through the middle region of Rauber's sickle (Fig. 12), we obtained two hemi-embryos (a left and a right one) containing each a half primitive streak (starting from the most median parts of Rauber's sickle) giving rise to a half mesoblast mantle and a half area vasculosa, which differentiate incompletely thus indicating mosaic development. This (mediosagittal) hemi-sectioning of the avian blastoderm is comparable with the unilateral destruction experiments of the first two ascidian blastomeres by CHABRY (1887) and CONKLIN (1905). Indeed, the ascidian two-cell embryo already presents a left-right asymmetry visualized by the natural mediosagittal cleavage plane through the caudal sickle-shaped Wnt gene expressing zone (SASAKURA et al., 1998), homologous with Rauber's sickle. 2. When the unincubated blastoderm is hemi-sectioned more obliquely going through a more lateral part of Rauber's sickle (sickle horn), two complete bilaterally symmetrical miniature embryos will form indicating so-called regulation phenomena (Fig. 13). We demonstrated that those two types of development are in reality due to the different spreading and concentration of Rauber's sickle tissue around the area centralis (CALLEBAUT et al, 2007). Embryonic regulation must thus not be considered as a kind of totipotent regeneration capacity of isolated parts of the unincubated avian blastoderm but depends on the spatial distribution of a kind of extraembryonic tissue (Rauber's sickle) build up by the late oblique uptake of γ ooplasm at the moment of bilateral symmetrization (CALLEBAUT, 1994; CALLEBAUT et al., 2000) forming an ooplasmic mosaic. Thus not only embryonic gene expression phenomena take place during early development but also uptake of extraembryonic preformed ooplasmic determinants play a fundamental role for the initiation of gastrulation, neurulation and cardiovascular development. So finally three ooplasms are respectively found in the three elementary tissues (not germ layers !) of the early, unincubated avian blastoderm (Figs 7; 8): the upper layer from which the embryo proper develops containing mainly β ooplasm (forming the embryonic stem cells), the Rauber's sickle containing γ ooplasm and the endophyll (primary hypoblast) containing δ ooplasm both forming the extraembryonic tissues. In mammals also the embryonic ectoderm (upper layer) constitute the real stem cells which under influence of the surrounding extraembryonic tissue transform in mesendodermal and neural cell lines (HADJANTONAKIS & PAPAIOANNAOU, 2001). So the general body plan (bilaterally symmetric) is established in the diblastic germ. This confirms, in the case of birds, the existence of a similar master plan for the early development as was proposed for all chordates by EYAL-GILADI (1997). According to the hypothesis of Eyal-Giladi, the speed at which axialization of the embryo proper takes place, depends on the translocation speed of oocytal determinants from the vegetal pole towards the future dorsocaudal side of the embryo. After arrival at their destination, the activated determinants form in all chordates, an induction center homologous to the amphibian "Nieuwkoop center" and the ascidian Wnt expressing sickle shaped region, which later organize the formation of the intraembryonic "Spemann's organizer". Thus in birds by using radioactive or trypan-blue induced fluorescence oocyte labelling we could demonstrate that a kind of evolutive preformism exists which follows more or less the main evolution of the animal kingdom: from a radial symmetric disposition in the premature oocyte (as exists in coelenterates) via a sickle-shaped diblastic bilateral symmetric germ to a triblastic one-axis-containing embryo (gastrulation induced in aves by Rauber's sickle). Finally, we observed the formation of the coelomic cavity with associated cardiovascular system (typical for coelomates; DOLLANDER and FENART, 1973) also induced by Rauber's sickle material (CALLEBAUT et al., 2002; 2004a). An ooplasmic influence on spatial patterning of the mouse blastocyst has been demonstrated by GARDNER (1997), by using the localization of the polar body as a marker for the animal pole. Also PIOTROWSKA and ZERNICKA-GOETZ (2001) showed that the sperm entry position products the plane of initial cleavage of the mouse egg and can define embryonic and abembry-

onic halves of the future blastocyst. In addition, the cell inheriting the sperm entry position acquires a division advantage and tends to cleave ahead of its sister.

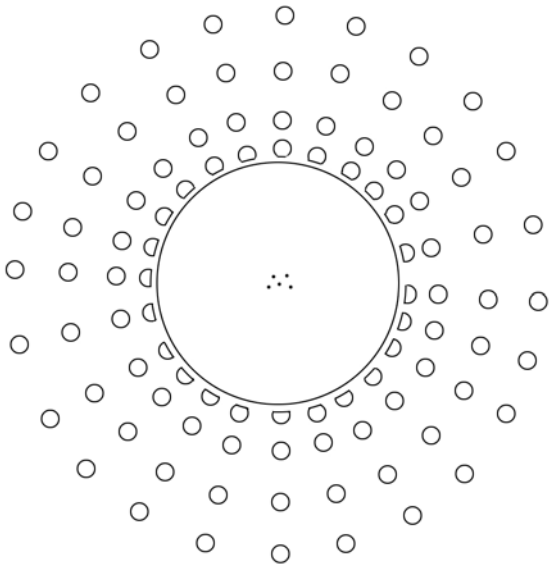


Fig. 9. – Schematic drawing of the ooplasmic radial symmetry in the premature quail (*Coturnix coturnix japonica*) oocyte, visible by the presence of RNA-rich subcortical cytoplasmic organelles (aggregates of mitochondria represented by small circles) (CALLEBAUT, 1972; D'HERDE et al., 1995).

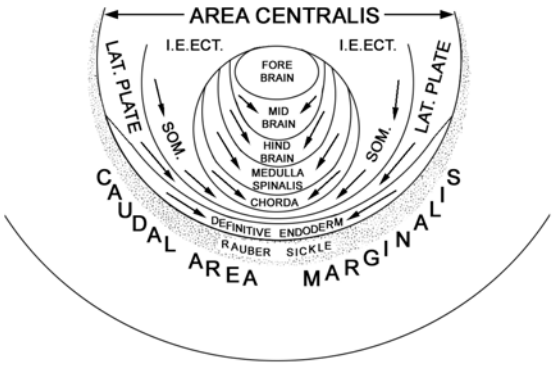


Fig. 10. – Schematic representation of the mean localization of the predisposed (not definitively committed) anlage fields (in good order but with possible partial overlapping of neighbouring parts) in the upper layer of the area centralis of a chicken (*Gallus domesticus*) unincubated blastoderm (slightly simplified after CALLEBAUT et al., 1996). Note the general eccentric sickle-shaped aspect of the anlage fields in the area centralis after the radially symmetry breaking eccentricity of the subgerminal ooplasms. There is an obvious parallelism between the sickle shape of the anlage fields in the UL and the ovoid central subgerminal ooplasmic layers (CALLEBAUT et al., 2000). The curved arrows on the anlage fields indicate the logically previsible converging movements of the upper layer during the ensuing gastrulation (WETZEL, 1929) and neurulation (BORTIER and VAKAET, 1992).

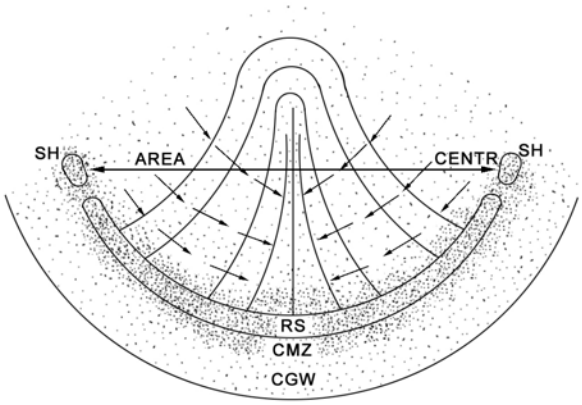


Fig. 11. – Combined schematic drawing representing: 1. the hypothetical diffusion of morphogens or signalling molecules indicated by small dots) emanating from Rauber's sickle (RS) and its sickle horns (SH) into the neighboring tissues of the avian blastoderm, i.e., into the area centralis (AREA CENTR), into the caudal marginal zone (CMZ) and into the caudal germ wall (CGW), where they can influence (induce or inhibit) ectopically placed structures (endophyll, Rauber's sickle fragments, sickle or junctional endoblast) to form or not to form a second streak; 2. the broad movements (indicated by curved arrows) of cell groups in the upper layer of the area centralis, in the direction of the median primitive streak (partially after WETZEL, 1929). The curved legs of the U-shaped lines indicate moving fronts of cell groups (corresponding to local temporal primitive streak anlagen) that will ingress after fusion into the final median primitive streak.

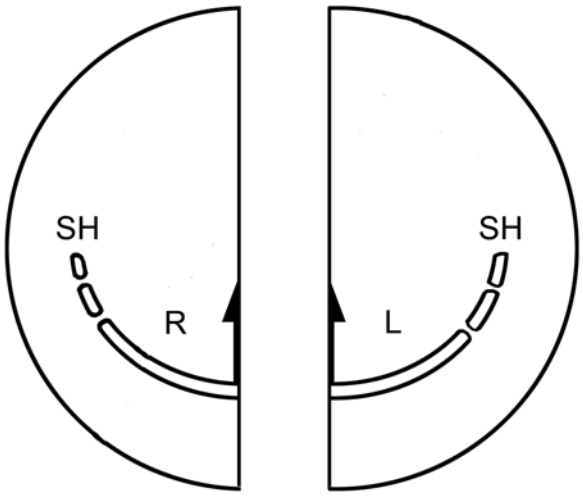


Fig. 12. – Schematic representation of the hemi-sectioning through the middle of the sickle of Rauber (double lined) of an unincubated blastoderm; both halves of the sectioned blastoderm are represented at some distance (ready for culture); SH: sickle horns of Rauber's sickle extending far cranially; R: right half blastoderm and L: left half blastoderm will each transform in a right and left half embryo, indicating mosaicism; the thick half arrows represent the formation of hemi-primitive streaks at the cut edge of the blastoderm after incubation.

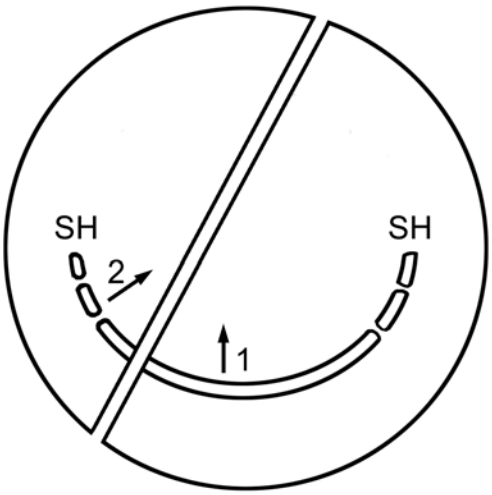


Fig. 13. – Schematic representation of the oblique hemi-sectioning through the lateral part of the Rauber's sickle of an unincubated avian blastoderm; the arrows 1 and 2 indicate the place where after culture a bilateral symmetric primitive streak will appear respectively under the influence of the middle part of the Rauber's sickle (double lined) or under influence of the remaining fragmentary sickle horn (SH), indicating regulation.

OOPLASMIC DETERMINANTS IN PRIMORDIAL GERM CELLS

NUSSBAUM (1880; 1901) and WEISMANN (1892) were the first to propose the preformation thesis in birds i.e. that the primordial germ cells have inherited and retained for long periods the yolk from the precursor oocyte from which their egg yolk ball and blastoderm are derived. In birds DANTSCHAKOFF (1908) observed so called "endodermal Wander Zellen" in the space between the upper layer (epiblast) and deep layer. The primordial germ cells (PGC's) are indeed only first unequivocally distinguishable in the 1-8 somite chicken blastoderm between epiblast and endophyll of the germinal crescent (SWIFT, 1914). BOUNOURE (1939) describes a kind of "germ plasm" in the vegetal pole of Anura eggs. By irradiation this germ plasm could be inactivated, giving rise to sterile individuals. Only cells which inherited part of this germ plasm gave rise to PGC's. It was thus the kind of ooplasm which determined the fate of the involved cells. The germ plasm thus contains so-called "Keimbahn determinants". Because PGC's in birds are found close to the endophyll and seem to immerge from it, VAKAET (1962) supposed that they were derived from the endophyll. DUBOIS (1967; 1969) also concluded that the endophyll is at the origin of the formation of PGC's in birds. The precursors of the PGC's which are often initially morphologically indistinguishable from the surrounding somatic cells in earlier stages, are called presumptive primordial germ cells (p PGC's). These divide mitotically to produce one PGC containing "Keimbahn determinants" and one somatic cell. Thus in general it was accepted that chicken germ cells originate from the primitive deep layer. By contrast, EYAL-GILADI et al., (1981) concluded by using chick-quail chimeras, made before primitive streak formation (i.e. stage XIII: 10-12h incubation), that avian PGC's were from epiblastic origin. Avian PGC's were then thought to arise through a gradual epigenetic process. However, in these older blastoderms the deep layer is no longer composed of endophyll but mainly formed by sickle endoblast, derived from Rauber's sickle (CALLEBAUT et al., 1997). Indeed the endophyll and associated PGC's are then already displaced cranially and adhere to the deep cranial part of the epiblast and to the there present hemicircular fibrous bands (ENGLAND, 1983). They will form part of the endophyllic crescent in older stages. The experiments of CUMINGE and DUBOIS (1992) seemed to confirm the thesis of Eyal-Giladi et al., but they also investigated similar old blastoderm stages which greatly differ from the unincubated blastoderm. By using trypan blue induced fluorescent labelling of the ooplasmic yolk layers of quail oocytes during their final post-lampbrush stage, I could demonstrate that primordial germ cells together with the endophyll contain yolk from the deep central region of the germ disc i.e. δ ooplasm from the superficial part of the nucleus of Pander (CALLEBAUT, 1984; 1987). So nearly 95 % of the PGC's can be labeled 6-7 days after one single injection of trypan blue to the mother quail. Oocytal yolk labeling, 1 to 4 days after an injection gives no labeling of the primordial germ cell yolk, but gives labeling of more superficial somatic cells which contain more superficial ooplasms (β or γ). The observed trypan blue induced fluorescent yolk labelling in the caudally in the area centralis localized endophyll of the unincubated quail blastoderm (CALLEBAUT, 1987) is in agreement with the observed localization of the pPG cells (also containing δ yolk) after transsection experiments (FARGEIX, 1967; ROGULSKA, 1968; DUBOIS and CROISSILLE, 1970) i.e. mainly in the caudal region of the unincubated blastoderm. The original deep and central localization of pPGC material has recently been confirmed by the use of a chicken vasa homologue (TSUNEKAWA et al., 2000). Chicken vasa protein forms part of the mitochondrial cloud in younger chick oocytes and localizes to the central cleavage furrows (which extend into the δ ooplasm of the nucleus of Pander) until stage IV (EYAL-GILADI and KOCHAV, 1976). At that moment 6 to 8 cells of the approximately 300 blastomeres containing germ, present vasa protein and are probably p PGC's. (TSUNEKAWA et al., 2000). The data of CALLEBAUT (1984) and TSUNEKAWA et al. (2000) thus indicate that a kind of deep preformation may be the mechanism for germ cell specification in birds. As in Xenopus, in the quail there are two known populations of oocytal mitochondria which become finally localized in the early embryo: one population becomes localized in the vegetal pole where it forms a component of the germ plasm in Xenopus (MIGNOTTE et al., 1987; TOURTE et al., 1984) and a component of the nucleus of Pander (δ ooplasm) in the quail (CALLEBAUT, 1984; D'HERDE et al., 1995). The other population of mitochondria is localized much more superficially and forms the obvious radially and concentrically disposed group around the germinal vesicle both in Xenopus (MIGNOTTE et al., 1987) as in the quail (Ticos: CALLEBAUT, 1972; 1983; D'HERDE et al., 1995). These mitochondria will populate the somatic tissues of the offspring (in Xenopus: DAWID and BLACKLER, 1972) (in quail: WATANABE et al., 1985). Our conclusion is in agreement with WOLPERT (1998) and EXTAVOUR and AKAM

(2003) that epigenetic germ cell development (derived from somatic stem cells) is an exception and that most animals use localized ooplasmic determinants to specify the germ line.

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Benefits and costs of semi-colonial breeding in the Montagu's Harrier *Circus pygargus*

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ABSTRACT. A total of 31 pairs of Montagu's Harrier *Circus pygargus* nesting on calcareous marshes near Che³m in eastern Poland were observed from 1991 to 1995 in "Bagno Serebryskie" nature reserve. This area has been included into Nature 2000 network as a special protection area for birds. Total time of observations was 1700 hours. The harriers were caught, ringed and individually marked by special colour wing tags. Observations each year started in April at the beginning of pair formation and continued until the start of post-fledging period in July. The size of a harrier nesting territory varied from 0.42ha to 2.25ha, averaging 1.14ha. Clumped territories were significantly smaller than solitary ones. The clumped territories were occupied earlier (as estimated from egg-laying dates) than solitary ones. Clutch size in clumped nests was larger than in solitary ones and the number of fledglings per nest was also higher in clumped territories. Semi-colonial nesting was safer in terms of predation risk. Copulation frequency was higher in semi-colonial than in solitary nests, suggesting a higher risk for extra-pair copulations. Males from clumped nests spent more time inside territories than males from solitary nests. Most of observed copulations were preceded by food pass.

KEY WORDS : *Circus pygargus*, semi-colonial breeding, copulation, breeding success, predation risk.

INTRODUCTION

Breeding density of the Montagu's Harrier *Circus pygargus* as other birds of prey is strongly dependent on habitat quality and prey availability (NEWTON, 1979). In optimal habitats most harriers' species nest in association or semi-colonies (HAGEMEIJER & BLAIR, 1997; SIMMONS, 2000). Semi-colonial breeding in birds has associated costs and benefits (BIRKHEAD & MOLLER, 1992). Main costs are competition for breeding sites, mates and food but benefits are evident: information about food or mates and decrease of predation risk.

Semi-colonial breeding helps in mate assessment of mates at the time of mate choice (WIĄCEK, 2004). In contrast, the risk of extra-pair copulation (EPC) is higher in semi-colonies than in solitary places (ARROYO, 1999), so cuckoldry may represent a high cost for birds in colonies (BIRKHEAD & LESSELLES, 1988; ARROYO, 1999). Two basic methods of paternity assurance occur in birds. The first method is mate guarding (BIRKHEAD, 1979), but in species such as raptors, with intensive courtship feeding, where females stay at the nest while males hunt far away from it, mate guarding interferes with foraging. The second strategy to decrease the risk of cuckoldry is frequent within-pair copulations (BIRKHEAD et al., 1987). Harrier males, similarly to other birds of prey, increase their paternity assurance by frequent copulations (BIRKHEAD & MOLLER, 1992; MOUGEOT et al., 2001). Therefore semi-colonial breeding birds such as Montagu's Harrier may have high copulation rates in comparison to birds breeding solitary (MOUGEOT, 2004) although KORPIMAKI et al. (1996) found that in some bird of prey species solitary nesting birds copulated more frequently than clumped birds.

In a study with Montagu's Harrier, losses caused by predators were lower in semi-colonies than in isolated nests (ARROYO et al., 2001). Safety of group- living birds depends on individuals participating in group defence of breeding places or nests. One of the ways of defence in semi-colonial breeding birds of prey is mobbing behaviour. Montagu's Harriers are medium-sized birds of prey, defending their breeding places by active antipredator behaviour individually or communally. Communal defence provides benefits to all members of the colony and semi-coloniality decreased the individual's costs of defence (ARROYO et al., 2001). Costs associated with mobbing behaviour include the time spent in the territory, risk of death during attacks or a decrease in physical condition.

This study sought to determine whether nesting in solitary or clumped territories modified harriers behaviour, in such aspects as time spent inside the territory, mate guarding, copulation frequency and breeding success.

STUDY AREA AND METHODS

From 1991 to 1995 aspects of the behaviour of 31 pairs of Montagu's Harrier were observed on calcareous marshes in the nature reserve: "Bagno Serebryskie" near Chełm (51°10'N, 23°37'E) in eastern Poland (Fig. 1). This nature reserve is a part of Special Protection Area for birds within Nature 2000 network. The area of the nature reserve was 376.6ha (BUCZEK & BUCZEK, 1996). The Sedge *Cladietum marisci* is the dominant vegetation type (*Cladium mariscus* dominant). The study area was surrounded by agriculture landscape. Over the study years, fifteen harriers (11 females and 4 males) from clumped territories were caught in special ornithological nets

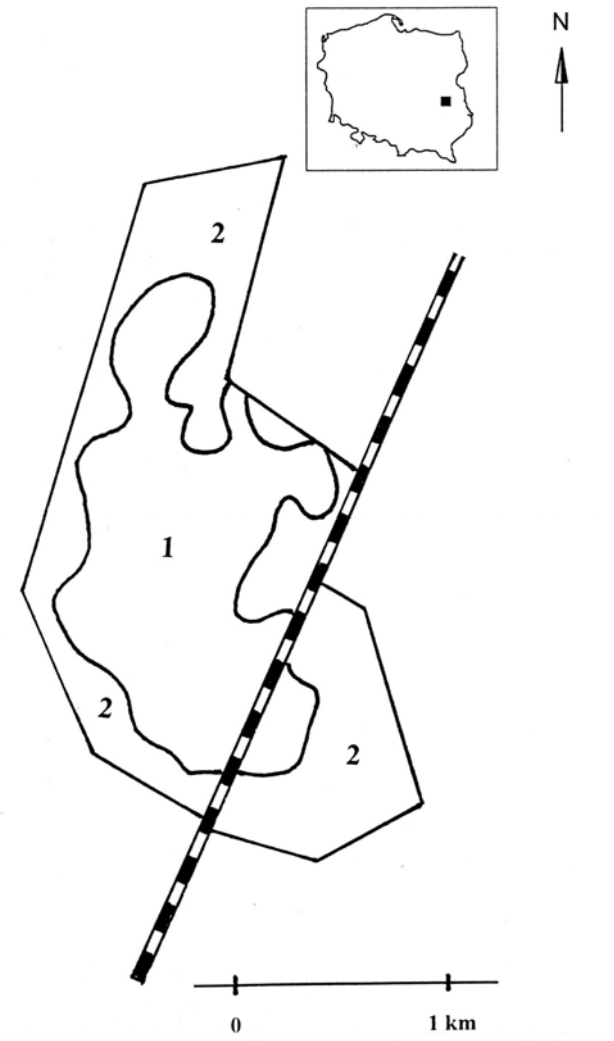

Fig. 1. – Map of the study area and its location in Poland.
1. Breeding area covered by the sedge *Cladietum marisci*.
2. Meadows around the marsh; black and white line = railway line along study area.

(BUSSE, 2000) using Eagle Owl *Bubo bubo* as a decoy (Table 1). These birds were ringed and individually marked using special coloured wing tags (KOCHERT at al., 1983). The colour markers did not modify the harriers' behaviour. Some unmarked individuals were individually recognized by differences in their plumage and the moulting stage (gapes in primaries, secondaries or tail feathers).

Observations started each year in the second half of April (between 16th and 20th) after the arrival of harriers at the breeding places. We recorded first egg laying and hatching dates in the studied pairs through nest visits, as well as number of eggs in each nest and growth rate of nestlings. Nests with fledglings were monitored every week.

Field observations were conducted throughout the pre-laying period, incubation, hatching and nestling periods until the beginning of the post-fledging period in July. The behavioural observations focused on early stages of the breeding season, mainly in the pre-laying period. Total time of observations in this period was 1700h. Observations in later periods of the breeding season were conducted mainly while visiting the nest and nestlings until they started to fly. Observations were performed from 100 to 150m away from nests, using 10x50 binocular and a spotting scope 20x77. Birds were observed from 7 a.m. till sunset (12 hours a day). The size of 27 of the 31 studied territories was estimated through Minimum Convex Polygon method (KENWARD, 1987), using observations of female location as data. We measured the time spent in the territory by both sexes with accuracy to 1s (by the use of a stopwatch). This data were presented as a percent of the time spend within territory. Copulations rates per female were estimated from the total number of copulations observed, whether successful or unsuccessful. Mating was described as successful when duration of copulation was near 5s. Shorter time of copulations with no contact of the cloacae was recorded as unsuccessful. Copulation rates were presented as number of copulations per hour observation. All cases of predator attack, nest detection and nest destruction by predators were also recorded.

The location of the territories, within the marshes during all the years of observation, was uneven. Some of these territories were located in high density, in close proximity to each other so that they bordered with one another, whereas the remaining ones were located within a certain distance from one another. A change in behaviour towards the nearest neighbour was the criterion distinguishing between "clumped" or "solitary" categories. Harriers in solitary territories did not provoke behavioural answers from nearest settled pairs of harriers, in contrast to birds in the clumped category where a reaction was immediate: pursuits, escorts or attacks (WIĄCEK, 2006). The distance between clumped territories was 40 to 146m, on average 78.4m (N=16). Solitary areas (N=15) was placed in the distance from 265 to 655m, on average 368m (Table 2). All the nests and distances between nests were measured by use a measuring tape. Analyses were made with nonparametric statistics (Mann-Whitney test and χ^2 test). All analyses were performed with Statistica 6.1.

TABLE 1

The breeding pairs of the Montagu's Harriers *Circus pygargus* in the nature reserve "Bagno Serebryskie" in the time of study.

Year	nesting pairs	observed pairs	clumped territories / observed/	isolated territories /observed/	marked birds	density per 100ha
1991	16	5	2	3	2♂	4.25
1992	11	7	4	3	5♀+1♂	2.92
1993	12	6	3	3	2♀+1♂	3.19
1994	9	8	4	4	2♀	2.39
1995	6	5	3	2	2♀	1.59

TABLE 2

The distance of the nearest neighbour between breeding pairs of the Montagu's Harrier *Circus pygargus* (CP) in the time of study.

Season	Clumped pairs	Isolated pairs
1991	CPA – 146m	CP1 – 295m
	CPB – 146m	CP4 – 265m
		CP2 – 285m
1992	CP8 – 56m	CPO – 325m
	CP7 – 40m	CP10 – 345m
	CP9 – 40m	CP13 – 325m
	CP3 – 126m	
1993	CPF – 67m	CPM – 275m
	CP£ – 67m	CPK – 310m
	CPT – 75m	CPL – 655m
1994	CP2 – 44m	CP4 – 280m
	CP3 – 64m	CP6 – 610m
	CP8 – 44m	CPZ – 485m
	CP1 – 60m	CPS – 410m
1995	CP4 – 110m	CPC – 335m
	CP13 – 60m	CPW – 320m
	CPU – 110m	

RESULTS

The size of observed territories varied from 0.42 hectares to 2.25 hectares, on average 1.14 hectare (N=27, SD=0.52). Clumped territories were smaller (from 0.42 to 1.4ha, on average 0.92ha, N=16, SD=0.44) than solitary ones (from 0.6 to 2.25ha, on average 1.47ha, N=11, SD=0.47), Mann-Whitney test Z=-2.76, p=0.005.

The conducted observations and nest monitoring enables to suggest that semi-colonial breeding in Montagu's Harrier provides pairs with higher nest safety. Among 16 observed pairs in clumped territories, only one case of destruction of the nest by a raptor was observed, while there were 5 cases among 15 solitary territories (χ^2 =43.859, df=1, p<0.001).

There were also significant differences in the breeding success of nesting birds in the two types of territories. Number of eggs in nests located in semi-colonies was higher (4.37 eggs per pair, N=16, SD=0.6), than in solitary territories (3.73 eggs per pair, N=15, SD=1.09). Those differences had further consequences in the number of hatched fledglings: 3.43 fledglings in clumped territories (N=16, SD=1.36) and 2.93 in solitary territories (N=15, SD=1.66). The number of flying fledglings in clumped nests was 2.56 per pair (N=16, SD=1.31), while in solitary ones 1.46 per pair (N=15, SD=1.35) and this difference was statistically significant (Mann-Whitney test Z=2.055, p=0.03). Clumped territories were occupied earlier than solitary ones. The date of laying of the first egg over all study years was six days earlier in clumped than in solitary territories (Z=1.97, p=0.04).

Overall copulation rate was 0.22 per hour (2.65 copulations a day) but breeding density influence copulation behaviour. Clumped pairs (N=16) had a higher copulation density than those in solitary (N=15) territories (Z=3.05, p=0.002), (Fig. 2). Two peaks of copulation were observed: first in mid-morning and second in the afternoon (Fig. 4). Most of 69 observed copulations (62%) were preceded by food pass. Copulation occurred more often after courtship feeding than without food (χ2=4.18, p<0.04).

All observed pairs built nest of similar sizes and located in similar areas and surroundings. Nevertheless, the internal diameter of clumped nests (N=16) was larger than in solitary territories (N=15), this difference was statistically significant (Z=2.21, p=0.03) and was associated with the higher number of eggs in these nests.

There were also significant differences in time budget for males among clumped and solitary territories (Fig. 3). During the pre-laying period, males (16) in clumped territories spent more time within the territory than did males (15) in solitary territories (Z=2.303, p=0,02). Additionally, females from solitary territories spent more time in the territory that did females from clumped territories but differences were not statistically significant.

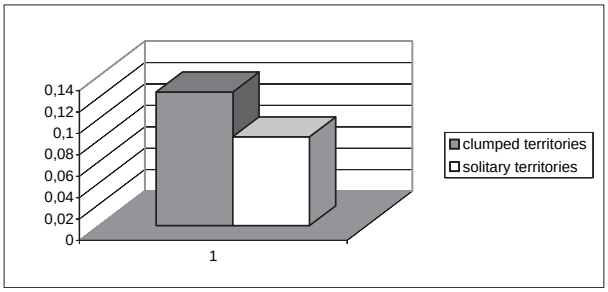

Fig. 2. – The copulation frequency as number copulations per hour observation (as medians)

DISCUSSION

Montagu's Harrier shows a distinct tendency to semi-colonial breeding (CLARKE, 1996) in the whole area of its occurrence (HAGEMEIJER & BLAIR, 1997). Territorial behaviour is mostly observed within the nest area while aggressive behaviour associated with hunting territories is not observed. The size of nesting territories observed in this study was smaller than territories occupied by Montagu's Harrier in an Italian population, where the average size of the territory was approximately 4 hectares (PANDOLFI & PINO D'ASTORE, 1992). However the habitat conditions of the Italian population differed from that in Poland. In Italy, harriers occupied dry habitats with grass and shrubs while in Poland birds were breeding in wet marshes with sedge *Cladium mariscus*. The small marshes in eastern Poland (study area 3.76km^2) created optimal breeding conditions for harriers building their nests in high densities (KROGULEC & LEROUX, 1994). Therefore the distance of nearest neighbour on calcareous marshes in eastern Poland was much shorter in comparison with that in most other areas (ARROYO et al., 2004) such as in a big agricultural area in Spain where longest distance between nearest neighbours recorded in clumped category was 600m (ARROYO, 1999).

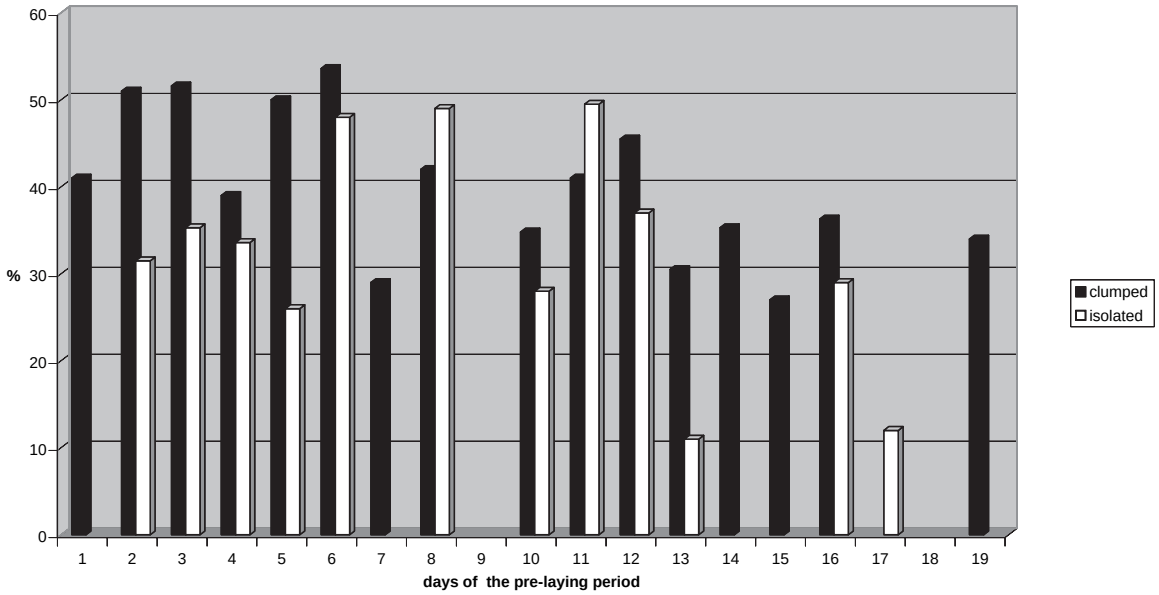

Fig. 3. – Time spend inside the territory by males

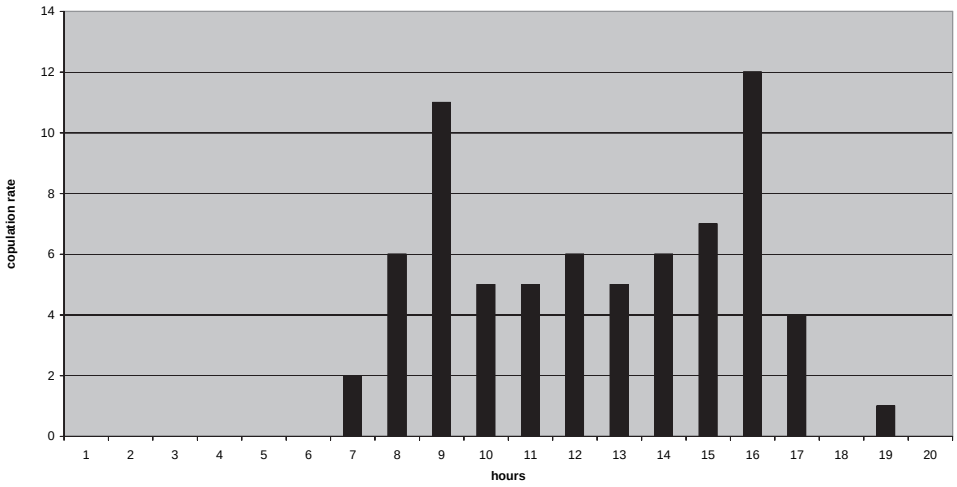

Fig. 4. – Daily pattern of the copulation behaviour

Semi-colonial breeding apparently increases the safety of nests located in close proximity. Based on the result of observations and experiments conducted in Spain and France, semi-colonial breeding decreases individual costs of nest defence and the risk of undertaking such defence (ARROYO at al., 2001). The bigger the colony of birds, the faster the detection of predators so Montagu's Harrier broods is probably safer. My results show that indeed probability of predation by a raptor in tightly clumped nests was significantly lower.

The clumped territories were occupied earlier than solitary ones. Earlier laying occurred in the territories with the most intensively displaying males indicating that males in better physical condition which were chosen first by females (SIMMONS, 1988; 1988a). The high level of sky-dancing points to high genetic quality of these individuals (WIĄCEK, 2004). The breeding place around the best sky-dancers, which are the first ones to settle, is probably considered as the safest one in the context of possible common defence against the predator and at the same time, it seemed more attractive to neighbouring pairs.

Settling near attractive males may have benefits for female in terms of EPC. Territories, taken the earliest, occupied by birds of the highest genetic quality showed larger clutches than isolated ones. The partners of best males, in good condition, are frequently mature females, usually laying the higher number of eggs (KROGULEC, 1992). The bigger size of these nests was the result of higher number of eggs from these females.

Semi-colonial breeding, and high density of territories in one place, increases the level of safety, but the level of aggression between individuals increase too, especially between females who spent most of the time in the territory (CRAMP & SIMMONS, 1980; CLARKE, 1996). Such situation provokes a lot of aggressive situations among

females settling in semi-colonies (SIMMONS, 2000; MOUGEOT et al., 2001).

Females from solitary territories spent more time in the territory than did females from clumped territories. This difference probably derived from a higher risk of predator attack in solitary territories where birds cannot rely on help in the form of common defence against the intruder. That is why they have to spend more time in the territory in order to defend it against C*orvidae* or a fox, the main predators of Montagu's broods in France and Spain (ARROYO et al., 2001) or Poland (WIĄCEK, 2007).

Semi-colonial breeding of Montagu's Harrier increases the risk of extra-pair copulation, in particular because the males of this species spend the majority of the time outside the territory (MOUGEOT et al., 2001; MOUGEOT, 2004). Mate-guarding and intensive courtship feeding during the female fertile period in Montagu's Harrier are difficult to carry out. Nevertheless, the fact that males from clumped nests spend more time in the territory than males in the separated territories may indicate a certain rudimentary form of mate-guarding. This tendency has been described by other authors as rare occurring in other birds of prey with intensive courtship feeding (PANDOLFI et al., 1998; SIMMONS, 2000), although has already been suggested for semi-colonial Montagu's Harriers (MOUGEOT et al., 2001).

A statistically significant difference in the number of copulations in semi-colonies and solitary territories, indicates that the main protection of the female against another male is frequent-within pair copulation which can dilute sperm from rival males and decrease the risk of cuckoldry (BIRKHEAD et al., 1987). The similar correlation between the number of copulations in separated territories and semi-colonies in a Spanish population of Montagu's Harrier, was described by ARROYO (1999). Copulation behaviour in this study was observed most often after food transfer than without food. The same correlation was observed in Spanish population where harrier copulated in two peaks before and afternoon (ARROYO, 1999).

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The Diet of the serotine bat
A Comparison between rural and urban environments

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ABSTRACT. The diet of four maternity colonies of serotine bats in Southern Belgium was investigated by analysing faecal pellets collected from beneath the roost throughout the activity season. Their diet is composed of *Coleoptera Melolonthidae (Melolontha sp*., *Amphimallon sp.*, *Rhizotrogus sp.*, *Serica brunnea)*, *Coleoptera Scarabaeidae* (*Aphodius sp*., *Geotrupes sp.)*, *Coleoptera Carabidae, Diptera Tipulidae, Diptera Chironomidae, Lepidoptera, Hemiptera Pentatomidae, Hymenoptera Ichneumonoidea Ophionidae*, *Trichoptera* and *Arachnida*.

The diet of an urban colony of serotine bats was broadly the same as the diets of three rural colonies. Though some qualitative and quantitative variations were observed between study sites, the main source of variation in the diet was the seasonal availability of potential prey.

The prominence of agriculture-dependant prey (chafers in mid summer and *Aphodius* beetles in late summer and autumn) was observed at all study sites. Consequently, dietary breadth and diversity is smaller during these periods.

KEY WORDS : Food, *Eptesicus serotinus*, *Vespertilionidae,* foraging tactic.

RESUMÉ. Le régime alimentaire de quatre colonies de reproduction de sérotines communes dans le sud de la Belgique a été étudié par l'analyse d'excréments récoltés dans les gîtes durant toute la période d'activité.

Le régime alimentaire se compose de *Coleoptera Melolonthidae (Melolontha sp*., *Amphimallon sp.*, *Rhizotrogus sp.*, *Serica brunnea)*, *Coleoptera Scarabaeidae* (*Aphodius sp*., *Geotrupes sp.)*, *Coleoptera Carabidae, Diptera Tipulidae, Diptera Chironomidae, Lepidoptera, Hemiptera Pentatomidae, Hymenoptera Ichneumonoidea Ophionidae*, *Trichoptera* et d'*Arachnida*.

La composition du régime alimentaire d'une colonie urbaine est largement semblable à celle de colonies situées en zone rurale. Des différences qualitatives et quantitatives existent entre les quatre sites d'études, mais la variation du régime alimentaire est principalement tributaire de la disponibilité saisonnière des proies potentielles.

Dans tous les sites d'étude, la sérotine commune témoigne d'une forte dépendance alimentaire envers des proies liées à l'activité agricole: les hannetons au début de l'été et les *Aphodius* en fin d'été et en automne. Il en découle que la largeur de niche alimentaire et la diversité alimentaire sont réduites à ces périodes.

MOTS CLÉS : Nourriture, *Eptesicus serotinus*, *Vespertilionidae*, tactique de recherche de nourriture.

INTRODUCTION

Bat populations are declining world-wide as a result of a growing number of factors, including habitat loss and fragmentation, disturbances to roosts, exposure to toxins, and introduced predators (RACEY, 1998). This makes it difficult to draw general conclusions about bat conservation, which may require species-specific conservation plans (FENTON, 1997). Understanding the natural history of species and developing hypotheses about foraging strategies requires basic information on food habits (LITVAITIS, 2000).

The serotine bat (*Eptesicus serotinus*) is found throughout much of Western Europe and often roosts in houses. Relatively abundant species, such as the serotine bat, are important for conservation because of their role in ecosystems and the research opportunities they offer.

The rural environment is an usual habitat for the serotine bat since it can take advantage of current farming practices (CATTO et al., 1995; RACEY, 1998). In cities (GAISLER & BAUEROVA, 1986; MICKLEBURGH, 1987; 1989; GAISLER et al., 1998), the serotine bat could however face difficulties in finding food because urban ecosystems do not provide the same insect concentrations (qualitatively and quantitatively) as rural habitats (TAYLOR et al., 1978).

Recently, more attention has been devoted to the urban ecology of bats (*Chiroptera*) because of their ecological importance and the habit of some species to roost in artificial structures (GEGGIE & FENTON, 1984; BENZAL & MORENO, 1989; MICKLEBURGH, 1987; 1989; KURTA & TERAMINO, 1992; GAISLER et al., 1998; GEHRT & CHELSVIG, 2003a; 2003b; WHITAKER et al., 2006).

Numerous studies have documented the food habits of the serotine bat in Great Britain (ROBINSON & STEBBINGS,

1993; CATTO et al., 1994; 1996; VAUGHAN, 1997), Netherlands (LABEE & VOUTE, 1983), Germany (KURTZE, 1982; DENSE, 1992), Luxembourg (HARBUSCH, 2003), Switzerland (BECK, 1995; GERBER et al., 1996) and Czech Republic (ZUKAL et al., 1997; GAJDOSIK & GAISLER, 2004) but none of these studies were conducted in urban surroundings, where habitat fragmentation and loss were likely to be of major importance.

We assumed that serotine bats could modify their trophic niche in order to adapt their dietary requirements to the urban availability of prey. In this way, they would exploit different insect resources from those in rural environments. In this study, data on the diet of an urban colony of serotine bats are presented and compared with similar data collected in rural habitats.

The goals of this paper are to (1) describe the diet of the serotine bat and its seasonal changes; (2) point out intra-specific dietary differences in relation to contrasting environmental conditions around the summer roost.

These issues have broad implications for other species and can be used to focus future research and conservation efforts.

STUDY SITES

Four colonies were studied in southern Belgium: three were in a rural landscape and one was in a city.

The first rural colony consists of circa 40 breeding females roosting in a house in Tintigny, Province of Luxembourg (UTM coordinates 31 U FR 81 06), a village situated in the Semois River valley in a landscape including pastures, and both coniferous and deciduous forests. On the south, it is bordered by beech forests and on the north by the southern slopes of the Ardennes. This colony dispersed in 1997 due to a visit by a stone marten, *Martes foina*. The second rural colony, similar in size, roosted approximately 3km from the first, in the church of Saint Vincent, Province of Luxembourg (UTM coordinates 31 U FR 78 05) in the same habitat type. A colony roosting in a house in Doische, Province of Namur (UTM coordinates 31 U FR 24 55), consisted of about 20 breeding females. This village is surrounded mainly by broad-leaved and coniferous forests, pastures, and arable land.

A colony of approximately 40 females roosted in an attic in Namur (UTM coordinates 31 U FR 32 91), a town of 100000 inhabitants, located in the Meuse River valley at its confluence with the Sambre River. The surrounding area is heavily urbanized.

METHODS

Polythene sheets were placed on the attic floor, beneath the roosting bats, from the end of April to October. Faecal pellets were collected every two weeks, air-dried and stored in plastic bags. Five periods are distinguished according to the physiological stage of the females (Table 1).

Data gaps are due to the absence or scarcity of bats in the roost during the sampling period. Sample periods during which bats were scarce (<3 individuals in the colony) are excluded from the study. Pellets were taken at random, using a random number generator, to reduce the probability of collecting pellets from the same individual. The sample size was assessed a posteriori by examining the variation of prey proportions relative to the number of analysed pellets (KERVYN, 1998; KERVYN, 2001). Serotine bats, in the roosts, used to crawl on the ridgebean rather than hang freely and this could have led to a weak contamination of faecal samples by older droppings.

TABLE 1

Subdivision of bat activity period into fortnights and periods.

Date	Development of the young	Fort-night	Physiological state of females	Period
April II		1	post hibernation	I
May I		2	post hibernation	I
May II		3	late pregnancy	II
June I		4	late pregnancy	II
June II	Birth	5	lactation	III
July I		6	lactation	III
July II	First foraging flights	7	lactation	III
Aug. I		8	post lactation	IV
Aug. II		9	post lactation	IV
Sept. I		10	post lactation	IV
Sept. II		11	pre hibernation	V
Oct. I		12	pre hibernation	V

Each faecal pellet was soaked in water on a microscope slide and teased apart under a binocular microscope using a pair of dissecting needles. Identification of insect pieces was facilitated by the descriptions of WHITAKER (1988), MCANEY et al. (1991), PIR (1994), and KERVYN (1995; 2001). Insect fragments were also compared with whole insect specimens collected on the bat's foraging grounds or with the entomological collection of the Zoological Museum of Liège. No attempt was made to estimate accurately the frequency of fragments or percentage volume of prey taxa within a dropping, because most fragments cannot be attributed to a single taxon. Moreover, the remains of a single prey are distributed among many droppings (ROBINSON & STEBBINGS, 1993).

Relevance and limits of this method were evaluated by several authors (KUNZ & WHITAKER, 1983; DICKMAN & HUANG, 1988; ROBINSON & STEBBINGS, 1993). Faecal analysis does not provide the exact composition of the ingested food. However, it allows an estimation of food composition, especially common prey items. Its use is valuable for seasonal or geographical comparisons of the diet. Results usually overestimate the proportion of large insects and of those leaving easily identifiable pieces even after ingestion and digestive transit. Soft bodied insects may be underrepresented.

Results are expressed in relative frequency of occurrence which represents the number of pellets containing the item among the sample of 40 pellets, divided by the total number of items.

Dietary diversity was calculated using the Shannon-Weaver index: $H'=-Sp_i.\log_2 p_i$ where p_i is the proportion of the i^{th} item and n is the total number of items (BREWER, 1994). Trophic niche breath was calculated as follow: $DB=((Sp_i^2)^{-1}-1)/(n-1))$ (HESPENHEIDE, 1974).

To detect possible temporal variations, a goodness-of-fit test (SOKAL & ROHLF, 1981) was performed for each individual colony, comparing the frequency distribution of prey items during successive fortnights. A Newman-Keuls test was used to identify the origin of the variations. Most analyses were performed on Minitab 10.1 for Windows (Minitab Inc., 1829 Pine Hall Rd State College PA 16801-3008 USA).

RESULTS

Diet Composition

Thirty-six faecal samples were collected: 9 at Doische in 1996, 11 at Tintigny in 1996, 4 at Saint-Vincent in 1996 and also in 1997, and 8 at Namur in 1998. Some fortnights were not studied because of movements of the colony inside the roost or to secondary roosts.

TABLE 2

Diet composition of the serotine bat over 12 fortnights (see Table 1) at four study sites (D96: Doische 1996, T96: Tintigny 1996, SV96: Saint-Vincent 1996, SV97: Saint-Vincent 1997, N98: Namur 1998).

Fortnight	Site and year	Melolontha sp.	Amphimallon sp. and Rhizotrogus sp.	Serica brunnea	Aphodius sp.	Geotrupes sp.	Carabidae	Tipulidae	Chironomidae	Lepidoptera	Ichneumonidae	Hemiptera	Trichoptera	Arachnidae	indéterminés	TOTAL
1	D96	5	34	0	0	0	0	2	0	0	2	0	0	0	8	51
	T96	29	0	1	8	0	0	3	0	3	1	2	0	0	2	49
	SV96	34	0	2	3	0	0	1	0	5	1	0	0	0	4	50
2	D96	7	35	0	0	0	0	5	1	2	0	0	0	0	4	54
	T96	36	0	0	4	0	1	1	0	2	0	0	0	0	1	45
	SV97	40	0	0	0	1	0	6	0	0	5	0	0	0	0	52
	N98	37	2	0	0	0	0	11	0	9	6	3	1	0	5	74
3	D96	33	17	0	0	0	1	5	0	0	1	0	0	1	0	58
	T96	40	0	0	0	0	0	11	0	0	9	0	0	0	0	60
	SV97	39	0	0	1	3	0	23	0	0	10	0	0	0	0	76
	N98	30	17	0	0	0	0	15	0	12	5	6	3	0	4	92
4	D96	15	3	0	0	0	0	1	0	0	3	0	17	0	2	41
	T96	36	0	0	0	0	1	32	0	6	25	0	0	0	3	103
	SV96	14	0	0	0	1	7	31	0	19	22	1	21	0	0	116
	N98	4	20	0	0	0	6	12	0	10	3	11	12	0	2	80
5	D96	1	3	0	0	0	0	4	0	0	1	0	35	0	5	49
	T96	29	0	2	1	0	0	30	0	19	11	0	0	0	1	93
	N98	2	27	0	0	0	1	8	0	8	2	2	5	0	4	59
6	D96	1	29	0	0	3	0	2	0	4	0	2	2	0	4	47
	T96	0	0	0	19	0	2	8	1	23	4	2	2	0	2	63
	N98	1	26	0	0	0	1	24	3	9	1	3	1	0	0	69
7	D96	14	16	0	1	1	0	3	5	4	0	0	6	0	3	53
	SV96	10	0	5	13	2	5	6	7	8	0	13	9	0	2	80
	SV97	0	0	3	39	0	0	20	0	14	1	8	16	0	2	103
	N98	0	26	0	2	0	7	10	0	15	0	18	1	0	2	81
8	D96	0	0	0	36	5	0	9	0	20	0	10	0	0	3	83
	T96	0	0	16	30	4	3	5	0	14	3	4	1	0	1	81
	SV97	0	0	7	32	8	3	17	0	9	2	18	0	0	7	103
	N98	0	11	0	9	0	1	2	0	15	0	35	1	0	1	75
9	T96	4	0	0	33	0	0	5	0	3	8	0	0	0	2	55
10	D96	0	0	12	38	6	0	5	0	3	6	0	0	0	4	74
	T96	0	0	0	40	1	0	2	0	0	7	1	0	0	2	53
	SV96	0	0	3	37	6	3	5	0	6	4	12	0	0	3	79
	N98	0	0	0	21	0	1	0	0	13	1	21	0	2	1	60
11	T96	0	0	0	40	6	0	1	0	0	6	0	0	0	2	55
12	T96	0	0	0	40	3	0	3	0	2	2	0	0	0	1	51

A total of 2467 insect fragments were recorded from 1440 droppings (Table 2). 2380 items were identified and 87 were not. The mean number of prey taxa per dropping was 1.61±0.84 (n=1440), with a maximum of 6.

Coleopterans accounted for the majority of identified prey: *Melolontha sp*. (19.4%), *Amphimallon sp.* and *Rhizotrogus sp.* (11.2%), *Serica brunnea* (2.1%), *Aphodius sp*. (18.8%), *Geotrupes sp.* (2.1%), and *Carabidae* (e.g. *Harpalus sp*., 1.8%). The second most frequently consumed group of insects belonged to the order *Diptera*: *Tipulidae* (13.8%), and *Chironomidae* (0.7%). Other prey were from the orders *Lepidoptera* (10.8%), *Hemiptera* (*Pentatomidae* 7.2%), *Hymenoptera* (*Ichneumonoidea*

Ophionidae 6.4%) and *Trichoptera* (5.6%), and non-insect Arachnids (0.1%) (Fig. 1).

Seasonal and spatial variations

Considered fortnight by fortnight, the results are significantly heterogeneous between the different colonies. The G values are always highly significant ($p<0.001$) indicating that the frequency distribution of the prey items vary from one colony to another, independently of the time of the year. Table 3 shows the prey categories involved in these significant changes and Table 4 the localities (colonies) deviating from random.f

TABLE 3

Partial values of G for composition of prey types.

ortnight	1	2	3	4	5	6	7	8	10
d.f.	**13**	**13**	**13**	**13**	**13**	**13**	**13**	**13**	**13**
Melolontha sp.	<0,001	<0,001	<0,025	<0,001	<0,001	ns	<0,001	ns	ns
Amphimallon sp. / Rhizotrogus sp.	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	ns
Serica sp.	ns	ns	ns	ns	ns	ns	ns	<0,001	<0,005
Aphodius sp.	ns	ns	ns	ns	ns	<0,001	<0,001	<0,005	<0,05
Geotrupes sp.	ns	ns	ns	ns	ns	ns	ns	ns	ns
Carabidae	ns	ns	ns	ns	ns	ns	<0,025	ns	ns
Tipulidae	ns	ns	<0,05	<0,001	<0,01	<0,005	ns	<0,025	ns
Chironomidae	ns	ns	ns	ns	ns	ns	<0,005	ns	ns
Lepidoptera	ns	ns	<0,01	<0,025	<0,01	<0,01	ns	ns	<0,005
Ichneumonidae	ns	ns	<0,05	<0,001	ns	ns	ns	ns	ns
Hemiptera	ns	ns	ns	<0,001	ns	ns	<0,001	<0,001	<0,001
Trichoptera	ns	ns	ns	<0,001	<0,001	ns	<0,025	ns	ns
Arachnidae	ns	ns	ns	ns	ns	ns	ns	ns	ns
unidentified	ns	ns	ns	ns	ns	ns	ns	ns	ns

TABLE 4

Partial values of G for food composition at four study sites ('–' means no data).

fortnight	1	2	3	4	5	6	7	8	10
d.f.	**2**	**3**	**3**	**3**	**2**	**2**	**3**	**3**	**3**
D96	<0,001	<0,001	ns	<0,001	<0,001	<0,005	<0,001	ns	<0,025
T96	<0,001	<0,025	ns	<0,001	<0,001	<0,001	–	<0,005	<0,05
SV96	<0,001	–	–	<0,01	–	–	<0,01	–	ns
SV97	–	<0,025	<0,025	–	–	–	<0,001	ns	–
N98	–	ns	<0,025	<0,001	<0,001	<0,005	<0,001	<0,001	<0,001

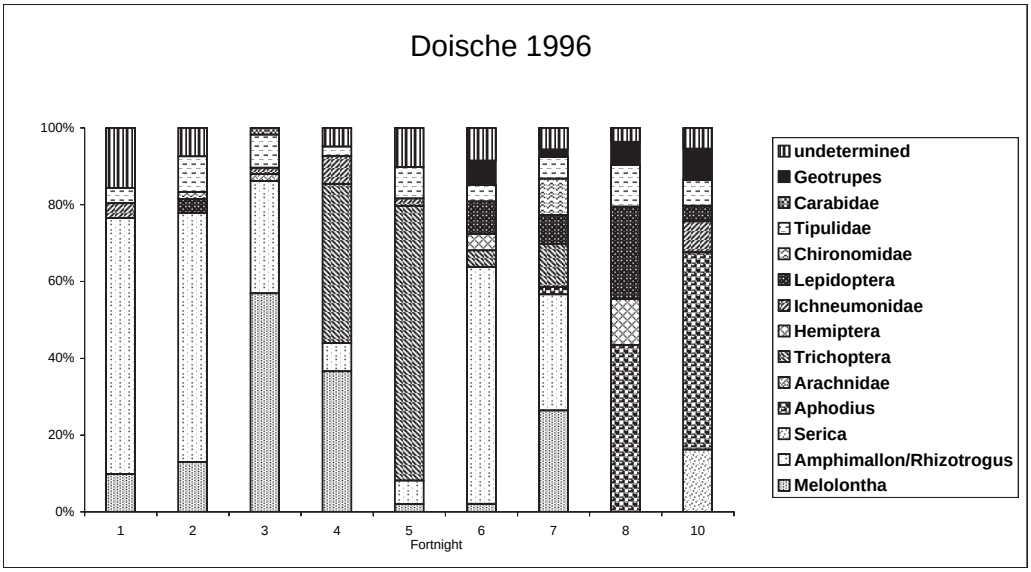
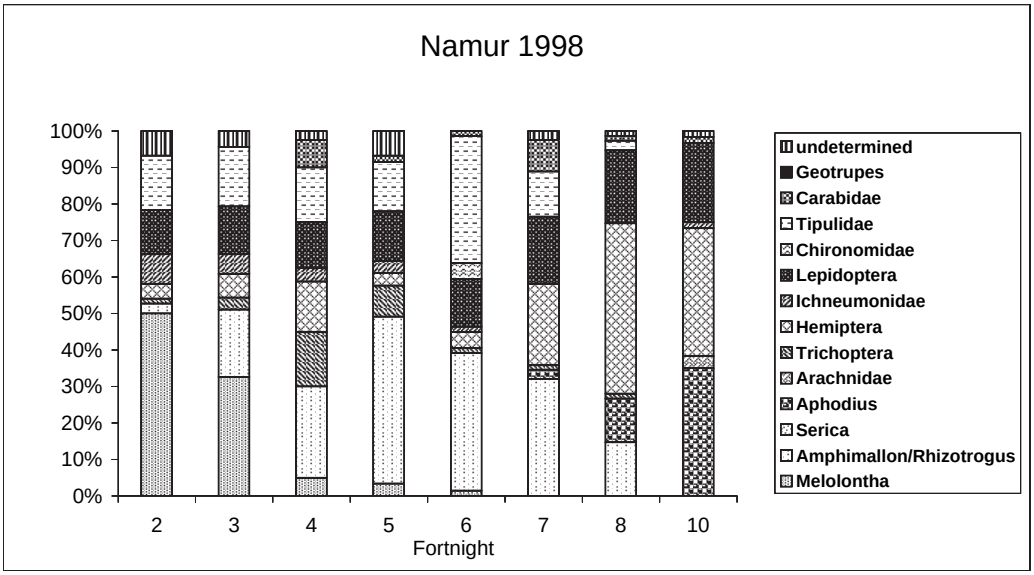
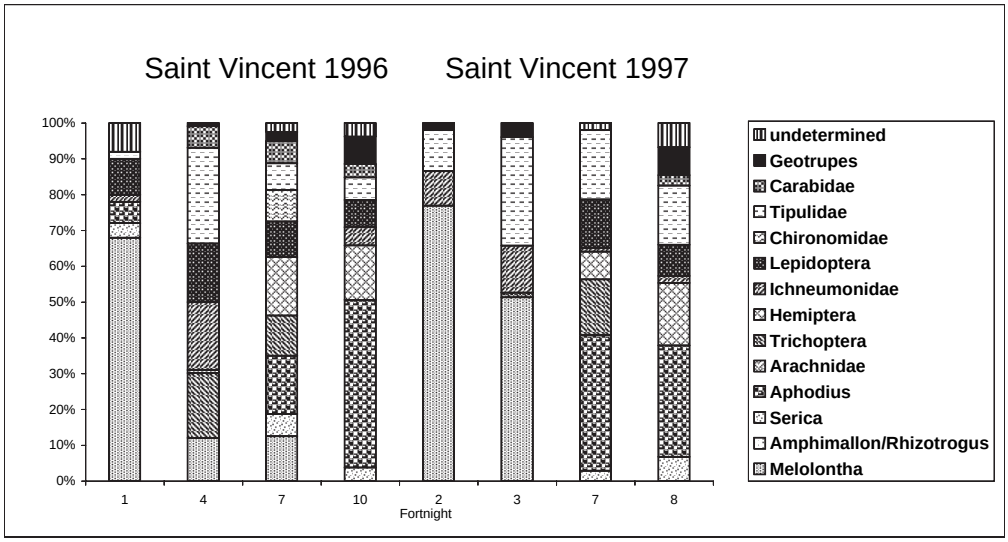
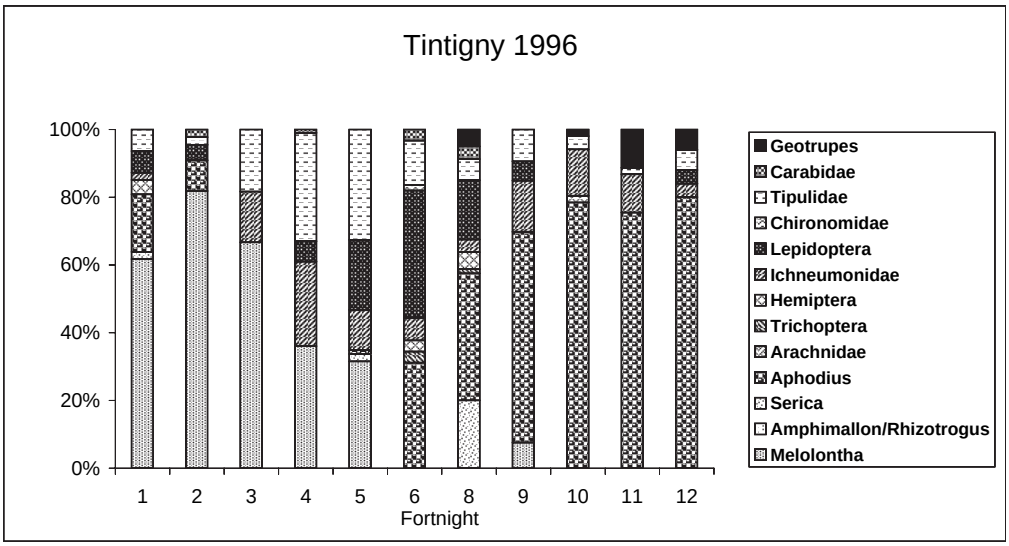


Fig. 1. – Seasonal variations of the food composition (relative frequency of occurrence) of the serotine bat in Tintigny, Saint-Vincent, Doische and Namur. Fortnights are explained in Table 1.


Namur 1998
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2
3
4
5
6
7
8
10
Fortnight
undetermined
Geotrupes
Carabidae
Tipulidae
Chironomidae
Lepidoptera
Ichneumonidae
Hemiptera
Trichoptera
Arachnidae
Aphodius
Serica
Amphimallon/Rhizotrogus
Melolontha


Saint Vincent 1996
Saint Vincent 1997
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
4
7
10
2
3
7
8
Fortnight
undetermined
Geotrupes
Carabidae
Tipulidae
Chironomidae
Lepidoptera
Ichneumonidae
Hemiptera
Trichoptera
Arachnidae
Aphodius
Serica
Amphimallon/Rhizotrogus
Melolontha


Tintigny 1996
100%
80%
60%
40%
20%
0%
1
2
3
4
5
6
8
9
10
11
12
Fortnight
Geotrupes
Carabidae
Tipulidae
Chironomidae
Lepidoptera
Ichneumonidae
Hemiptera
Trichoptera
Arachnidae
Aphodius
Serica
Amphimallon/Rhizotrogus
Melolontha

At each colony, the G value is highly significant ($p<0.001$), indicating the diet changes dramatically. Prey changes by sites are illustrated in Table 5 whereas periods (fortnights) deviating from random are in Table 6.

TABLE 5

Total values of G for the prey types versus the four study sites.

site	D96	T96	SV96	SV97	N98
d.f.	104	143	39	39	104
G-test	<0,001	<0,001	<0,001	<0,001	<0,001

TABLE 6

Partial values of G for food composition by fortnight ('-' means no data).

site	D96	T96	SV96	SV97	N98
d.f.	**8**	**11**	**3**	**3**	**8**
1	<0,001	<0,01	<0,001	-	-
2	<0,001	<0,001	-	<0,001	<0,001
3	<0,001	<0,001	-	<0,001	<0,001
4	<0,001	<0,001	<0,001	-	<0,025
5	<0,001	<0,001	-	-	<0,005
6	<0,001	<0,001	-	-	<0,001
7	ns	-	<0,025	<0,001	<0,005
8	<0,001	<0,001	-	<0,001	<0,001
9	-	<0,01	-	-	-
10	<0,001	<0,001	<0,001	-	<0,001
11	-	<0,001	-	-	-
12	-	<0,001	-	-	-

Chafers made up most of the diet during late April – May at all study sites (Fig. 1). *Rhizotrogus – Amphimallon* predominate in Doische, whilst *Melolontha sp*. was the main prey elsewhere. During late June – July, *Rhizotrogus – Amphimallon* predominated again in Doische and was present in Namur. Tipulids were found mainly in May and June but also in September. Ophionids were taken in May and June but in small quantities. *Trichoptera* were consumed in June – July, especially in late June in Doische where they accounted for more than two thirds of the diet. Lepidopterans were mainly consumed in late June – July, except in Doische. Chironomids were eaten in July in moderate quantities. *Hemiptera* were encountered, mainly in Namur, in late July – August. This order is mainly represented by *Pentatomidae,* especially *Pentatoma* sp. *Serica brunnea* was identified in the diet in August – September in Saint-Vincent, Tintigny and Doische. *Aphodius* beetles appeared in July and were a major part of the diet until the end of the season at all sites. A weak contamination by older droppings could explain the presence of *A. rufipes* in the samples of April-May. *Geotrupes* and Arachnids were rarely found and do not show a seasonal trend.

Dietary breadth and diversity

Shannon-Weaver diversity indices were not significantly different between study sites for each of the first four periods (F-test at a=0.05: $F_1=1.29$; $df_1=6$; $p_1=0.480$; $F_2=3.79$; $df_2=7$, $p_2=0.151$; $F_3=1.08$, $df_3=9$, $p_3=0.455$; $F_4=1.13$, $df_4=8$, $p_4=0.455$). The fifth period was not included because of the small sample size (n=2).

An F-test indicated the diversity was significantly different (F-test at a=0.05: F=4.22, df=35, p=0.008) among the five periods. A Newman-Keuls test (a=0.05) showed that the first and the last periods were significantly different from the others (Fig. 2).

Similar results were obtained for dietary breadth indices (F-test at a=0.05: $F_1=1.51$; $df_1=6$; $p_1=0.436$; $F_2=4.99$; $df_2=7$, $p_2=0.109$; $F_3=5.12$, $df_3=9$, $p_3=0.051$; $F_4=0.59$, $df_4=8$, $p_4=0.688$) (F-test at a=0.05: F=2.90, df=35, p=0.038).

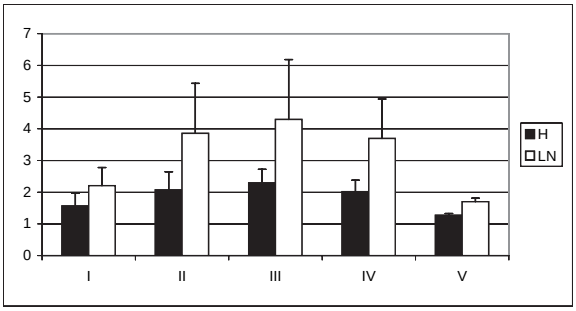


Fig. 2. – Shannon-Weaver diversity index and dietary breadth index in relation to the serotine bat activity periods. Periods are defined in Table 1.

DISCUSSION

Composition

The diet composition was similar to that reported by other authors (KURTZE, 1982; LABEE & VOUTE, 1983; ROBINSON & STEBBINGS, 1993; CATTO et al., 1994; BECK, 1995; GERBER et al., 1996; VAUGHAN, 1997; HARBUSCH, 2003). The most striking result is the lack of qualitative differences between the diet of urban and rural colonies of serotine bats.

A goodness of fit test showed quantitative differences between the four study sites. However, the difference between the urban study site and the three rural sites is not more important than within the three rural sites, except the two last fortnights (8 and 10) where *Hemiptera* are more frequent in the diet in Namur and *Aphodius* beetles are less important (Fig. 1).

In Doische, summer chafers were eaten instead of cockchafers elsewhere. Similarly trichopterans were more consumed in Doische than by the other colonies, reducing the proportion of lepidopterans and tipulids.

Influence of the phenology of prey

Seasonal variations observed in the diet of the Serotine bat can easily be explained when considering the phenology of its main prey. Cockchafers (*Melolontha sp.*) are a major prey in May and June, i.e. at the peak of their flight activity (KERVYN, 1996; GERBER et al., 1996; HARBUSCH, 2003).

The food item *Rhizotrogus sp. – Amphimallon sp.* shows a bimodal pattern in Doische corresponding to the respective emergence of *Rhizotrogus sp.* (late April – May) and *Amphimallon solstitialis* (June – July). Tipulids, mainly represented by the large *Tipula* genus, emerge from the soil in late May – June (*Tipula oleracea*)

and again in July and August (*T. paludosa*) (COULSON, 1959; 1962). The same pattern of occurrence of this prey item (*T. oleracea*) was also observed in the diet of *Myotis myotis* in southern Belgium (KERVYN, 1996).

Consumption of caddisflies (*Trichoptera)* in June – July reflects emergence of these insects observed in the field. Predation of chironomids also reflects local availability of swarms.

Serica brunnea are known to fly in late summer (DU CHATENET, 1986). This small chafer is also consumed at that time by the greater horseshoe bat and the serotine in neighbouring Luxembourg (PIR, 1994; HARBUSCH, 2003).

Since most identified hemipterans were *Pentatoma rufipes*, the occurrence of the hemipterans in the diet in late summer is explained by its phenology (VILLIERS, 1945).

Aphodius beetles are represented by *A. rufipes*, a large species that emerges in late summer (DESIÈRE, 1974). This prey is observed during that period in all dietary studies of the serotine in Western Europe (LABEE & VOUTE, 1983; CATTO et al., 1994; BECK, 1995; GERBER et al., 1996).

The phenology of lepidopterans in the diet is hard to interprete because there are many species that are impossible to distinguish from one other in bat droppings.

KURTZE (1982) and ROBINSON & STEBBINGS (1993) reported a peak in predation of *Geotrupes* in spring and in July.

Previous studies revealed the predation of other insects. *Hymenoptera* were observed (BECK, 1995), especially *formicids* by GERBER et al. (1996) and ZUKAL et al. (1997). The predation of *hydrophilids* (LABEE & VOUTE, 1983), of the burying beetle *Necrophorus humator* (ROBINSON & STEBBINGS, 1993; CATTO et al., 1994) and of the chafer *Polyphylla fullo* has also been reported (GERBER et al., 1996). CATTO et al. (1994) also report the incidental identification of *Neuroptera*, *Plecoptera* and *Aphidae.*

Dietary diversity

Dietary diversity is greatly reduced in early spring and late autumn because of a heavy dependence upon cockchafers – summer chafers and *Aphodius* beetles respectively. A higher diversity from late May to early September can be due to a higher diversity of available insects, to increased foraging periods during the night by lactating females (DENSE, 1992; CATTO et al., 1995, pers obs.), or to the presence in the colony of individuals of various physiological states (non reproductive, pregnant, lactating, young) (CATTO et al., 1994) or that forage in different habitats. This seasonal pattern of food diversity contrast with those obtained by HARBUSCH (2003) and is very different from the one observed in *Rhinolophus ferrumequinum*, a similarly-sized bat eating the same prey types, but focusing on lepidopterans during late pregnancy (JONES, 1990; RANSOME, 1996). Food availability should be regarded as a major source of variability in dietary diversity (HARBUSCH, 2003). Horseshoe bats have a specialised echolocation system enabling them to prey on lepidopterans. Their echolocation clicks (frequency range near 80KHz) fall indeed outside the hearing possibilities of the moths, ranging from 15 to 60KHz, whereas the frequency of the serotine bat signals ranges around 25KHz.

Differences among study sites

Intraspecific geographic variation in bat diets likely reflects geographic variation in the availability of insects (BELLWOOD & FENTON, 1976; WHITAKER, 1995; GERBER et al., 1996; AGOSTA & MORTON, 2003; BRACK & LAVAL, 2006).

However, in this study, chafers and *Aphodius* beetles were invariably, at all study sites, the key prey eaten respectively in spring and in autumn.

Two hypotheses can explain the absence of difference between rural and urban bat diets. On the one hand, insect prey could be present in the city (parks, urban gardens), in close vicinity (residential areas), or could disperse from the countryside towards the cities. On the other hand, serotine bats could compensate for local urban food shortage by foraging further away, in unbuilt areas.

Both hypotheses are valid, depending on the prey concerned. For instance, summer chafers (*Amphimallon solstitialis*) were seen flying along tree-lined streets and were hunted by foraging serotine bats. Parks, cemeteries, fallow lands, and football playing fields in urban areas are not ploughed or sprayed with insecticide. They are the urban equivalent of unimproved pasture and a good source of chafers. So are also many gardens. However, *Aphodius* beetles are closely associated with cow-dung and are therefore quite rare in cities. Therefore, their presence in the food of urban serotine bats suppports the hypothesis that bats travel to rural areas to forage.

Foraging strategy and habitats

Serotines take advantage of insect populations emerging in a short period of time in the night as well as over the season. A large prey item (e.g. chafer, tipulid, *Hemiptera*,..) could be energetically beneficial These insects fly noisily and can therefore be detected at a distance of up to 10 meters (pers. obs.). Passive acoustic detection is undoubtedly a cue used to locate them, especially just prior to take off (ROBINSON & STEBBINGS, 1993, pers. obs.). This does not exclude use of echolocation to avoid obstacles, locate precisely a flying noisy insect or identification of other potential prey (e.g., lepidopterans).

The ecology of cockchafers, summer chafers, tipulids and ichneumonids indicates that the serotine bat is likely to forage from May to July along broad-leaved forest edges, in orchards, and over hay meadows and pastures (CATTO et al., 1996; SCHMIDT, 2000). Lepidopterans frequent the same habitats, and are found in the vicinity of street lamps (CATTO et al., 1996; SCHMIDT, 2000). *Aphodius* beetles are strongly associated with cow-dung and are eaten in autumn by serotines foraging over grazed pastures. The importance of *Aphodius* in the diet may explain why BARTONICKA & ZUKAL (2003) failed to find foraging serotines in towns from the end of August on. In the urban study site of Namur, the nearest grazed pasture is 2.5km from the roost. This distance is therefore a minimal activity radius for this colony. Swarms of insects (chironomids, trichopterans) along stream and small riv-

ers also attract foraging serotine bats (LABEE & VOUTE, 1983, pers. obs.).

Conservation

Cockchafers and summer chafers emerge in large quantities and are consumed not only by the serotine bat (CATTO et al., 1994), but also by other large and rare bats such as *Rhinolophus ferrumequinum* (JONES, 1990; PIR, 1994), and *Myotis myotis* (KERVYN, 1995). Our results indicate that these insects are key-species for serotine bats since they are the first – and nearly the only – prey taken between and after the last hibernation bouts. Current farming practices, especially ploughing and sowing of pastures, destroy large quantities of larvae of these preys.

Aphodius beetles can also be considered key prey species since they are almost the only prey eaten in autumn. This illustrates a close association between this bat and human activities, in addition to roost sites (CATTO et al., 1995; RACEY, 1998). This relationship is fragile since use of antihelminthic drugs affects development and survival of dung beetles (WALL & STRONG, 1987; MADSEN et al., 1990; RANSOME, 1996).

The main conclusion is that, when in cities, the serotine bat does not adapt its diet to other prey but instead uses a restricted array of prey, probably energetically important large flying insects and/or insects available in large quantities.

We suggest that the absence of key-prey in the proximate surroundings of the colony induces, for the serotine bat, longer commuting flights and could consecutively decrease fitness – highlighted for the greater horseshoe bat (RANSOME, 1996) – or force the colony to use roosts located closer to profitable foraging grounds. Further studies taking fitness into account (e.g. reproductive success of adults and juvenile survival) should be completed to evaluate the impact of key-prey habitat loss or fragmentation near urban colonies.

CONCLUSION

The food composition of an urban colony of *E. serotinus* is broadly the same as in rural environment. Some qualitative and quantitative variations of food composition are observed among study sites. The main source of variation in the diet – both in rural and urban environments – is the phenology of available prey. Altough dietary diversity is higher in mid summer, the serotine bats prey on a restricted set of insects in early summer (chafers) and in late summer and automn (*Aphodius* beetles).

In Namur, some prey are present within the city (*Amphimallon*), whereas other prey can only be found outside a radius of minimum 2.5km (*Aphodius rufipes*), supporting the hypothesis that bats are likely to forage outside the city.

This study confirms adaptation of the serotine bat to an anthropogenic environment, but because it feeds mainly on key prey species from agricultural lands, including species dependant upon cattle husbandry (Cockchafers – summer chafers, and *Aphodius* beetles) it is also potentially sensitive to current farming practices.

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Species assemblages and habitat preferences of Ostracoda (Crustacea) in Lake Abant (Bolu, Turkey)

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ABSTRACT. Aquatic habitats are threatened by human activities in Turkey. These threats can lead to changes in ecological conditions and community composition of ostracod species. Overall, reduction in habitat quality may increase the number of tolerant (e.g., generalist) species in relation to the number of native (e.g., specialist) species. This phenomenon is called *pseudorichness* and is also characterized by a critical increase in the total numbers of colonial bacteria. A total of 16 ostracod species were encountered in Lake Abant (Bolu, Turkey) and its environs between 2001 and 2003. Two species (*Psychrodromus fontinalis, Eucypris pigra*) found outside the lake are new records for the region. About 80% of the species in this area have a cosmopolitan distribution throughout the Holarctic region. Four major clustering groups (UPGMA) were recognized based on species occurrences and ecological preferences. Each species was distinctly tolerant to different ecological variables though cosmopolitan species tended to have wider tolerance ranges compared to sensitive species. About 82% of the relationship between species and environmental variables was explained with Canonical Correspondence Analysis ($P<0.05$). Accordingly, temperature, dissolved oxygen and conductivity of water were found to be the three most influential factors affecting species composition. Spearman correlation analysis showed a significant positive relationship ($P<0.01$) between the number of individuals and both dissolved oxygen and the number of species. Generally, to provide long-term conservation of the Lake Abant Nature Park, immediate attention is needed for the wastewater treatment, and help from international organizations.

KEY WORDS : conservation, Lake Abant, Ostracoda, pseudorichness, limnoecology, tolerance.

INTRODUCTION

Among the 16 nature parks in Turkey, Lake Abant Nature Park (LANP) in Bolu (about 1196.5 ha) is one of the most famous. The park includes about 1221 plant and animal species, of which at least 60 are endemic, including 1 rat subspecies (*Muscardinus avellanarius abanticus* Kıvanç, 1983), 1 fish subspecies (*Salmo trutta abanticus* Tortonese, 1954), ca. 50 plant species (e.g., *Crocus abantensis* T. Baytop & B. Mathew 1975), and at least 3 invertebrate species (e.g., *Zerynthia* (*Allancastria) caucasica abanti* Koçak, 1975). However, as with many other natural areas, anthropogenic disturbance (e.g., pollution, habitat fragmentation) threatens species diversity within the park. In the case of LANP, such factors cause a rapid reduction in the quality of the lake water and its environment, causing a decline in the number of native species and an increase in the numbers of cosmopolitan species.

When species assemblages are determined by the ecological preferences and tolerance levels of species, changes in ecological conditions can affect species composition (Dügel & Kazancİ, 2004). Thus, one may expect to observe a corresponding decline in both water quality and the "quality" of the species assemblages when the numbers of cosmopolitan species increase. The meaning of "reduction of water quality" refers to the "changes in standard ecological conditions and/or health of lake water and environment". This process is explained by the "pseudorichness hypothesis", in which the total number of species tends to increase in the short-term while the ratio of specialist species to cosmopolitan species tends to decrease (Külköylüoğlu, 2004). However, the levels of decline in species composition may not be recognized with short-term studies, even when species composition includes cosmopolitan species with high tolerance ranges (Yilmaz & Külköylüoğlu, 2006). In such a case, one may mistakenly conclude no (or little) significant effect of environmental changes (e.g., due to anthropogenic disturbance) on community composition. This may lead to acceptance of the null hypothesis when it is actually false, or a Type II error (Hurlbert, 1984; Zar, 1999). The consequences of this error are well described in literature, but almost nothing is known about it, concerning the use of ostracods as ecological indicators.

Ostracods are widely distributed in all types of aquatic environments, from fresh to saline waters, in which they show different tolerances and preferences to various ecological variables. If ecological preferences and tolerance levels of individual species are known, the past, current and future habitat conditions can be estimated (Delorme, 1991; Külköylüoğlu & Dügel, 2004; Külköylüoğlu, 2005a; 2005b). Therefore, ostracods are useful as indicators of water quality in different aquatic bodies (Bromley & Por, 1975; Külköylüoğlu, 1999; Külköylüoğlu & Vinyard, 1998; Mezquita et al., 1999). Consequently, knowledge of species' current habitat requirements and ecological preferences can also be used to reconstruct past and future ecological conditions. Additionally, ostracods are sensitive to changes in different environmental variables, which can thus affect species composition as a whole. Detection of such changes in community composition can be used to interpret conditions in any aquatic body within a broader context. This may also allow estimation of possible future changes in species composition.

However, certain (i.e. cosmopolitan) species tend to have wide tolerance levels, and therefore they can resist changes in water conditions, at least for a wider range than specialists. In such cases, negative environmental effects on species richness of disturbed habitats may not be recognized due to dominancy of common species. Although it is critically important to know the role of cosmopolitan species in community assemblages, our knowledge is far from complete for ostracods, and many other taxa as well. To better understand the relationship between conservation and the pseudorichness hypothesis along with the role of cosmopolitan ostracods, KÜLKÖYLÜOĞLU (2004) proposed two critical questions: i) what kind of environmental conditions do individual ostracod species prefer?, and ii) which ecological factor(s) affect(s) their occurrence most? Although these questions can be applied to different taxonomic groups, they are not easy to answer without detailed studies.

The aim of this study consists of three parts: (1) documenting the species composition of ostracods in Lake Abant, (2) characterizing the relationships between ecological preferences and tolerance levels of ostracods, and (3) highlighting the conservation status of Lake Abant Nature Park, along with its environmental problems.

MATERIALS AND METHODS

Study Area

Lake Abant (Fig. 1) is a monomictic lake (Maximum depth (Zmax): 18m; Elevation: 1345m a.s.l.), which is located in Lake Abant Nature Park (40º36'702" N 31º16'721" E) about 30km west of the city Bolu (Turkey). The lake was formed as a result of land sliding at the northeastern ends (ERINÇ et al., 1961; ORBAY et al., 1994) after tectonic activities about 8000-10000 years ago (NEUGEBAUER et al., 1997). Since this time climatic activities have changed the lake environment. However, the most influential change occurred 3500-4000 years ago, when the first humans settled around the lake (WOLDRING et al., 1986; BOTTEMA et al., 1993). One of the recent effects of human activities occurred about 50 years ago, when the lake surface area was extended from 115ha to 125ha (IRMAK, 1947; AKŞIRAY, 1959) to provide suitable shallow habitats for the juveniles of common rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)). This fish, however, reduced the numbers of the endemic fish (*S. t. abanticus*) in the lake. Besides these two, there are five more fish species in the lake: *Tinca tinca* L., 1758 (tench), *Barbus plebejus* (Bonaparte, 1839) (barbus), *Alburnoides bipunctatus* (Bloch, 1782) (chub), *Gobio gobio* (Linnaeus, 1758) (gudgeon), and *Leuciscus cephalus* (Linnaeus, 1758) (freshwater chub) (HOŞ, 2005).

Sampling

Eight physicochemical variables commonly used in studies of aquatic habitats (WRIGHT et al., 1989; RUSE, 1996; MAZLUM et al., 1999; MOUNY & DAUVIN, 2002; KÜLKÖYLÜOĞLU & DÜGEL, 2004), were measured monthly from October 2001 to October 2003: pH, redox potential (Standard Hydrogen Electrode (SHE) [mV]), dissolved oxygen (DO [mg/L]), percent oxygen saturation (%Sat), water (T(w)) and air temperature (T(a) [ºC]), electrical conductivity (EC [µS/cm]), and salinity (S [ppt]). Total dissolved solids (TDS [mg/l]) were measured in the laboratory following standard methods (APHA, 1988). pH and redox potential were measured with a Hanna model HI-98150 pH/ORP meter, while other variables were measured with a YSI-85 model oxygen-temperature meter at each station. Ostracod samples were collected at a water depth of 100cm with a plankton net (0.25mm mesh size) from 10 randomly selected stations around the lake side and fixed in 70% ethanol *in situ* after measuring the ecological variables. In the laboratory, after filtering the samples over four different sieves (0.25: 0.50: 1.0: 2.0mm in mesh size), ostracods were hand-sorted and preserved in 70% ethanol. Subsequently, specimens were mounted in lactophenol solution. Systematic keys of MEISCH (2000) were used for identification. Microbiological analyses followed standard methods (APHA, 1988). Bathymetric measurements were obtained with a Skipper 603 model echosounder (Fig. 2). Geographical data (elevation and coordinates) were recorded with a geographical positioning system (GPS 45) unit.

Statistical Analyses

Canonical Correspondence Analysis (CCA), a gradient analysis technique, was used to examine the relationship between predictor variables (e.g., environmental factors) and the response variable (species). Results of CCA were tested with a Monte Carlo randomization method, which randomly reassigns the values for the species data to the values for the environmental variables. Since some predictor variables can be closely related, arch effects or multicolinearity may exist (TER BRAAK, 1986; 1987). To

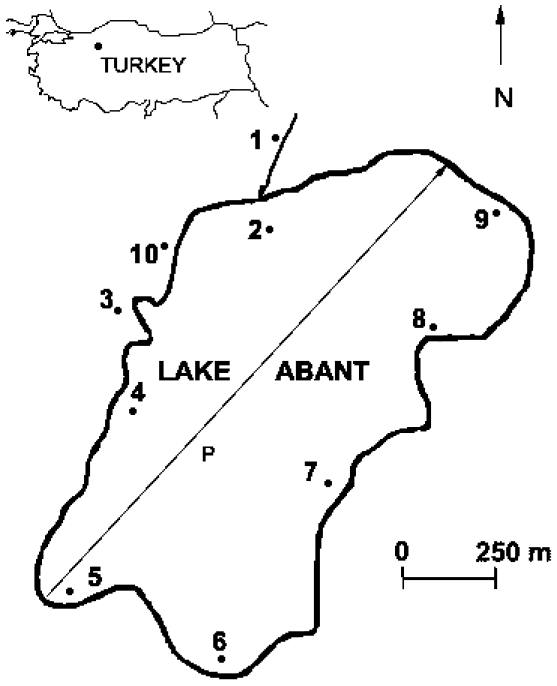

Fig. 1. – The ten sampling stations around Lake Abant. Stations 1, 3, and 10 are located outside the lake. "P" presents the bathymetric line, depicted in Fig. 2, along the longest distance of the lake.

decrease the effects of multicolinearity, the numbers of response variables were kept higher than the numbers of predictor variables and the rare species were down-weighted.

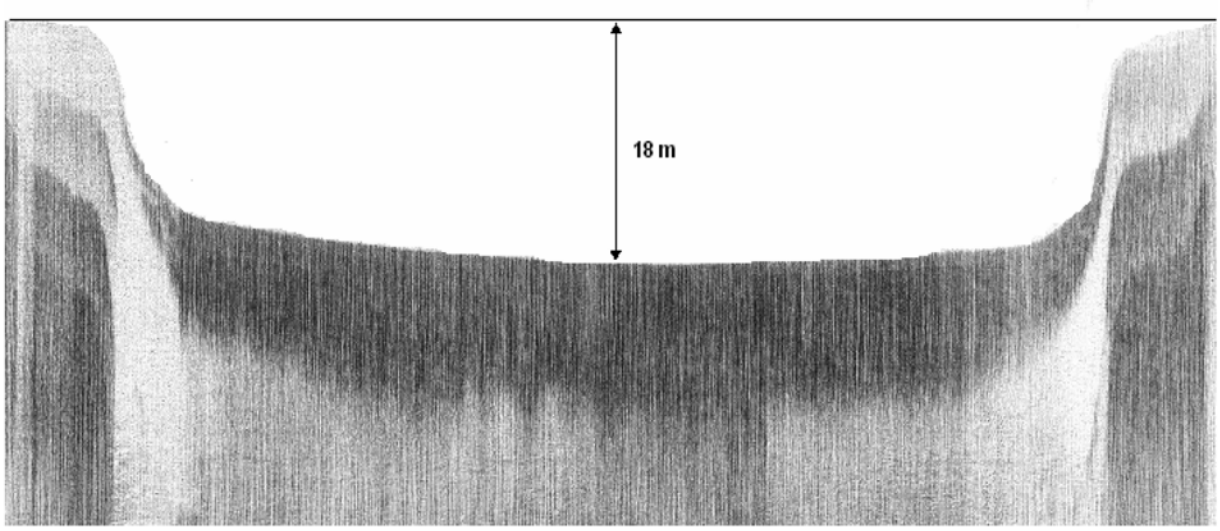

Fig. 2. – Bathymetry of the lake, showing a maximum depth of 18 meters. This is the first bathymetric picture for this lake.

To estimate both tolerance (t_k) and optimum (u_k) values of the six most abundant species, five environmental variables (pH, Eh, DO, Temp., EC, Sal.) were used in a weighted averaging method. Unweighted Pair Group Mean Averages (UPGMA) along with Jaccard's similarity tests were used with log transformed data to display clustering relationships among eight species based on binary data of species' occurrence (presence/absence). Species with only one occurrence were eliminated from the analysis to increase the power of the test. A two-tailed Spearman rank correlation test was used to examine the relationships among six environmental variables, the six most common species, numbers of individuals, and total numbers of species. Hill's N_2 is a kind of diversity index and calculated here as the reciprocal of Simpson index (HILL, 1973). This indicates effective number of occurrences instead of measurement of diversity (JUGGINS, 2001). (All statistical analyses were conducted using the Multivariate Statistical Package (MVSP) version 3.1 (KOVACH, 1998), SPSS version 6.0 and CALIBRATE 1.0 (JUGGINS, 2001).

RESULTS

Reduction in the quality of Lake Abant water was monitored between 2001 and 2003. Human activities, particularly wastewater discharge into the lake, were the main reason for the decline in water quality. As a result, ostracod species composition was negatively affected, although the mean number of species (3.61 species) was slightly above the average number of species (3.2) found in other lakes of Turkey (Table 1). In total, 16 ostracod species were found in Lake Abant (Bolu, Turkey) and its environs: *Cypridopsis vidua* (O.F. Müller, 1776), *Candona neglecta* (Sars, 1887), *Darwinula stevensoni* (Brady & Robertson, 1870), *Cypria ophtalmica* (Jurine, 1820), *Candona candida* (O.F. Müller, 1776), *Physocypria kraepelini* G.W. Müller, 1903, *Ilyocypris bradyi* Sars, 1890, *Heterocypris incongruens* (Ramdohr, 1808), *Notodromas monacha* (O.F. Müller, 1776), *Pseudocandona compressa* (Koch, 1838), *Eucypris pigra* (Fischer, 1851), *Herpetocypris chevreuxi* (Sars, 1896), *Psychrodromus olivaceus* (Brady & Norman, 1889), *Psychrodromus fontinalis* (Wolf, 1920), *Cypris pubera* O.F. Müller, 1776, *Leucocythere* sp. (see Table 2). Of these species, 80% have a cosmopolitan distribution, at least within the Holarctic region, while two species (*P. fontinalis* and *E. pigra*) are new records for the region. Based on the species occurrence data, the UPGMA dendrogram (Fig. 3) revealed four major clustering groups. The first group includes two cosmopolitan species (*C. ophtalmica*, *C. neglecta*), while the second group consists of one cosmopolitan (*C. candida*) and one non-cosmopolitan species (*P. compressa*), and the third group has two cosmopolitan (*I. bradyi, D. stevensoni*) and one rare species (*N. monacha*). The fourth group covers a single cosmopolitan species *C. vidua*. There was a significant positive relationship ($P<0.01$) between the number of individuals and both dissolved oxygen and the number of species (Table 3). The first axis of the CCA diagram (Fig. 4, Table 4) explained 82% of the relationship between 12 species and six environmental variables ($P<0.05$, F-ratio=5.36). Water temperature, dissolved oxygen, and conductivity were the three factors that most influenced species composition.

The ecological requirements of each species were found unique and species displayed differences in their tolerances to different ecological variables (Table 5). Cosmopolitan species tended to have wider tolerance ranges compared to less common species.

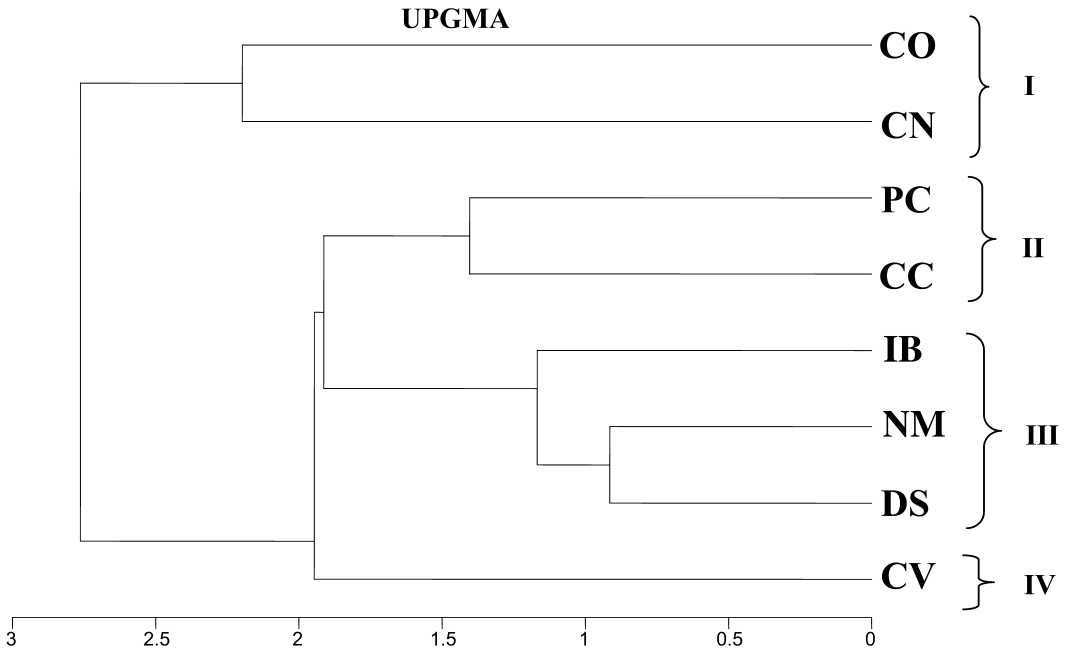

Fig. 3. – UPGMA dendrogram showings four clustering groups (I-IV) for the eight most abundant species, which occurred at least two times in this study.

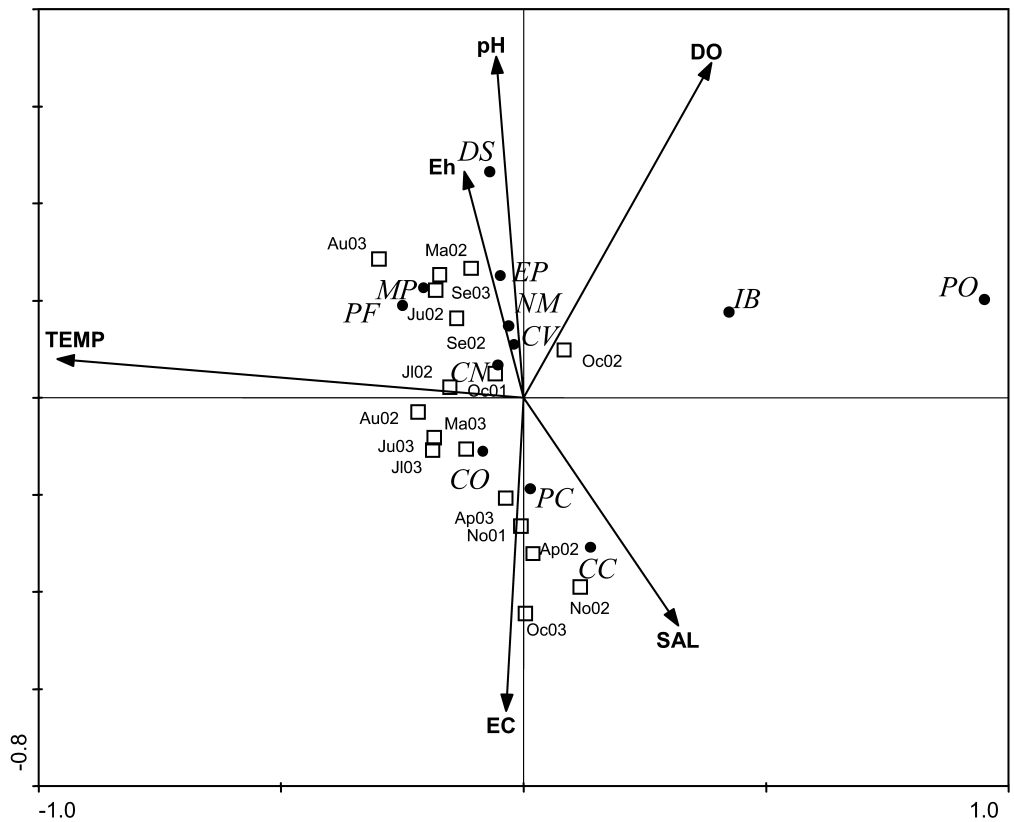

Fig. 4. – CCA diagram, showing the relationships between six environmental variables and 12 species. For abbreviations see Tables 1 and 2.

TABLE 1

The mean values of eight variables measured in Lake Abant between October 2001 (Oc01) and October 2003 (Oc03), completing 20 months of sampling. Abbreviations: pH; Eh (mV) (Standard Hydrogen Electrode or SHE), redox potential; DO (mg/L), dissolved oxygen; Temp (°C), water temperature; EC (µS/cm), electrical conductivity; TDS (mg/L), Total Dissolved Solids; %Sat, oxygen saturation; Sal (ppt), salinity; N_{ind}, Number of individuals; Spp, Numbers of Species. Species codes (Code) are given in Table 2. Codes with bold represent species found from the stations in the lake, others outside of the lake.

Date	pH	Eh	DO	%Sat	Temp	EC	TDS	Sal	N_{ind}	Spp	Code
Oc01	7.86	167.08	8.13	89.96	15.8	260.78	174.72	0.14	68	7	**CV, DS, CN, CO, NM, CC, PC**
N01	7.67	175.57	7.66	69.26	10.4	261.84	175.43	0.13	62	6	**CV, CN, CO, NM, CC, PC**
D01	7.47	187.83	7.58	55.52	1.74	157.38	105.44	0.1			-
Ap02	7.83	166.65	10.50	95.04	12.3	256.07	171.56	0.12	4	1	CV, CN, CO, CC, **PC**
My02	7.97	166.24	9.75	107.40	20.6	267.42	179.17	0.11	2	2	CN, CO, CC, **NM, HC**
Ju02	7.97	165.10	8.96	108.50	23.4	274.84	184.15	0.12	27	5	**CV, CN, CO, NM, LE**
Jl02	7.73	162.90	6.42	89.90	23.6	226.93	152.05	0.11	8	3	**DS, CN, CO**
Ag02	7.96	157.09	8.03	94.52	19.8	218.88	146.65	0.1	5	3	**CN, CO, PC**
S02	7.90	160.68	7.62	81.16	16.0	248.94	166.79	0.11	28	3	**CV, CN, CO**
Oc02	7.91	164.44	10.6	84.80	10.1	253.89	170.11	0.12	83	9	**CV, CN, CO, NM, CC, PC, HI, IB, PK**
N02	8.06	154.99	10.7	86.98	5.1	264.23	177.04	0.12	9	3	CC, **CV, CN, PC**
D02	8.25	145.69	14.5	99.70	0.1	265.30	177.75	0.1			-
Ja03	7.90	175.67	20	141.50	1.68	151.73	101.66	0.13	47	3	**CN, IB, PO**
Ap03	7.95	167.19	7.31	72.42	16.5	268.61	179.97	0.12	11	4	CC, **CV, CN, CO, PC**
My03	7.99	165.53	5.24	56.49	18.9	281.91	188.88	0.11	10	3	**CN, CO, PC**
Ju03	7.97	179.28	4.06	46.81	20.8	286.19	191.75	0.12	3	1	CN, **CO**
Jl03	7.87	171.31	3.62	38.92	19.0	287.92	192.91	0.13	4	1	CN, **CO**
Ag03	na	na	4.78	56.70	23.0	252.13	168.93	0.12	6	2	CV, CO,
S03	7.98	163.45	5.26	53.06	16.2	259.21	173.67	0.13	15	4	CN, **CV, CO, PC, IB**
Oc03	7.78	171.69	4.57	40.29	9.04	274.46	183.89	0.12	15	5	**CV, CN, CO, CC, PC**
Ave	7.90	166.76	8.26	78.44	14.2	250.93	168.13	0.12	22.61	3.61	
Max.	8.30	187.80	20	141	23.6	287.90	192.90	0.1	83	9	
Min.	7.50	145.70	3.6	38.9	0.1	151.70	101.70	0.1	2	1	
StDev.	0.2	9.29	3.9	26.5	7.55	37.12	24.87	0.0	25.2	2.15	

TABLE 2

The 16 species collected from 10 stations around Lake Abant. (*): species with a wide distribution, at least in Holarctic region.

Species		Code	Station No
Cypridopsis vidua	*	CV	1, 2, 3, 4, 6, 8, 9
Darwinula stevensoni	*	DS	2, 6
Candona neglecta	*	CN	1, 2, 3, 6, 8, 9
Candona candida	*	CC	3, 5, 8, 9
Cypria ophtalmica	*	CO	2, 3, 5, 6, 7, 8, 9
Notodromas monacha		NM	4, 8, 9
Pseudocandona compressa	*	PC	1, 3, 4, 5, 6, 8, 9
Eucypris pigra	*	EP	1
Herpetocypris chevreuxi	*	HC	4, 10
Heterocypris incongruens	*	HI	6, 10
Psychrodromus olivaceus	*	PO	8
Psychrodromus fontinalis		PF	1
Physocypria kraepelini	*	PK	8
Ilyocypris bradyi	*	IB	2, 6, 8
Cypris pubera	*	CP	10
Leucocythere sp.		LE	8

TABLE 3

Spearman rank correlation matrix for six environmental variables, number of individual (N_{ind}), number of species (Spp) and six dominant ostracod species. High correlations ($P<0.05$) are indicated in bold face. Codes are the same as in Tables 1 and 2.

	pH	Eh	DO	Temp	EC	Sal	N_{ind}	Spp	CV	CN	CO	NM	CC	PC
pH	1													
Eh	**-0.56**	1												
DO	0.19	-0.35	1											
Temp	0.12	-0.09	-0.50	1										
EC	0.39	0.14	-0.38	0.24	1									
Sal	-0.13	0.38	-0.13	-0.04	0.25	1								
N_{ind}	-0.29	0.08	**0.75**	-0.27	-0.22	0.33	1							
Spp	-0.25	0.04	0.51	-0.36	0.16	0.45	**0.88**	1						
CV	0.13	0.13	0.36	0.31	0.02	-0.04	0.58	0.09	1					
CN	-0.19	0.10	0.36	-0.54	-0.11	-0.07	**0.56**	0.51	**0.73**	1				
CO	-0.46	0.12	0.41	0.16	-0.06	0.22	**0.67**	0.51	0.54	0.11	1			
NM	0	-0.23	-0.65	0.44	-0.74	**0.87**	-0.19	0.70	-0.80	-0.75	-0.51	1		
CC	-0.25	0.13	0.20	-0.13	0.05	0.41	0.11	-0.49	0.81	-0.32	**0.84**	0	1	
PC	**-0.63**	0.36	0.25	-0.34	-0.41	**0.82**	0.53	**-0.86**	0.10	0.17	0.54	0.32	0.12	1

TABLE 4

Main results of Canonical Correspondence Analyses revealing a significant ($P=0.006$, $F=2.197$) result for all canonical axes after 499 permutations in a Monte Carlo test.

Summary					
Axes	1	2	3	4	Total inertia
Eigenvalues:	0.379	0.313	0.082	0.036	4.905
Species-environment correlations:	0.821	0.673	0.415	0.301	
Cumulative percentage variance					
of species data:	7.7	14.1	15.8	16.5	
of species-environment relation:	45.2	82.6	92.3	96.6	
Sum of all eigenvalues					4.905
Sum of all canonical eigenvalues					0.838

TABLE 5

Optimum (Opt), tolerance (Tol) and the effective number of occurrence (Hill's N_2) of the seven most frequently occurring species related to the six environmental variables, selected because of their influences on species occurrence. First six species are ostracods and the last (MP) is an isopod, *Micronecta pusilla* (Horváth, 1895). Analyses included species that occurred more than three times in a total of 71 samplings. (for abbreviations see Tables 1 and 2).

				pH		Eh		DO		Temp		EC		Sal	
Species	**Count**	**Max**	**N_2**	**Opt**	**Tol**	**Opt**	**Tol**	**Opt**	**Tol**	**Opt**	**Tol**	**Opt**	**Tol**	**Opt**	**Tol**
CV	22	16	10.27	7.86	0.32	-48.57	19.18	8.80	4.53	14.64	5.953	239.25	106.30	0.12	0.06
CN	36	101	7.21	7.70	0.28	-38.65	16.33	7.45	4.33	17.97	5.879	320.69	110.69	0.12	0.06
CO	28	7	18.50	7.49	0.39	-28.87	21.16	4.24	3.20	16.43	4.710	336.70	127.68	0.16	0.09
NM	6	5	3.67	7.97	0.44	-53.89	25.09	9.05	3.95	14.65	4.736	221.90	72.96	0.11	0.03
PC	16	19	6.56	7.34	0.52	-20.83	29.86	5.41	3.70	12.24	3.738	329.88	103.44	0.16	0.06
CC	7	29	4.65	7.19	0.54	-13.96	29.22	5.59	5.32	11.23	6.789	403.98	60.46	0.21	0.06
MP	4	2	3.60	7.84	0.18	-46.52	8.61	8.19	3.45	21.15	3.795	230.02	95.92	0.10	0.06

DISCUSSION

Our results showed that each species has its own specific requirements and tolerance levels to different environmental factors. For example, two almost cosmopolitan species (*C. ophtalmica*, *C. neglecta*) found in the first cluster group had similar optima and tolerance levels to six different variables. Moreover, *C. neglecta* had a higher optimum (7.45mg/L) and tolerance (4.33mg/L) to dissolved oxygen than *C. ophtalmica* (4.24mg/L and 3.20mg/L). Similarly, MEZQUITA et al. (2005) reported that *C. ophtalmica* collected from different water bodies in Spain had slightly higher optimum estimates (9.1mg/L) than *C. neglecta* (8.8mg/L), although there was no significant difference in the tolerance values (2.3mg/L and 2.1mg/L, respectively). Although these optimum estimates are higher than our findings (Table 5), the tolerance values are both lower. Such differences may imply a large range of tolerance for both species found in a variety of similar, organically-rich, habitats. Recently, NAGORSKAYA & KEYSER (2005) reported these two species in ten different water bodies of Belarus, where *C. ophtalmica* showed

the highest (0.62) and *C. neglecta* a medium index of commonality (0.13). In a previous investigation, MEISCH (2000) found that both *C. neglecta* and *C. ophtalmica*, both common in organically-rich polluted waters, can tolerate low levels of oxygen values below (3mg/L). *Cypria ophtalmica* was the most abundant species in Lake Caicedo (Spain) where the species was highly tolerant to hypoxic conditions throughout the year (MARTIN-RUBIO et al., 2005). Having a relatively high environmental tolerance index value (ETI=0.77), *C. ophtalmica* showed a wide range of tolerance to water temperatures from 2.1 to 20.1°C in the United States (CURRY, 1999). In a shallow lake (Lake Fehér) of Hungary, KISS (2002) observed *C. ophtalmica* frequently in a reed belt community characterized by a large range of water temperature (1.1-23.2°C), pH (5.76-8.01), and dissolved oxygen (0.0-10.79mg/L). According to RAMDANI et al. (2001), *C. ophtalmica* was the only species found throughout the year in an acidic lake (Megene Chitane) in Tunisia. ROSSETTI et al., (2004) collected *C. ophtalmica* from freshwater wetlands with eutrophic conditions in northern Italy, where it was one of the most common species, surviving a wide range of salinities (from ca. 300 to >900µS/cm), temperatures (up to 35°C), and acidities (usually >7, up to 9.67). In contrast, *C. neglecta* was reported from Lake Edku (Egypt), a freshwater lagoon (BIRKS et al., 2001). A 25-months-long sampling at an organically-enriched small reedbed in Turkey yielded *C. neglecta* in waters with a pH of 7.16-8.65, dissolved oxygen levels of 4.13-15.44mg/L, and a temperature range of 2.13-27.3°C (KÜLKÖYLÜOĞLU, 2005a). The species was most common from November to August in a relatively cold limnocrene spring with low oxygen levels (3.28mg/L) (KÜLKÖYLÜOĞLU, 2003), but its occurrence was negatively correlated to water temperature (KÜLKÖYLÜOĞLU & YILMAZ, 2006). In the present study, *C. neglecta* was revealed near the center of the CCA diagram, which implies the absence of direct effects of environmental variables on its occurrence (Fig. 4). Furthermore, *C. ophtalmica* was located on the negative side of the temperature axis. These findings support the above-cited studies, demonstrating that both species have high tolerances to different environmental variables.

The second group of the UPGMA dendrogram consists of two species (*C. candida*, *P. compressa*) with a broad geographical distribution. In general, *C. candida* can live in a variety of aquatic habitats, such as springs (SÄRKKÄ et al., 1997). According to HARTMANN & HILLER (1977), the species cannot tolerate water temperature above 18ºC during summer. However, more recent studies have revealed the opposite (DELORME, 1991; KÜLKÖYLÜOĞLU & VINYARD, 2000; YILMAZ & KÜLKÖYLÜOĞLU, 2006). The present study reveals that the species has a higher tolerance to water temperature (6.79) and pH (0.54) than the other species (Table 5) found in the littoral zones. Since the species can survive in diverse habitats with extensive geographical distribution, it can be assumed that its tolerance to some of the environmental factors is much higher than previously thought. On the other hand, *P. compressa* seems to have a limited geographical distribution in the Holarctic region (MEISCH, 2000). ROSSETTI et al. (2004) documented the species from freshwater wetlands in northern Italy, along with another common species *C. ophtalmica,* which can be found in similar eutrophic conditions (see above). The authors stated that *P. compressa* occurred less frequently than *C. ophtalmica* in Italy, but its occurrence was most likely related to higher trophic conditions and elevated ionic content. In the present study, *P. compressa* showed the highest tolerance value (29.85) for redox potential (Table 5) with a strong negative correlation to pH ($P<0.05$, $r=-0.63$) and positive correlation to salinity (Table 3). The close relationship between redox potential (due to reduction in water molecules) and changes in ionic contents of waters may suggest that *P. compressa* has much higher tolerance and higher optimum values than previously thought.

The third group of the UPGMA dendrogram consists of two cosmopolitan (*I. bradyi, D. stevensoni*) and one rare species (*N. monacha*). In general, the first two species are very common in different aquatic bodies, but *N. monacha* is found mostly in open waters and seems to have seasonal preferences for specific habitats with certain conditions. Recently, MEZQUITA et al. (2005) reported the tolerance and optimum values for *I. bradyi* (7.81-0.49 for pH, 8.8-2.2mg/L for DO, 14.5-1.8°C for water temperature, and 3.01-0.30mS/cm for electrical conductivity) and *D. stevensoni* (7.74-0.40 for pH, 8.4-2.1mg/L for dissolved oxygen, 16.4-1.2°C for water temperature, and 3.09-0.39mS/cm for electrical conductivity), which are in accordance with earlier records (KÜLKÖYLÜOĞLU, 2000). Despite the fact that both species can have different occurrence patterns throughout the year, they were encountered only three and two times during our study (Tables 2 and 3). Because of their limited occurrence patterns, our results cannot provide unequivocal knowledge about their tolerance and optimum values in Lake Abant at this moment. However, it is well known that both species can tolerate substantial changes in different environmental variables in a variety of habitats, usually preferring cold and well-oxygenated stagnant waters (BRONSHTEIN, 1947; KÜLKÖYLÜOĞLU & VINYARD, 2000; MEISCH, 2000; ROCA et al., 2000; KÜLKÖYLÜOĞLU, 2005c). A rare Holarctic species, *N. monacha*, mostly prefers littoral zones of lakes and ponds rich with aquatic plants. In Germany, it was recorded from May to August from shallow aquatic bodies (HILLER, 1972), while FINN (2005) calculated the optimum and tolerance values for temperature (21.4 and 0.9°C, respectively) of the species during summer. Similarly, KISS (2001) reported the species from a small, slightly acidic (pH 6.66) and shallow (ca. 90cm depth) Lake (Kõhegyi) in Hungary, with dense aquatic macrophytes, where juveniles accounted for 90% of the population in August. KISS (2002) also reported that *N. monacha* was the most common ostracod that occurred from April to October in the reed belt of a small Lake (Fehér, Hungary), with large ranges of water temperature (1.1-23.2°C), pH (5.76-8.01), electrical conductivity (411-2410µS/cm), and relatively low dissolved oxygen (0-10.79mg/L). The species was encountered from October-November and May-June during our study. Overall, it seems that *N. monacha* occurs throughout the year (except the winter months from December-February) in habitats having suitable environmental conditions. Our results agree with those of earlier reports that the species has the highest optimum for dissolved oxygen (9.05) and pH (7.97), while it has a strong positive correlation with

salinity (Tables 3; 5). There is not much known about the ecological preferences of the species, but findings suggest that it prefers relatively cool, well-oxygenated habitats covered with aquatic plants.

The fourth clustering group in the UPGMA dendrogram consists of only one species, *C. vidua*, a well-known cosmopolitan species with a wide range of tolerances to different environmental conditions, also found almost all year around. Indeed, during the present study, it was also found in almost all stations, even outside of the lake (Tables 1; 2). In the eastern Iberian Peninsula, MEZQUITA et al. (2005) collected *C. vidua* from different types of aquatic habitats and reported optimum and tolerance values of 7.89-0.48 for pH, 7.9-2.2 for DO, 15.9-1.5°C for temperature, and 3.10-0.41mS/cm for electrical conductivity, respectively. In comparison, the values in our stations were: 7.86-0.32 for pH, 8.8-4.5 for DO, 14.6-5.9°C for temperature, and 239.24-106.30µS/cm for electrical conductivity. These results support the earlier studies of *C. vidua* that document its ability to tolerate large changes in water conditions.

Among the remaining five taxa (*P. kraepelini, H. incongruens, P. olivaceus, H. chevreuxi, Leucocythere* sp.), the first two species are cosmopolitan, whereas the rest have a more limited geographical distribution in the Holarctic region. While *P. kraepelini* usually prefers large aquatic bodies, such as lakes and ponds, *H. incongruens* with a cosmopolitan distribution can be found in different types of habitats such as ditches, ponds and troughs, where it survives high levels of pollution. However, two other species (*P. olivaceus*, *H. chevreuxi*) are generally common in springs or waters associated with spring discharge. Indeed, recently, in Spain, *H. chevreuxi* was collected in August from a shallow pond fed by groundwater, with relatively high dissolved oxygen concentration (17mg/L) (ROSSETTI et al., 2004). This finding agrees with our study in that there are some water inputs from the springs to the sampling sites (stations 4 and 8) where this species was collected (Fig. 1, Table 2). According to FINN (2005), *H. chevreuxi* had high optimum estimates (20.4) but low tolerance (0.3) to water temperature in different types of aquatic habitats of northeast Germany. In the present study, it is interesting that we found this species in a small, shallow pond (ca. 20cm) nearby the lake (Table 1), where temperature (30.3°C) and pH (9.3) values reached maximum levels with relatively high oxygen saturation (12.39mg/L). To our knowledge, these are the highest levels measured for this species so far. On the other hand, it is probable that the occurrence of this species in such extreme conditions of temperature and pH might be only temporary. Therefore, at the moment, one should not generalize the tolerance of *H. chevreuxi* to such extreme conditions for long time intervals. Just as for *H. chevreuxi*, ecological information about *P. olivaceus* is also scarce. Based on previous works (e.g., GÜLEN, 1985; BALTANÁS, 1992; BALTANÁS et al., 1993), we know that it generally prefers cold and well-oxygenated waters. KÜLKÖYLÜOĞLU & YILMAZ (2006) showed that *P. olivaceus* had relatively high optimum (120.16µS/cm) and tolerance (14.76µS/cm) values for electrical conductivity (referring to salinity). In our study, about 26 individuals were collected in very cold (1.67°C under ice-cover) lake water. This suggests that this species may have much higher tolerance and optimum levels to at least some of the main environmental factors than previously estimated. Nevertheless, this cannot be generalized at the moment due to a lack of data. Additionally, because of their scarce occurrence, detailed information on the ecological preferences of three other species (*P. fontinalis, E. pigra, C. pubera*) found at stations outside of the lake is not available, thus general conclusions cannot be drawn concerning their ecological preferences. The first two species are new for the region and tend to prefer cool and well-oxygenated waters, whereas *C. pubera* can have greater tolerance and optimum estimates in different aquatic habitats.

The negative effects of human activities (e.g., pollution, habitat fragmentation) are well documented throughout the world. The eventual consequences of such activities cause habitat destruction and changes in water quality. Thus, even slight changes in water quality can influence the suitability of a habitat for (e.g., ostracod) species communities. Such changes in lake water quality, eventually, will reduce ecological conditions, decreasing the survival chances of specialist ostracods, while increasing the persistence of cosmopolitan species with a wide tolerance range. This is because such fluctuations in habitat conditions can provide better opportunities for generalists and thus increase their dominance over specialist species. Therefore, relatively higher mean numbers of ostracod species in Lake Abant were probably due to an increase in the number of cosmopolitan species. This phenomenon is called pseudorichness; it also occurs in other taxonomic groups such as phytoplankton and colonial bacteria. Pseudorichness hypothesis suggests an overall decrease in the quality of certain habitats (KÜLKÖYLÜOĞLU, 2004). Presumably, the concept of pseudorichness can be generalized to different ecological communities where it can help us to understand past, current, and future water and habitat quality. Results show that the current water quality of Lake Abant has changed from oligotrophic to mesoeutrophic, during which species turnover favors generalist species over specialists. As a result, if anthropogenic activities continue in the same way, such problems will threaten overall species diversity, its functional significance, and habitat quality.

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Expression of Collapsin Response Mediator Protein-4 (CRMP-4) in Plastic Brain Areas of Adult Songbird Brain

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ABSTRACT. Collapsin response mediator protein-4 (CRMP-4), a member of CRMP family, is involved in neuronal differentiation and regulation of synaptic rearrangement. Neurite growth and axonal reorganization still continue in the adult songbird brain. It is probable for CRMP-4 to play an important role during these processes. To address this issue, we first obtained CRMP-4 cDNA from the brain of songbird (*Lonchura striata)* by use of RT-PCR and 3' rapid amplification of cDNA ends (3'RACE). We then examined the expression of CRMP-4 mRNA and protein in some plastic brain regions, including the hippocampus, song control nuclei and the cerebellum. Double immunohistochemistry was employed to identify the phenotypes of CRMP-4 expressing cells. The results indicated that: 1) a cloned fragment of CRMP-4 cDNA (798bp) showed high similarity with those comparable cDNA of reported species, confirming the evolutionary conservation of CRMP-4 sequence; 2) both CRMP-4 mRNA and protein were found to be dominantly located in the hippocampus, song control nuclei and the cerebellum among the whole brain; 3) some CRMP-4 positive cells were also labeled by the antibody for HuD (immature neurons), but not for NeuN (mature neurons), and GFAP (astrocytes). The presence of CRMP-4 in the plastic regions suggests that CRMP-4 might be concerned with the neural plasticity of songbird brain.

KEY WORDS : CRMP-4; RT-PCR; *in situ* hybridization; immunohistochemistry; songbird brain

INTRODUCTION

The collapsin response mediator protein-4 (CRMP-4) is a member of the TOAD (turned on after division, 64kDa)/Ulip (Unc-33-like phosphoprotein)/CRMP/TUC/DRP (dihydropyrimidinase-related protein) family of homologous cytosolic phosphoproteins. Unc-33 in *C. elegans* shares sequence homology with CRMP-4 in humans (DRP-3) (HAMAJIMA et al., 1996), rats (TUC-4b) (MINTURN et al., 1995; QUINN et al., 2003), mice (mUlip1) (BYK et al., 1995), chickens (CRMP-4A and CRMP-4B) (YUASA-KAWADA et al., 2003) and *Xenopus* (nsp-1) (BYK et al., 1998), indicating high conservation throughout the evolutionary process. Unc-33 is regarded to play a role in the control of neurite elongation and axonal guidance, according to a finding that neurons have abnormal neuritic outgrowth in the Unc-33 *C. elegans* mutants (LI et al., 1992; MCLNTIRE et al., 1994). Recently, it has been reported that phosphorylation of CRMP-4 can destabilize actin bundles, contributing to the collapse of the actin cytoskeleton (ROSSLENBROICH et al., 2005). CRMP-4 has been studied by immunohistochemistry in the brains of adult rats (NACHER et al., 2000) or lizards (NACHER et al., 2002). The data show that CRMP-4 occurs in newly generated neurons during adulthood, which include those in the dentate gyrus and the subventricular zone migrating through the rostral migratory stream to the olfactory bulb of the rat (MINTURN et al., 1995; NACHER et al., 2000), and those in the medial cortex of the lizard (NACHER et al., 2002). In addition, CRMP-4 has been reported to appear in the cerebral cortex, the piriform cortex and the hypothalamus of the rat (NACHER et al., 2000), or the dorsal and lateral cortices of the lizard (NACHER et al., 2002). It also appears in the other brain regions where no neurogenesis or neuronal migration is detected, but neurite outgrowth, synaptic reorganization or lesion-induced structural plasticity occurs (NACHER et al., 2000; 2002). More recently, CNOPS et al. (2006) revealed spatio-temporal expression profiles of CRMP-4 during visual cortex maturation in the cat, suggesting a potential role of CRMP-4 in the formation of functional network. These data all indicated that CRMPs are involved in neuronal differentiation, neurite growth and regulation of axonal pathfinding in synapse formation (BYK et al., 1998; QUINN et al., 1999, 2003; WANG & STRITTMATTER, 1996).

In songbirds, neuron generation still continues in some adult brain regions such as the hippocampus (ABSIL et al., 2003; BARNEA & NOTTEBOHM, 1994; HOSHOOLEY et al., 2005), some song control nuclei (ABSIL et al., 2003) and the cerebellum (RAO & SHRIVASTAW, 1976). Many studies have further shown that these areas experience significant neural plasticity (hippocampus: CLAYTON & KREBS, 1994; SADANANDA & BISCHOF, 2004; song control nuclei: FOSTER & BOTTER, 1998; BRAINARD & DOUPE, 2000; MOONEY, 2000; the cerebellum: CONSOLE-BRAM et al., 1996; CORVETTI & ROSSI, 2005). For example, the neural plasticity-related genes ZENK (JARVIS et al., 2000; RIBEIRO & MELLO, 2000; BAILEY & WADE, 2005), c-fos (BAILEY & WADE, 2005), c-jun (NASTIUK et al., 1994; RIBEIRO & MELLO, 2000) and BDNF (LI et al., 2000; RIBEIRO & MELLO, 2000) are induced in the hippocampus

and some song control nuclei in the adult songbird brain, following the stimulation of birdsongs.

To our knowledge, no report has been concerned with the distribution of CRMP-4 in the brain of bird. Given the established role for CRMP-4 in neurite outgrowth or synaptic rearrangement, we wanted to know the potential roles of CRMP-4 in the adult songbird brain. To approach this issue, we first obtained CRMP-4 cDNA from the brain of songbird (*Lonchura striata)* by using RT-PCR and 3' rapid amplification of cDNA ends (3'RACE). We then examined the expression of CRMP-4 mRNA and protein in the several plastic brain areas i.e., the hippocampus, song control nuclei and the cerebellum. We also used several immunohistochemistry markers to identify the phenotypes of CRMP-4 positive cells.

MATERIALS AND METHODS

Animals and tissue preparation

The adult male Bengalese finches (*Lonchura striata*) at 12–24 months of age were bred and raised in the breeding colony at Beijing Normal University (Beijing, China). The birds were kept in standard cages (50×62×38cm) under a 14/10hr light/dark cycle at 19-24°C. Seed and water were available ad libitum, supplemented with a mixture of cooked eggs and baby cereal every 2-3days. All experiment procedures were carried out in accordance with the guidelines of Beijing animal protection committee. The birds were deeply anaesthetized by 20% ethyl carbamate (50μL/g body weight). After perfusion with 0.9% saline and then with 4% cold paraformaldehyde in 0.1M phosphate-buffer (pH 7.4), the brains were collected and postfixed 6hr in the same fixative at 4°C. Then the brains were immersed in 30% sucrose at 4°C overnight and cut into 10μm thick sagittal sections on a freezing microtome (CM 1850, Leica).

RNA extraction, reverse transcription–polymerase chain reaction (RT-PCR) and 3' rapid amplification of the cDNA ends (3' RACE)

Since the whole sequence of CRMP-4 cDNA is not yet available in the songbird, we first wanted to get by using RT-PCR and 3'RACE. Total RNA was extracted using the Trizol reagent (GIBCO). 1μg total RNA, 1μL of 50mM poly dT12–18, 4μL of 5 X first strand buffer, 2μL of 0.1M dithiothreitol, 1μL of 50mM dNTPs and 1μL of Moloney-murine leukemia virus reverse transcriptase (GIBCO) were incubated at 37°C for 1hr by using the little genius thermocycler (Japan). PCR was carried out in 25μL reaction mixtures containing 2μL of the first strand cDNA, 1μL 10mM of sense and antisense primers, 1μL 50mM dNTPs and 2U of Taq DNA polymerase (SNBC). Thermocycling was performed using the following protocol: (1) 95°C for 5min, (2) designated cycles of 95°C denaturation for 45sec, 55°C annealing for 45sec and 72°C extension for 45sec, and then (3) 1 cycle at 72°C final extension for 10min before cooling to 4°C. We then employed the 3'-RACE-PCR method using the FirstChoice RACE Kit (Ambion). The RACE procedure was carried out according to the protocol. Primer sets were designed according to the chicken CRMP-4B mRNA sequence (GenBank No: AF301553, the whole length was 2202bp). For 3'-RACE, the outer primer was 5'-gag tac aac atc ttt gaa ggg atg g-3' at positions 1319-1344, and the inter primer was 5'-ggc atg tat gat gga cct gtg ttt ga-3' at positions 1520-1546. The product of 3'-RACE was 798 bp. 3'RACE product shared high sequence similarity (97%) over its entire length with an even longer zebra finch (*Taeniopygia guttata*) clone (GenBank No: CK308729).

In situ hybridization

Digoxigenin-labeled riboprobes were produced by in vitro transcription of specific DNA fragments obtained by PCR amplification of plasmid vectors containing the cDNA for CRMP-4 in a pGEM-T Easy vector (Promega). For PCR amplification, primer pairs were directed against the vector sequences flanking both oppositely located RNA polymerase promoter sites to amplify the insert with both promoters for transcription of both strands using the same DNA preparation (pGEM-s, 5'-TATA GAA TAC TCA AGC TA AAG CAA AGG AAG AAA AAT GG-3'; and pGEM-a, 5'- GGG TGG AGA TGA AGG AGA TG-3'). The labeled probes were approximately 434-base fragments corresponding to the chicken CRMP-4 mRNA (GenBank No: AF301553, nucleotide position 1781–2214, 3' untranslated sequence) and Bengalese finch CRMP-4 cDNA sequence (submitted to NCBI and GenBank No. DQ402134, nucleotide position 262-786, 3' untranslated sequence). Blast NCBI analysis confirmed that none of the cDNA sequence used in the present study showed significant homologues with other CRMP family members or other molecules. Ten-μm-thick sagittal sections were mounted on slides. Digoxigenin-labeled probes were generated by labeling with digoxigenin-11-UTP (Roche Molecular Biochemicals) using SP6 or T7 RNA polymerase (Promega) in a 10μL transcription mixture containing 1μg linearized DNA; 1μL 10 X transcription buffer; 0.5μL 0.2mM dithiothreitol; 1μL of 10mM stocks of ATP, GTP, CTP and digoxigenin-11-UTP; 10U ribonuclease inhibitor; and 10U appropriate RNA polymerase. The transcription mixtures were incubated for 2hr at 37°C. The temperature for hybridization was adjusted according to the GC content of each probe and was selected for high stringency: 52°C for CRMP-4 probe.

Endogenous AP activity was quenched with 0.2M HCl followed by PBS rinses. Proteinase K (GIBCO) digestion was carried out at 37°C, followed by postfixation in 4% freshly depolymerized paraformaldehyde. After PBS rinses the sections were acetylated with 0.25% (v/v) acetic anhydride (Sigma) in a 0.1M triethanolamine (Sigma), pH 8.0 and 0.9% NaCl and then rinsed. After a wash in sterile dH_2O, the sections were incubated with prehybridization solution (50% formamide, 2 X SSC, 0.05g/mL dextran sulfate, 1 X Denhardt's, and 0.1mg/mL salmon sperm DNA). Digoxigenin labeled RNA probe was diluted in prehybridization buffer (1μg/mL). Sections were incubated overnight. After washed with 2 X SSC and 0.05% Tween-20, 0.1M Tris-HCl, and 0.15M NaCl (TTN), the sections were preincubated in 0.5% (w|v) BSA and incubated with a sheep polyclonal anti-digoxigenin antibody $F(ab)_2$ fragment conjugated with alkaline

phosphatase (Roche Molecular Biochemicals, 1: 1000). The sections were washed in TTN and next in Tris, pH 9.4, NaCl: $MgCl_2$ (20: 1), and colour-developed in the presence of nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) (Promega) for 48hr. Negative controls were prepared using the corresponding sense probes.

Protein extraction and Immunoblot analysis

The whole brain of an adult male bird was lysed in 50mM Tris (pH 7.4), 150mM NaCl, 1% Triton X-100 and 0.1mM phenyl methane sulfonyl fluoride. The samples were separated by 12% SDS-PAGE and electro-transferred onto a polyvinylidene-difluoride (PVDF) membrane (Millipore). Membranes were incubated overnight at 4°C in TBS–Tween (10mM Tris–HCl, pH 7.5, 150mM NaCl, 0.1% Tween 20) containing 5% dry milk. Blots were then incubated with TUC4 performed with peroxidase method with washes after each step.

Immunohistochemistry

The sections were processed for immunohistochemistry as follows: Sections were first incubated in 3% normal goat serum and then incubated with TUC4 (Chemicon, 1: 1000) against the synthetic peptide sequence YDG-PVFDLTTTPK corresponding to amino acids 499–511 from rat CRMP-4 and differing only by one amino acid for the chicken CRMP-4B sequence. This antibody has been shown to recognize exclusively CRMP-4 protein and does not cross-react with other CRMPs. According to the product protocol and previous report (NACHER et al., 2002), the primary anti-sera can cross-react with CRMP-4 into a relatively broad variety of amniotes including *Xenopus*.

The secondary antibody was a biotinylated goat anti-rabbit IgG (Jackson, 1: 400) followed by an avidin-biotin-peroxidase complex (ABC; Vector Laboratories, 1: 150). Colour development was achieved by incubating with 3, 3'-diaminobenzidine 4-HCl (DAB, Sigma). Each step was followed by PBS washing. The specificity of the immunoreactions was checked with reference to the guidelines suggested by SAPER & SAWCHENKI (2003); namely as negative controls, by omitting the primary antisera. For these negative controls, all other immunohistochemical procedures were the same as that described above. In addition, we compared our labeling results with previous demonstrations of which brain areas exhibit or lack CRMP-4 labeling (NACHER et al., 2000; 2002). The findings of all of these control procedures indicated that the labeling reported here was specific.

For double labeling, the sections were treated with 3% normal serum from the same animals as those used in the production of secondary antibodies and reacted with another antibody. The following antibodies were used as the primary antibodies: mouse IgG polyclonal anti-HuD (Molecular Probes, 12.5μg/mL), mouse IgG monoclonal anti-NeuN (Chemicon, 1: 500) and mouse IgG monoclonal anti-GFAP (Chemicon, 1: 200). According to the protocols provided by product companies and our previous work, all these antibodies could be used in songbirds. As the secondary antibodies, the following antibodies were used: FITC-conjugated goat anti-rabbit IgG (Jackson, 1: 200), Rhodamine-conjugated goat anti-mouse IgG (Jackson, 1: 200), FITC-conjugated goat anti-mouse IgG (Jackson, 1: 200) and Cy3-conjugated donkey anti-rabbit IgG (Jackson, 1: 200).

Photography and construction of double coloured fluorescent images

Images were obtained with CoolSNAP colour digital camera (Photometrics) attached to an Olympus microscope (BH-2). Fluorescence signals were detected with Spot digital camera (Diagnostic Instruments) attached to an Olympus fluorescent microscope (1X70) at excitation/emission wavelength of 550/570nm (Rhodamine or Cy3, red) and 492/520nm (FITC, green). The images were corrected for brightness and contrasted by Adobe Photoshop 8.0 (Adobe Systems).

RESULTS

Western blot analysis for CRMP-4 in adult avian brain

Only a band (64kDa) corresponding to TUC4 was shown among total proteins by Western blots (Fig. 1).

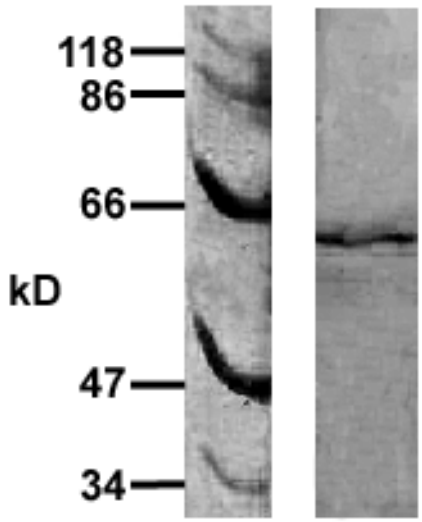

Fig. 1. – Antibody for collapsin response mediator protein-4 (CRMP-4) detects a band of approximately 64kDa on Western blot of adult Bengalese finch brain homogenates.

CRMP-4 labeling *in situ* hybridization and immunohistochemistry

We determined the localization of CRMP-4 through *in situ* hybridization and immunohistochemical staining in the brain of adult male Bengalese finch. For song control nuclei, we examined two nuclei in the motor pathway, i.e., HVC and robust nucleus of arcopallium (RA), several nuclei in the anterior forebrain pathway, i.e., lateral magnocellular nucleus of anterior nidopallium (LMAN), Area X in the basal ganglia, and the nucleus dorsolateralis

anterior, pars medialis (DLM) and an auditory area in the caudal medial nidopallium (NCM) (NOTTEBOHM et al., 1982; VATES et al., 1996). No detectable labeling was observed above background levels by hybridization with the complementary sense probe or in immunohistochemistry controls.

Distributional patterns of CRMP-4 mRNA and protein were similar in the examined plastic regions, as well as in the other brain areas. They were dominantly located in the examined plastic regions among the whole brain. However, this was more obviously reflected from CRMP-4 protein, than that from CRMP-4 mRNA (Figs 2-5). Although positive labeling for CRMP protein was dominantly present in most of studied plastic areas, it was also evident in their surrounding regions for several studied plastic areas such as RA (Fig. 3D) and DLM (Fig. 4B).

Hippocampus

The avian hippocampal complex consists of a medially situated hippocampus (Hp) and parahippocampal area (APH) (COLOMBO & BROADBENT, 2000). A few labeled cells for CRMP-4 mRNA and protein were observed in both subdivisions (Fig. 2). The density of labeled cells in immunohistochemistry was not as high as that in *in situ* hybridization. Although positively labeled cells also appeared in areas underlying the Hp, they were not as robust as in the Hp, especially for those cells positive for CRMP-4 protein (Fig. 2).

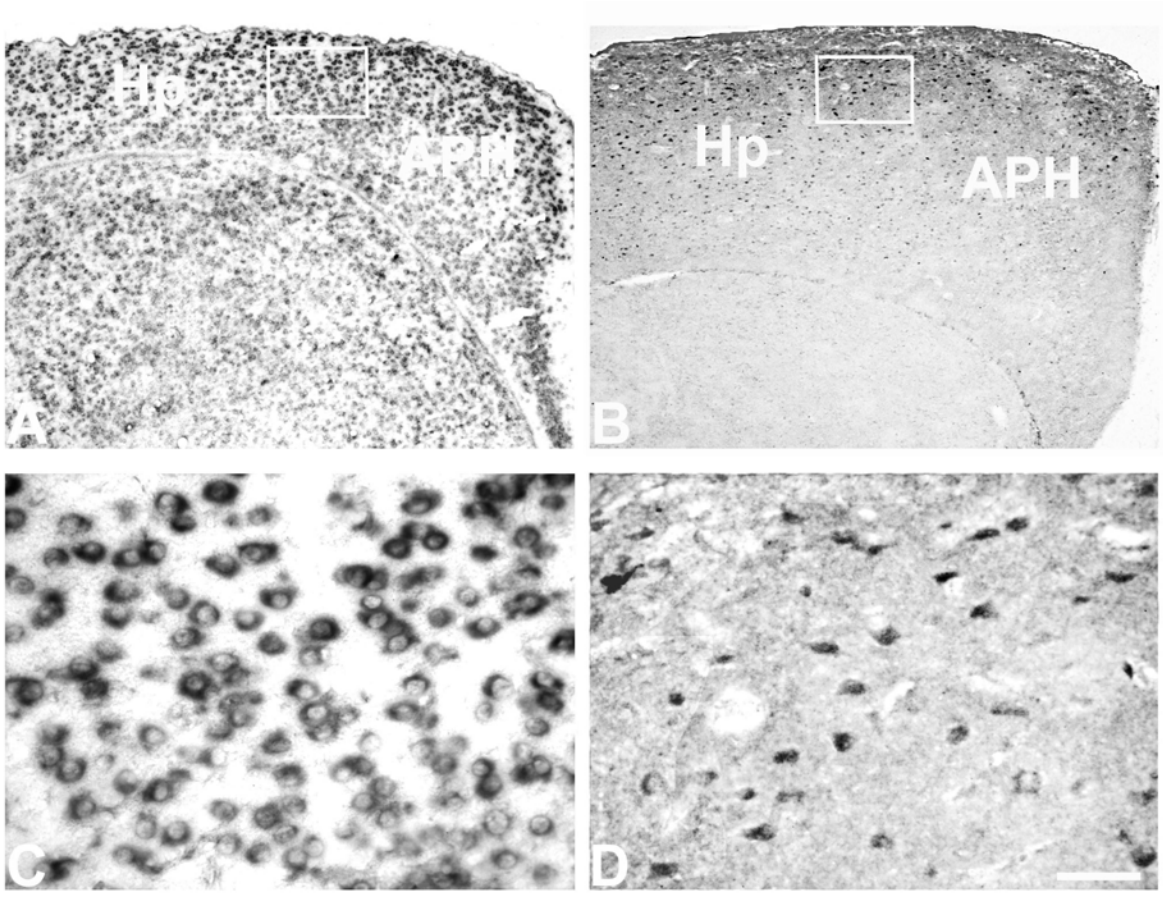

Fig. 2. – The location of CRMP-4 mRNA (A and C) and protein (B and D) in sagittal sections of the hippocampus in the adult Bengalese finch. The boxes in A and B are further illustrated in C and D, respectively. Scale bar: A and B, 150μm; C and D, 30μm.

Song Control Nuclei

CRMP-4 mRNA and protein both appeared in HVC (Figs 3A; 3B), RA (Figs 3C; 3D), LMAN (Figs 3E; 3F), Area X (data not shown), DLM (Figs 4C; 4D) and NCM (Figs 4E; 4F). Some positively labeled cells of HVC and NCM showed two or three basal, thin processes, with two spiny processes emerging from the two poles of the cell bodies (Fig. 4). There were almost no expanding branches around the somata in RA, LMAN, and DLM (Figs 3; 4).

Cerebellum

The cerebellum cortex is a trilayered region, and consists of the molecular layer, the Purkinje layer and the internal granule layer (PALAY & CHAN-PALAY, 1974). The labeling for CRMP-4 mRNA and protein was observed in almost all of the Purkinje cells (Figs 5A; 5B). Labeling of CRMP-4 protein appeared in the somata and dendrites of Purkinje cells. CRMP-4 mRNA labeling could also be seen in the other areas of cerebellar cortex including the

inner granule layer, and to a less extent in the molecular layer, while CRMP-4 protein was both expressed in the inner granule layer and in the molecular layer.

Other Brain Areas

Although positive cells for CRMP-4 mRNA and protein dominantly appeared in the above areas, they also occurred in other brain areas. These areas included some auditory nuclei such as the magnocellularis (NM) (Figs 5C; 5D) in the pons, and the nucleus ovoidalis (OV) in the diencephalon (Figs 5E; 5F), a visual relay nucleus center in the diencephalon (the nucleus rotundus), and some areas in the hypothalamus or the brain stem areas (data not shown).

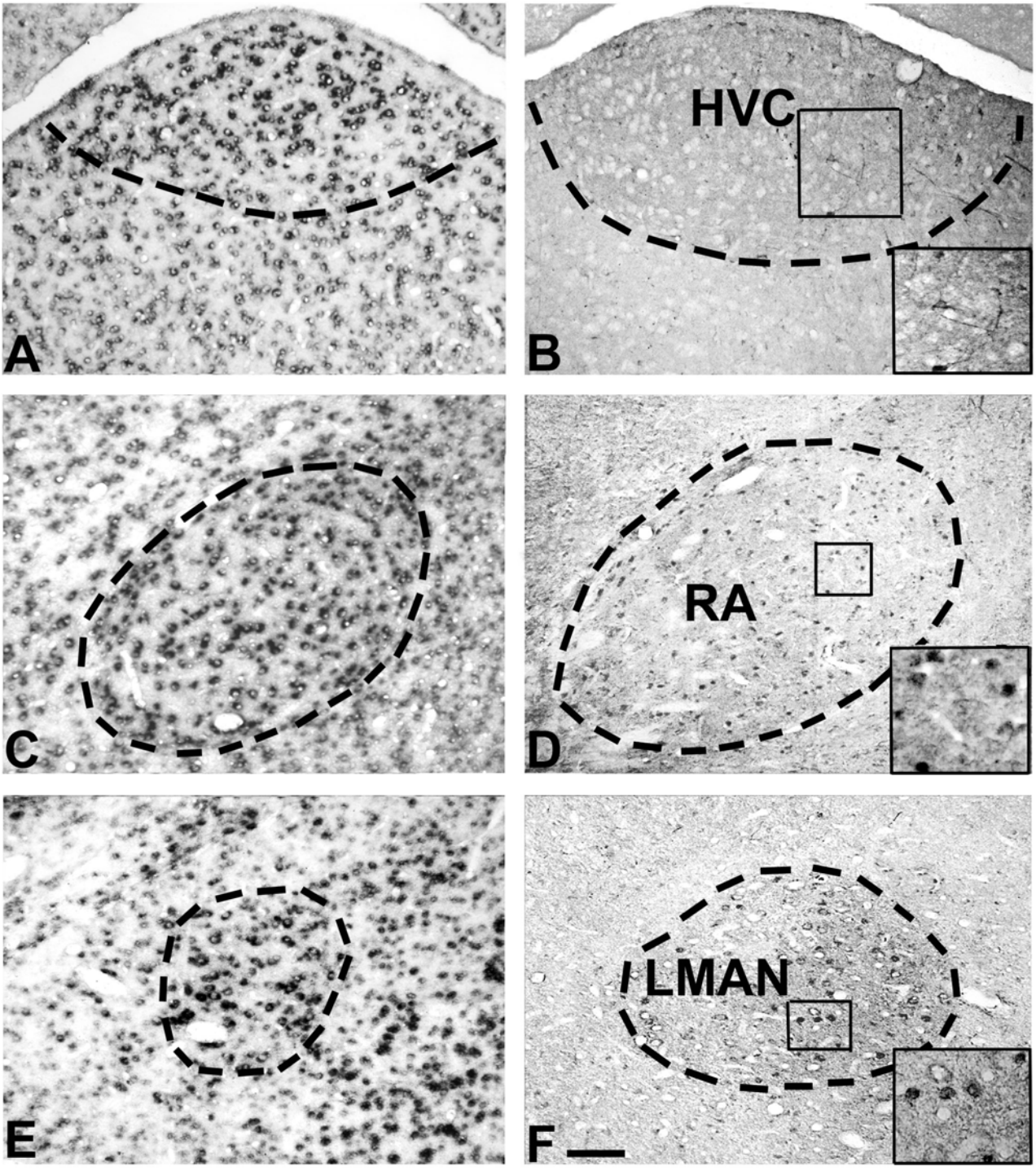

Fig. 3. – The location of CRMP-4 mRNA (A: HVC, C: RA, E: LMAN) and protein (B: HVC, D: RA, F: LMAN) in sagittal sections of song control nuclei of the adult songbird brain. Insets: showing CRMP-4-immunoreactive cells. Scale bar: 75μm; 30μm for insets.

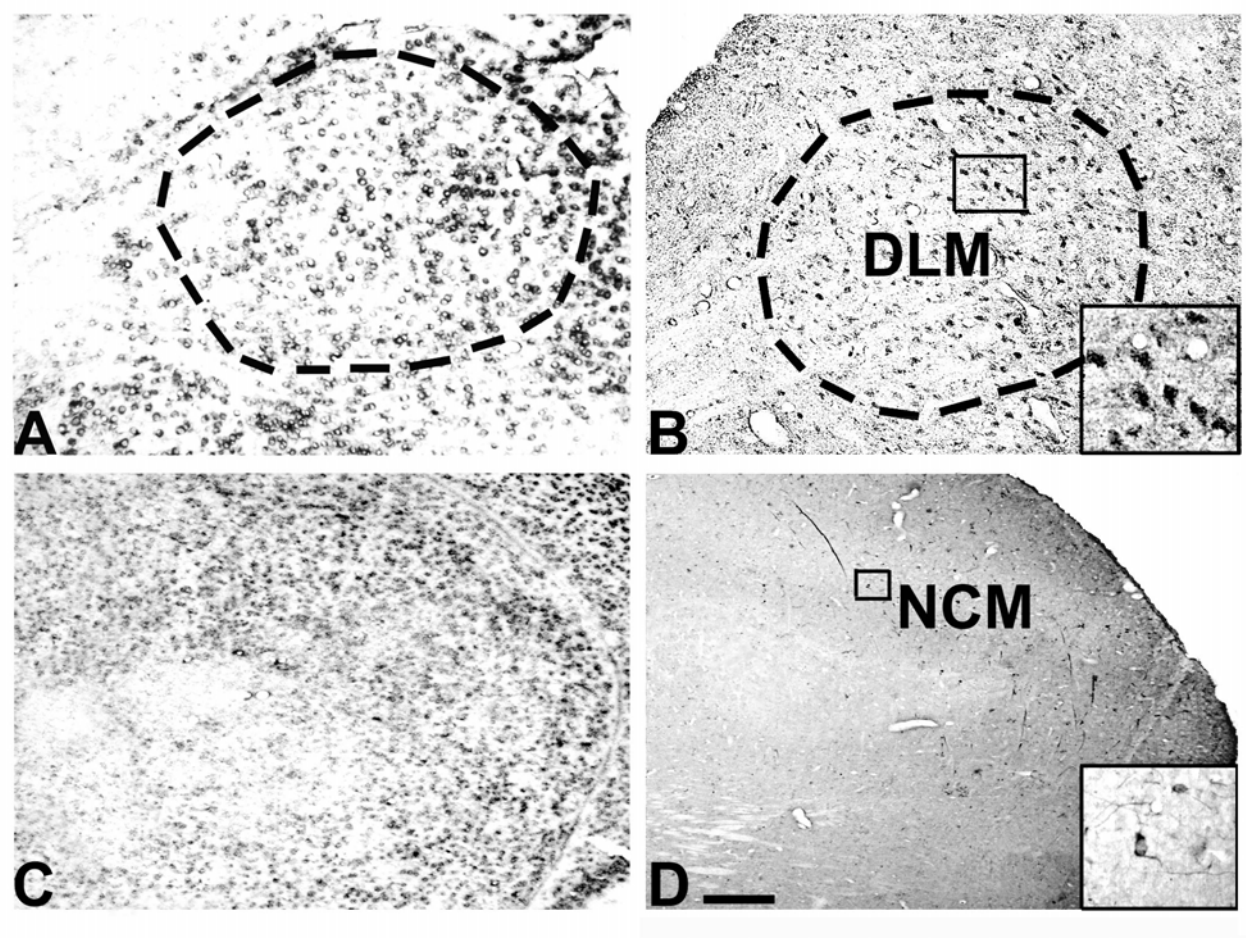

Fig. 4. – The location of CRMP-4 mRNA (A: DLM, C: NCM) and protein (B: DLM, D: NCM) in sagittal sections of song control nuclei of the adult songbird brain. Insets: showing CRMP-4 immunoreactive cells. The hippocampus in D was lost. Scale bar: A and B, 75μm; C and D, 150μm; 30μm for insets.

Double immunohistochemistry

No CRMP-4 immunolabeled cell bodies were found co-expressing with NeuN (Fig. 6A) and GFAP (Fig. 6B) immunoreactivity. A subset of CRMP-4-positive cells was also labeled for neuron marker, HuD. Cells expressing both CRMP-4 and HuD were mainly located in the hippocampus, HVC and NCM (Fig. 7).

DISCUSSION

The present study obtained a fragment of CRMP-4 cDNA with adequate length from the brain of Bengalese finch by employment of RT-PCR and 3'RACE. Both *in situ* hybridization and immunohistochemistry showed that CRMP-4 was located in the hippocampus, song control nuclei and the cerebellum. The double labeling study indicated that some CRMP-4 cells also expressed HuD, but not NeuN and GFAP. In what follows, we will compare the present results with those reported previously, and discuss the physiological implication of CRMP-4 distribution in the brain of songbird.

The sequence of CRMP-4 and its phylogenetic conservation

Although we did not get a complete CRMP-4 cDNA, an adequate length of CRMP-4 cDNA was obtained in the present study. The fragment of CRMP-4 cDNA showed high homology with CRMP-4B cDNA of chicken and other amniotes: human, rat and *Xenopus* (Byk et al., 1995; 1998; Hamajima et al., 1996; Li et al., 1992; Minturn et al., 1995; Nacher et al., 2000; Quinn et al., 2003; Yuasa-Kawada et al., 2003). Our study therefore confirmed again the phylogenetic conservation of CRMPs family (Hamajima et al., 1996).

Location of CRMP-4 in the brain of songbird and its possible physiological function

To our knowledge, the present study first mapped the distribution of CRMP-4 in the adult brain of amniote simultaneously by two approaches i.e., *in situ* hybridization and immunohistochemistry. Since the cDNA sequence corresponding to the CRMP-4 mRNA probe showed no similarities with other CRMPs members or other molecules, we were sure that our results for *in situ* hybridization were specific.

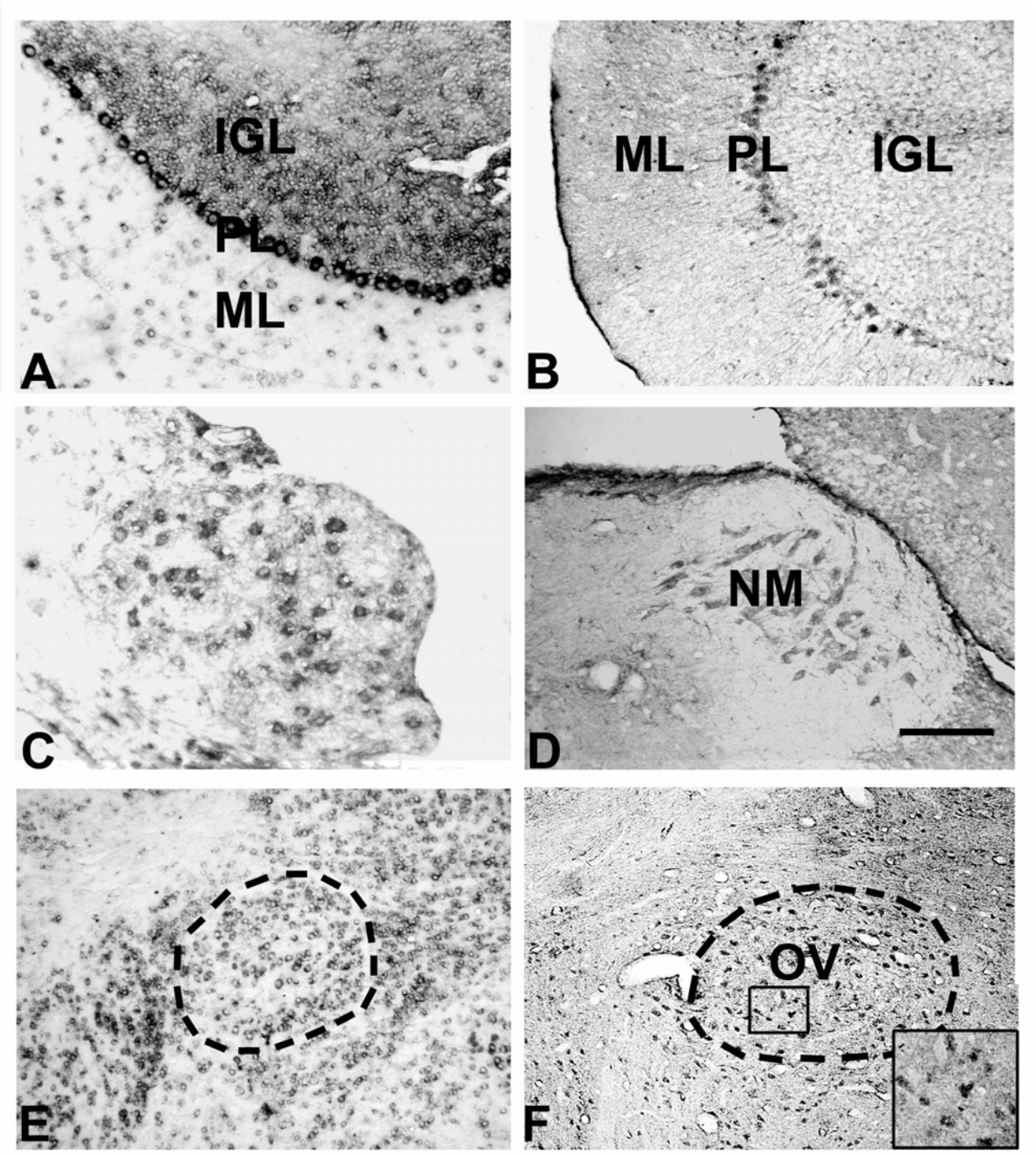

Fig. 5. – A and B: Cells expressing CRMP-4 mRNA (A) or protein (B) in the cerebellum. CRMP-4 mRNA appears in the Purkinje cell layer (PL), the inner granule layer (IGL), and to a less extent in the molecular layer (ML). In contrast, CRMP-4 protein mainly occurs in the PL. C- F: Cells expressing CRMP-4 mRNA (C and E) or protein (D and F) in an auditory nucleus in the brainstem (C and D), and the auditory nucleus in the diencephalon (E and F). Inset in F: showing immunoreactive cells for CRMP-4 protein. Scale bar: 50μm, A-D; 100μm, E and F; 50μm for inset in F.

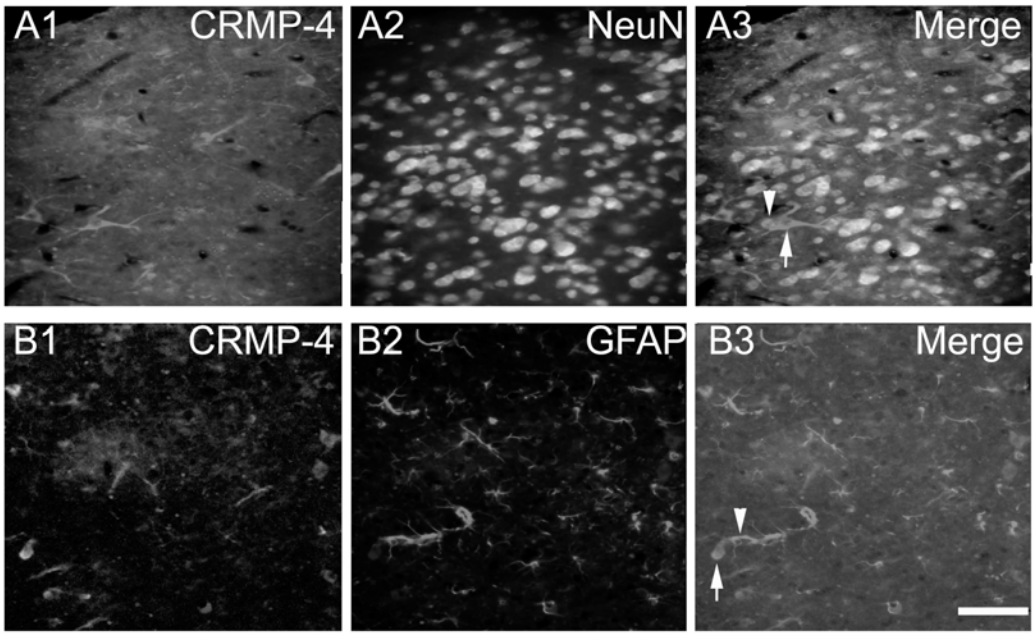

Fig. 6. – Immunofluorescence photomicrographs demonstrating double labeling by anti-CRMP-4 and other antibodies (NeuN and GFAP) in sagittal sections of the caudal medial nidopallium (NCM) in the adult Bengalese finch. Left panels showing cells positive for CRMP-4. Middle panels showing cells positive for another antibody. Right panels showing merged images. A: CRMP-4-positive cell (arrow) devoid of NeuN and NeuN-immunolabeled neuron (arrowhead) negative for CRMP-4. B: labeling cell for CRMP-4 (arrow) not positive for GFAP immunostained astrocyte (arrowhead). For panels in A and B, dorsal is up and caudal is left. Scale bar, 50μm.

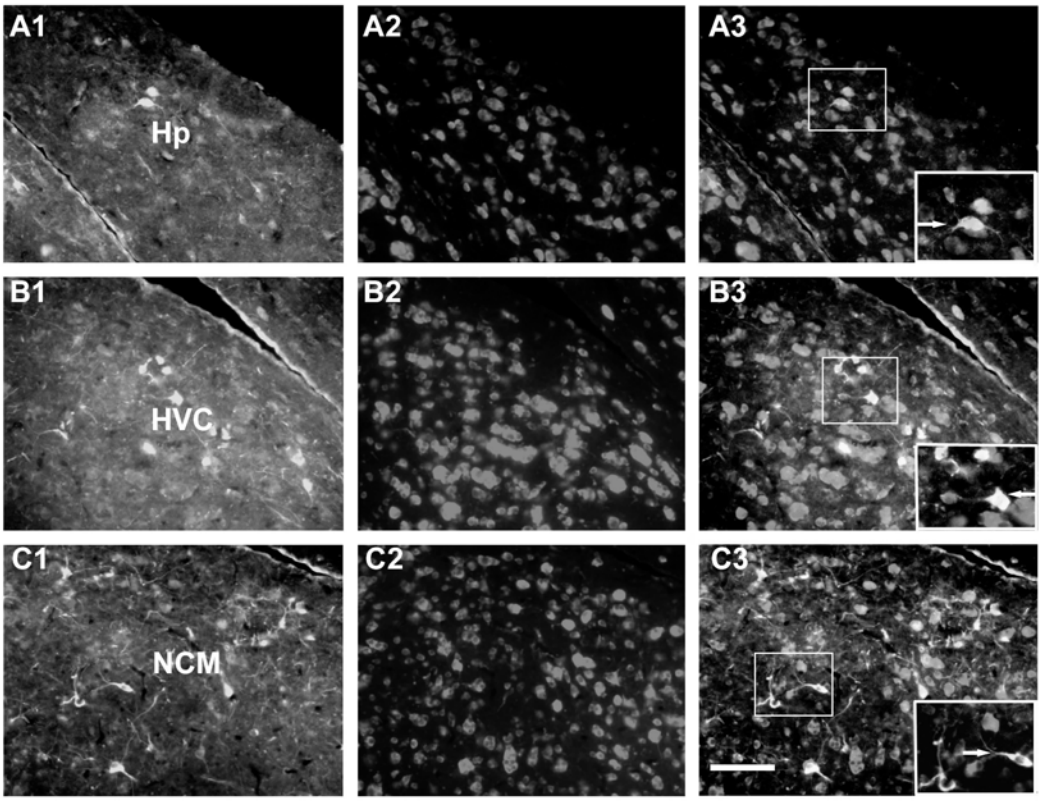

Fig. 7. – Immunofluorescence photomicrographs showing double labeled cells by anti-CRMP-4 and HuD antibodies. Left panels showing cells positive for CRMP-4. Middle panels showing cells positive for HuD. Right panels showing merged images. A: labeling in hippocampus. B: labeling in HVC. C: labeling in the caudal medial nidopallium. Insets: double labeled cells (arrows). For panels in C, dorsal is up and caudal is right. Scale bar, 50μm; 32μm for insets.

We also believed that our immunohistochemistry was specific for CRMP-4. First, the same antibody has been confirmed to be specific in the rat (MINTURN et al., 1995; NACHER et al., 2000), lizard (NACHER et al., 2002) or cat (CNOPS et al., 2004). Second, a single band corresponded to CRMP-4 was identified in our immunoblot analysis as in the rat (MINTURN et al., 1995; NACHER et al., 2000), lizard (NACHER et al., 2002) and cat brain tissue (CNOPS et al., 2004). However, as shown in the result, although the distribution patterns of CRMP-4 mRNA and protein were similar, more cells positively labeled for CRMP-4 mRNA were seen in some of examined areas in the present study (Figs 2-5). This may be due to the reason that CRMP-4 protein was not translated in some cells positive for CRMP-4 mRNA.

It is interesting to find that CRMP-4 is present in the medial cortex of lizard (NACHER et al., 2002), and the hippocampus of rat (NACHER et al., 2000) or Bengalese finch. This supports the view that reptilian medial cortex, and the avian or mammalian hippocampus are evolutionarily homologous from embryological perspectives, neural connection patterns and neurochemistry (see reviews in COLOMBO & BROADBENT, 2000). The presence of CRMP-4 in these homologous areas confirms again the view that the family of CRMP-4 is conserved throughout the phylogenetic scale (BYK et al., 1995; MINTURN et al., 1995; NACHER et al., 2000; 2002; QUINN et al., 2003).

The location of CRMP-4 in the hippocampus, song control nuclei and the cerebellum of Bengalese finch agrees with the previous reports that CRMP-4 expression appears in brain regions where adult neurogenesis and/or neurite outgrowth occur (NACHER et al., 2000; 2002; CNOPS et al., 2006). As mentioned above, many studies have shown that the hippocampus, song control nuclei or the cerebellum in the songbird undergo neuronal generation or synaptic remodeling during development or in adulthood (hippocampus: BARNEA & NOTTEBOHM, 1996; CLAYTON & KREBS, 1994; PATEL et al., 1997; song control nuclei: ABSIL et al., 2003; BRAINARD & DOUPE, 2000; FOSTER & BOTTER, 1998; MOONEY, 2000; cerebellum: CONSOLE-BRAM et al., 1996; CORVETTI & ROSSI, 2005). The presence of CRMP-4 in plastic brain areas suggests that CRMP-4 might be an indicator of continuous synapse growth or sparse fiber replacement, or might be involved in the axon guidance and synapse reorganization in creating a complex pattern of functional neuronal connectivity, to match the responses of the adult brain to physiological or external stimuli. It is also probable in some brain areas that somata and dendrites expressing CRMP-4 protein may do nothing but maintain their presented neural pathways. In other words, CRMP-4 protein is not only involved in axonal outgrowth but also in some other "plastic" events. This may be the reason why CRMP-4 was also detected in other brain areas such as auditory or visual areas and some hypothalamic areas, besides the traditionally regarded plastic brain regions.

Our double labeling study indicates that some CRMP-4 cells also express HuD, but not NeuN and GFAP. HuD is an RNA-binding protein that has been shown to induce neuronal differentiation (FUJIWARA et al., 2006) and plays a role in synaptic plasticity through stabilizing mRNAs associated with ribosomes both in somas and dendrites (BOLOGNANI et al., 2004). It has been reported that HuD expression increases during brain development, nerve regeneration, and learning and memory (SMITH et al., 2004; BOLOGNANI et al., 2004). These data suggest that CRMP-4 may take effect on the synaptic plasticity with the participation of HuD.

Our study showed that only immature neurons (HuD labeled cells), not mature ones (NeuN labeled cells), expressed CRMP-4. This was consistent with previous reports that CRMP-4 protein is located in postmitotic neurons that completed their final mitosis and the transient expression during the first postnatal weeks in the rat (MINTURN et al., 1995). These data support the argument that CRMP-4 can be regarded as immature cell marker, as the reports in the adult rat hippocampus after seizure (PARENT et al., 1997) and ischemia (LIU et al., 2003) or in the cerebral dysplasia (KERFOOT et al., 1999). However, recently reported results in the mature cat visual cortex indicate that CRMP-4 is not a reliable immature neuronal marker, at least not at all developmental stages or in all brain regions (CNOPS et al., 2006).

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An account of *Alciopina, Torrea*, and *Rhynconereella* (Polychaeta: Alciopidae) of the western Caribbean Sea

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ABSTRACT. Seven species of *Alciopina* Claparède & Panceri, *Torrea* Quatrefages, and *Rhynconereella* Costa were collected during five oceanographic cruises off the east coast of the Yucatan Peninsula, western Caribbean Sea. Descriptions and illustrations are provided here with an identification key. The most frequent species were *Rhynchonereella petersii* (Langerhans, 1880); *Alciopina parasitica* Claparède & Panceri, 1867; and *A. paumotanus* Chamberlin, 1919; which comprised 85% of the alciopid numbers collected during this survey. These three genera currently contain nine species, of which seven were obtained in our samples from the western Caribbean. These are the first records of the Alciopidae in the western Caribbean Basin and in Mexican waters of the Atlantic Ocean.

KEY WORDS : marine zooplankton, alciopid taxonomy, plankton

INTRODUCTION

Only a few groups of polychaetes have colonized the water column; among these, the highly specialized alciopids predominate (Støp-Bowitz, 1981; 1996; Wu & Lu, 1993). According to the results of the cladistical analysis and classification proposed by Rouse & Fauchald (1997) and modified by Glasby et al. (2000), the alciopid polychaetes belong to the clade Palpata, Aciculata, Phyllodocida. Alciopids were long considered a subfamily of the Phyllodocidae; however, as noted by Rouse & Pleijel (2001), there are no conclusive cladistical studies establishing that this group is nested within the Phyllodocidae, although both groups have important similarities. Wu & Lu (1993) addressed the phylogenetic relationships within the family, and agreed with Dales (1957) and Rice (1987) in inferring the origin of alciopids from a phyllodocid-like ancestor. Members of the Alciopidae are characterized by extreme development of the lateral eyes, a feature that modifies the structure of the entire cephalic area. Among other adaptations for the planktic life, alciopids show transformed podial cirri into natatorial appendages. Also, the cirriform processes of the pharynx have numerous gland cells used to catch small prey items (see Uschakov, 1972; Rouse & Fauchald, 1997).

All the members of this family are marine holoplanktic forms, easily recognized by their enormous sphaerical, lensed eyes (e.g., Pleijel & Dales, 1992); the unusual development of these organs probably relates to visual predatory behaviour. They are raptorial forms feeding on copepods, euphausiids, and other zooplankters. Alciopids occur in tropical and temperate waters of the world oceans, and are infrequently found in neritic environments. Most are epipelagic, dwelling in the upper layers (0–100m); however, some species migrate more widely through the water column, to depths exceeding 500m (Støp-Bowitz, 1981). Some species have been regarded as indicators of water masses (Orensanz & Ramírez, 1973).

Currently, the family contains 34 nominal species (Dales & Peter, 1972; Støp-Bowitz, 1996). Records of Alciopidae in the Atlantic Ocean can be found in the surveys by Treadwell (1943), Støp-Bowitz (1948; 1991; 1992; 1996), Renaud (1956), Tebble (1960), Rioja (1958), Day (1967), Orensanz & Ramírez (1973), Rice (1987), Pleijel & Dales (1992), and Núñez et al. (1993). Hitherto, this group has been surveyed only sporadically in the northwestern tropical Atlantic region. Twenty-six species of Alciopidae are known from the Atlantic Ocean; of these, up to 15 have been reported from the tropical northwestern Atlantic (see Salazar-Vallejo, 1992; 1996). In large oceanic areas in this region, such as the Caribbean Basin, the alciopid fauna remains poorly known. In this contribution we present a first taxonomic account and illustrated records of seven species of alciopids from the westernmost sector of this tropical basin. Descriptions of these species and a key for their identification are also provided.

MATERIALS AND METHODS

Five oceanographic cruises were carried out in oceanic waters in the Mexican Caribbean, the westernmost sector of the Caribbean Basin. These surveys were performed in February, March, May, August, and November 1991 on board of vessels of the Mexican Secretaría de Marina. Zooplankton samples were collected following a station plan that included 22 sampling sites during each cruise (Fig. 1). Sampling was performed by surface (0–10m) oblique trawls with a standard, square-mouthed plankton net (0.33mm mesh). This gear allowed collection of large and middle-sized pelagic polychaetes. Temperature and salinity were recorded at each sampling site. The mean volume of water filtered by the net per haul ranged between 100 and 160m^3; density values were obtained from these data. Samples were collected both in daylight (06: 00–18: 00h) and at night (19: 00–05: 00h). Samples were fixed and preserved in a buffered 4% formalin solu-

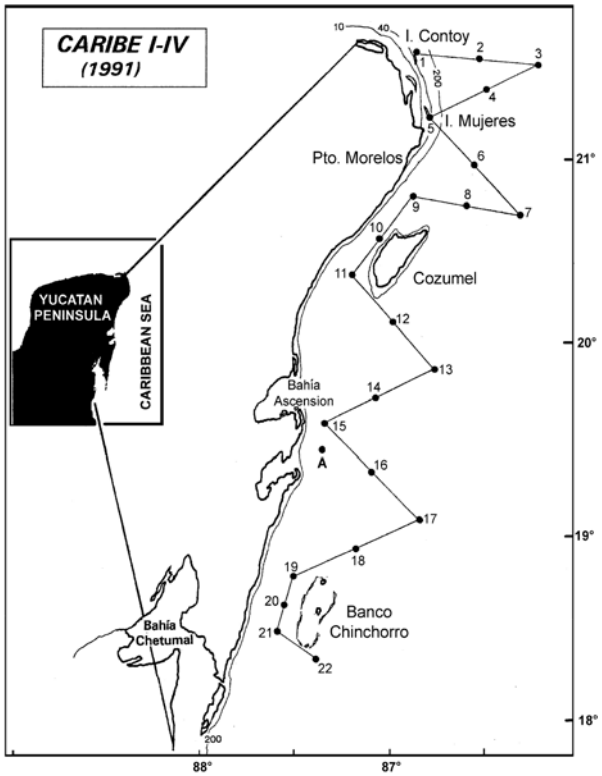

Fig. 1. – Surveyed area in the western Caribbean Sea, showing sampling stations.

tion. Alciopid polychaetes were sorted from the original sample and then transferred to 70% ethanol for long-term preservation. Additional material was collected during a cruise of the R/V "Edwin Link", carried out during August 1990 off the Caribbean coast of Mexico. Specimens were identified following the keys and illustrations published by CHAMBERLIN (1919), FERNÁNDEZ-ÁLAMO (1983), STØP-BOWITZ (1948; 1996), DAY (1967), and ORENSANZ & RAMÍREZ (1973). The specimens were deposited in the Collection of Zooplankton (ECO-CHZ) held at El Colegio de la Frontera Sur (ECOSUR), in Chetumal, Mexico. Type material of the species treated in this report was sought, unsuccessfully, from curators or databases of various institutions. We were able to compare our specimens with the original illustrations and descriptions of the alciopids found in this survey.

RESULTS

In this section we present revised diagnoses of the genera and species recorded in this survey, illustrations of the taxonomically relevant characters of each species, and a key for their identification. The abbreviations used in the section of material examined indicate the cruise name (C for the "Caribe" cruises, EL for "Edwin Link" cruise). In case of the Caribe cruises the C followed by the number (I–V) of the cruise and by the station number (A and 1-22, as in Fig. 1), the number and sex of the specimens examined, date and hour of collection, geographic position of the station, and catalogue number.

Systematics

Family Alciopidae Ehlers, 1864

Genus *Alciopina* Claparède & Panceri, 1867

Diagnosis: Body short. Prostomium rounded, produced anterior to eyes. One ventral pair of palps and three antennae, one dorsal pair plus a median one, the latter represented by a small process. Pharynx short, with marginal papillae. Eyes large, laterally directed. Six pairs of tentacular cirri, arranged as follows: first ventral pair arising from cirrophores on the lower surface of eyes, first and second segments with one dorsal and one ventral pair, third segment with single ventral pair. Fourth segment with or without chaetigerous lobe. Succeeding segments well-developed, with dorsal and ventral foliaceous cirri; chaetigerous lobes without cirriform appendages. Chaetae simple, capillary, acicular on anterior segments. Genital papillae on parapodia 9-18. Segmental glands from first chaetigerous segment. Two known species.

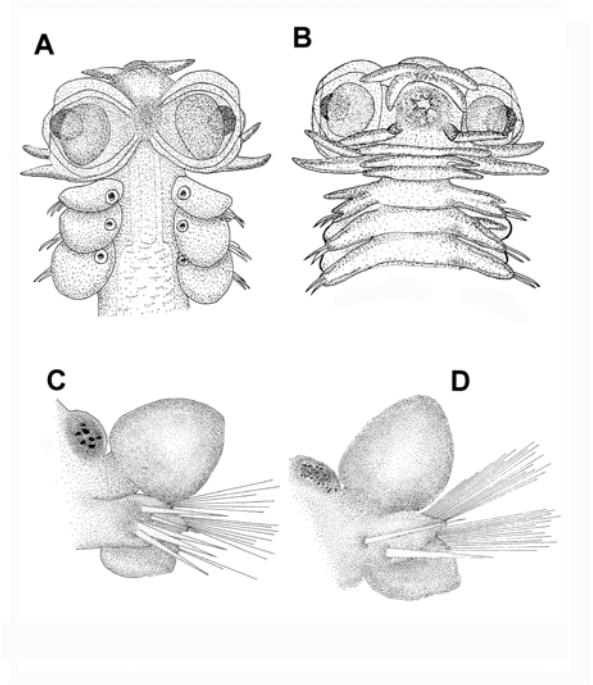

Fig. 2. – *Alciopina parasitica* Claparède and Panceri, 1867. A, anterior part of body, dorsal view; B, anterior part of body, ventral view; C, parapod from anterior part of body; D, parapod from middle part of body.

Alciopina parasitica Claparède & Panceri, 1867

(Figs 2 A–D)

Alciopina parasitica CLAPARÈDE & PANCERI (1867: 8); CLAPARÈDE (1868: 253, with figure); GRANATA (1911): 57, with figure); STØP-BOWITZ (1948: 32); DALES (1957: 128; 1963: 502); DAY (1967: 177); DALES & PETER (1972: 71); ORENSANZ & RAMÍREZ (1973: 35); FERNÁNDEZ-ÁLAMO (1983: 105).

Alciopina panceri BUCHHOLZ (1869: 95, with figure).

Corynocephalus albomaculatus LEVINSEN (1885: 327, with figure); APSTEIN (1900: 14, with figure).

C. albo-maculatus Fauvel (1916: 68; 1923: 208, with figure; 1939: 284; 1951: 293); Wesenberg-Lund (1939: 36, with figure); Zei (1956: 53); Rullier (1965: 872).

Material examined: CI-A (1♂), 11-02-91, 22: 05,19°13'05" N, 87°23'04" W, ECO-CHZ- 02486; CI-11 (1♂,1♀), 07-02-91, 06: 05, 20°23'00" N, 87°09'00" W, ECO-CHZ- 02487; CI-15 (1♂), 11-02-91, 5: 50, 19°34'03" N, 87°20'04" W, ECO-CHZ-02488; CII-14 (1♂), 06-03-91, 20: 04, 19°44'01" N, 87°02'07" W, ECO-CHZ- 02489; CIII-17 (1♀), 09-05-91, 23: 01, 19°04'00" N, 86°51'00" W, ECO-CHZ-02490.

Diagnosis: Body short, yellowish. Total length: 4–7mm, width: 1.0mm. 20 parapodia in complete specimens, 17–31 in incomplete specimens. Dorsal antennae robust, short; ventral palps relatively longer than dorsal (Fig. 2B). Median antenna represented by protuberance between eyes (Fig. 2A). First ventral pair on short cirrophore with cirri 0.3 times as long as dorsal ones; succeeding ventral pairs 0.6 times as long as dorsal. Chaetigerous lobes absent on first parapodia, with dorsal cirri larger than ventral ones, all foliaceous (Fig. 2B). Following segments with foliaceous dorsal and ventral cirri; cirriform appendages absent on chaetigerous lobes. Acicular and simple capillary chaetae on parapodia 1–3; in parapodia 4–5 acicular chaetae are predominant, capillary chaetae present from parapod 4, most abundant from parapod 8 (Figs 2C; D). Segmental glands small, from first pair of parapodia, represented by distally pigmented unbranched projections. Males with ventral genital papillae at base of parapodia 9–13.

Type locality. Gulf of Naples (Italy).

Distribution. Tropical and subtropical waters of the Atlantic, Mediterranean, eastern tropical Pacific. First record from the Caribbean Sea.

Alciopina paumotanus (Chamberlin, 1919)

(Fig. 3)

Corynocephalus paumotanus Chamberlin (1919: 141), Treadwell (1943: 37), Ushakov (1957: 277, with figure); Berkeley & Berkeley (1958: 400; 1964: 125).

?*Corynocephalus gazellae* Apstein (1893: 148; 1900: 15).

Material examined: CI-A (1♂), 11-02-91, 22: 05, 19°13'05" N, 87°23'04" W, ECO-CHZ-02491; CI-11 (1♂), 07-02-91, 06: 05, 20°23'00" N, 87°09'00" W, ECO-CHZ-02492; CI-15 (1♂), 11-02-91, 05: 50, 19°34'03" N, 87°20'04" W, ECO-CHZ-02493; CII-14 (1 spec., sex undet.), 06-03-91, 20: 04, 19°44'01" N 87°02'07" W, ECO-CHZ-2494; CIII-17 (1♂, 1♀), 09-05-91, 23: 01, 19°04'00" N 86°51'00" W, ECO-CHZ-02495.

Diagnosis: Body flattened dorso-ventrally, widest at middle section. Total length: 3–7mm, width: 0.5–1mm. 25 parapodia in single complete specimen, 9–32 in incomplete specimens. Dorsal antennae short, ventral palps robust, longer than antennae, with pigment spots at base (Fig. 3B). Median antenna poorly developed (Fig. 3A). Pharynx retracted in Caribbean specimens (Fig. 3B). Parapodia with large, foliaceous dorsal cirri, chaetigerous lobes elongated, devoid of cirriform appendages, with single, low acicule. Ventral cirri foliaceous, of nearly the same size as chaetigerous lobes. Simple acicular and capillary chaetae present. Parapodia 1–3 with 4–5 acicular chaetae, succeeding parapodia with one chaeta; parapodia 7–8 with capillary chaetae, most abundant from parapod 13 (Figs 3C–E). Segmental glands small, behind dorsal cirrus, with distal end pigmented. Males with ventral genital papillae on base of parapodia 10–14, with digitiform pigmented projections, larger than in *A. parasitica*.

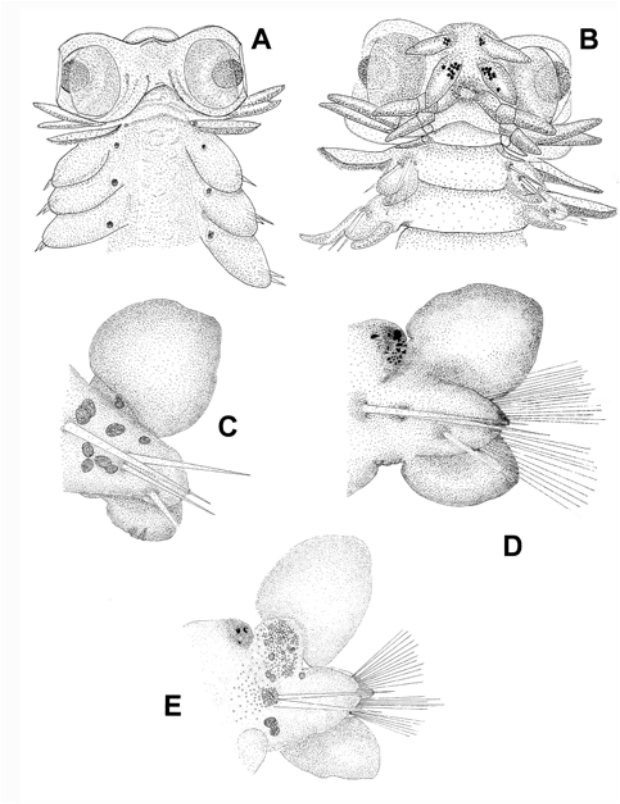

Fig. 3. – *Alciopina paumotanus* (Chamberlin, 1919). A, anterior part of body, dorsal view; B, anterior part of body, ventral view; C, parapod 6; D, parapod 15; E, parapod 23.

Type locality: Paumotu Islands (tropical Pacific).

Distribution: Pacific, North Atlantic.

Remarks: This is the first record of species in the western Caribbean Sea. There is one previous record from the easternmost sector of the Caribbean Basin (Treadwell, 1943), based on specimens collected during the Carnegie Plankton Expedition (1928).

Genus *Torrea* Quatrefages, 1850

Diagnosis: Body elongated, cylindrical. Prostomium not produced anteriorly from eyes. Pharynx large, with distal end bearing pair of lateral horn-like processes with or without marginal papillae. Eyes large, directed laterally. Three pairs of tentacular cirri on ventral surface of first three segments, one pair on each segment, first pair arising from base of eyes. Segments 4 and 5 with chaetae, with reduced chaetigerous lobes; females with large dorsal cirri on both segments to form a receptaculum seminis. Parapodia normal, with foliaceous dorsal and ventral cirri; cirriform appendage absent in chaetigerous lobes. All chaetae compound capillary with long appendages. Segmental glands pigmented. Single pair of anal cirri. Two species known.

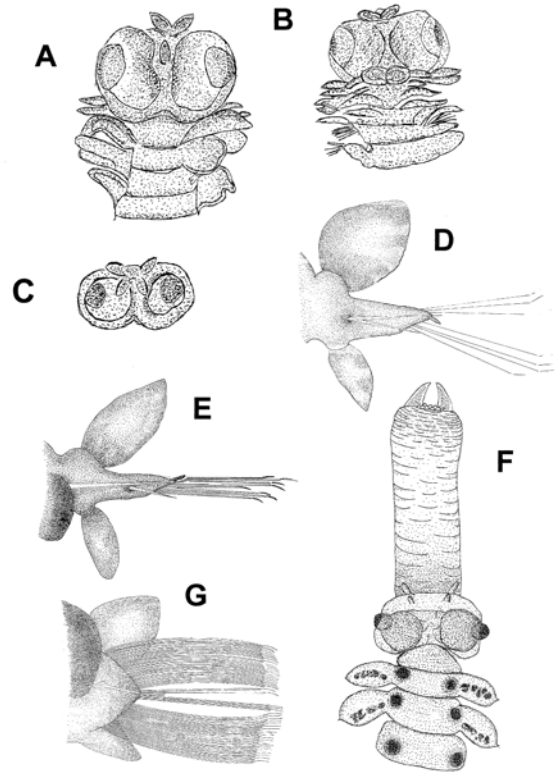

Fig. 4. – *Rhynchonereella moebii* (Apstein, 1893). A, anterior part of body, dorsal view; B, anterior part of body, ventral view; C, anterior part of body, frontal view; D, parapod 12. *Rhynchonereella gracilis* Costa, 1862. E, parapod from middle part of body; *Torrea candida* (delle Chiaje, 1828): F, anterior part of body, ventral view; G, parapod from middle part of body.

Torrea candida (delle Chiaje, 1828) Benham, 1929

(Figs 4 F; G)

Alciopa candida DELLE CHIAJE (1828: 88); KRÖHN (1845: 174).

Alciopa vittata HERING (1860: 11; 1892: 747, with figure).

Liocapa vertebralis COSTA (1864: 165, with figure); EHLERS (1864: 181); COSTA (1867: 55); CLAPARÈDE (1868: 252).

Liocapa candida LEVINSEN (1885: 333, with figure).

Torrea vitrea QUATREFAGES (1865: 159, with figure)

Asterope candida CLAPARÈDE (1870: 108, with figure); GREEFF (1876: 62, with figure); APSTEIN (1900: 7); LO BIANCO (1904: 50, with figure); FAUVEL (1907: 8, with figure; 1916: 65; 1923: 202, with figure; 1932: 16; 1939: 283); IZUKA (1914: 3, with figure); FYTE (1952: 10); ZEI (1956: 52).

Torrea candida BENHAM (1929: 186); MONRO (1930: 82; 1936: 119); WESENBERG-LUND (1939: 32, with figure); STØP-BOWITZ (1948: 22); DALES (1955: 435; 1957: 111); TEBBLE (1962: 194); GRICE & HART (1962: 302); IMAJIMA & HARTMAN (1964: 73); DAY (1967: 188); HARTMAN (1968: 315, with figure); USHAKOV (1972: 189); ORENSANZ & RAMÍREZ (1973: 4).

Material examined: EL 2775 (1), 20-08-90, Banco Chinchorro, 18°45.4' N, 87°15.80' W, ECO-CHZ-02496.

Diagnosis: A single specimen was obtained from our samples. Total length: 5.5mm. Specimen damaged, with 32 parapodia. Observable species characters: ventral palps twice as long as dorsal antennae (Fig. 4F), pharynx as in genus. First pair of tentacular cirri arising from cirrophore, larger than the other two pairs. Female specimen with dorsal cirri of first two parapodia transformed into seminal receptacles, with intensely pigmented dorsal fringe (Fig. 4F). Parapodia normal, with foliaceous dorsal cirri; ventral cirri smaller. Chaetae noticeably long (Fig. 4G). Segmental glands subrectangular, intensely pigmented.

Type locality: Naples Bay (Italy).

Distribution: Mediterranean, tropical and subtropical waters of the Atlantic, Indian, and Pacific. First record of this species in the Caribbean Sea.

Genus *Rhynchonereella* Costa, 1862

Diagnosis: Body elongated, slender. Prostomium produced anteriorly to eyes. Pharynx short, with marginal papillae, no lateral horn-like processes. Three antennae, one median, two dorsal; two ventral palps. First three segments with 4–5 pairs of tentacular cirri. First segment with ventral pair, second and third segments each with one dorsal and one ventral pair. Anterior parapodia well-developed; parapodia normal, with foliaceous dorsal and ventral cirri; chaetigerous lobes with cirriform appendage. Chaetae of three types, compound capillary and simple or compound acicular, at least on anterior parapodia. Five species known.

Rhynchonereella gracilis Costa, 1862

(Fig. 4E)

Rhynchonereella gracilis COSTA, (1862: 168, with figure); STØP-BOWITZ (1948: 36); DALES (1956: 293; 1957: 131, with figure; 1957: 662); HARTMAN (1969: 171); TEBBLE (1962: 396, with figure; 1968: 33); BERKELEY & BERKELEY (1964: 126); IMAJIMA & HARTMAN (1964: 72; 1964: 72); DAY (1967: 189); DALES & PETER (1972: 69); USHAKOV (1972: 199); ORENSANZ & RAMÍREZ (1973: 45); FERNÁNDEZ-ÁLAMO (1983: 93); PLEIJEL & DALES (1992: 156).

Callizona nasuta GREEFF (1876: 72, with figure); APSTEIN (1891: 133; 1893: 148, with figure); FAUVEL (1923: 215, with figure); ZEI (1956: 54); USHAKOV (1957: 279); BERKELEY & BERKELEY (1960: 790); RULLIER (1965: 871).

Callizona japonica IZUKA (1914: 7).

Material examined: CIV-5 (1 spec., sex undet.) plus fragment of middle section of body, 06-08-91, 07: 45, 21°10'09" N, 86°46'02" W, ECO-CHZ-02497.

Diagnosis: Caribbean specimens damaged, incomplete, length: 3mm, 13 parapodia. Pharynx not observable in Caribbean specimen. Five pairs of tentacular cirri. First pair of parapodia with dorsal and ventral foliaceous cirri, chaetigerous lobes and chaetae absent on this parapod. Dorsal cirri twice as long as ventral ones. Parapodia normal, with foliaceous dorsal cirri; smaller ventral cirri lanceolate. Chaetigerous lobes digitiform, with cirriform appendages. Chaetae compound capillary plus one or two simple ventral acicular chaetae on anterior and median

parapodia (Fig. 4E). Segmental glands posterior to dorsal cirrus; pigmentation weaker on anterior part of body, strongest medially.

Type locality: Bay of Naples (Italy).

Distribution: Mediterranean, tropical and subtropical waters of the Atlantic, Indian, and Pacific. First record in the Caribbean Sea.

Rhynchonereella moebii (Apstein, 1893)
Støp-Bowitz 1948

(Figs 4A–D)

Callizona möbii Apstein (1893: 147; 1900: 16, with figure); Wesenberg-Lund (1939: 40, with figure); Rullier (1965: 871).

Callizona moebii Fauvel (1923: 213, with figure).

Rhynchonereella möbii Støp-Bowitz (1948: 34); Dales (1957: 131; 1960: 484); Grice & Hart (1962: 302); Tebble (1962: 396, with figure); Berkeley & Berkeley (1964: 126).

Rhynchonereella moebii Day (1967: 189); Dales & Peter (1972: 70); Fernández-Alamo (1983: 102); Støp-Bowitz (1996: 176).

Material examined: CI-6 (1 spec., sex undet.), 06-02-91, 19: 05, 20°57'04" N, 86°26'03" W, ECO-CHZ-02498; CIV-17 (1 spec., sex undet.), 07-08-91, 23: 40, 19°04'00" N, 86°51'00" W, ECO-CHZ-02499.

Diagnosis: Caribbean specimens incomplete, total length: 2–3mm, with 14–18 parapodia. Dorsal and ventral antennae robust (Fig. 4C). Median antenna digitiform (Fig. 4A). Pharynx not observed in Caribbean specimens, but cylindrical, slender, slightly wider distally in species. Five pair of tentacular cirri. First pair of tentacular cirri short, arising from large cirrophores, not reaching margin of eyes, second and fourth pairs elongated, latter noticeably thicker than former. Third pair small, lanceolate, fifth pair foliaceous (Fig. 4D). Dorsal and ventral cirri relatively larger on first 4 pairs than those on other parapodia. Chaetae of two kinds, simple acicular, distally curved, abundant on first 4–5 parapodia (2–7 chaetae on each, then decreasing to one on each posterior parapod); compound capillary chaetae from parapodia 4 or 5, reaching slightly beyond distal end of acicular chaetae (Fig. 4D). Segmental glands represented by oval protuberances on postero-dorsal position of parapod.

Type locality: Messina, Sicily, Italy.

Distribution: Mediterranean, tropical and subtropical waters of the Atlantic, Indian, and Pacific. First record from the Caribbean Sea.

Rhynchonereella petersii (Langerhans, 1880)
Støp-Bowitz, 1948

(Figs 5A–C)

Alciopa (Halodora) petersi Langerhans (1880: 312, with figure); Ehlers (1913: 465).

Callizona setosa Apstein (1900: 18, with figure); Southern (1910: 5); Fauvel (1923: 14, with figure); Wesenberg-Lund (1939: 43); Ushakov (1957: 281).

Rhynchonerellla petersii Støp-Bowitz (1948: 34); Dales (1957: 133; 1963: 502); Tebble (1962: 398); Grice & Hart (1962: 302); Berkeley & Berkeley (1964: 126); Hartman (1964: 61); Bhaud (1966: 436); Guille & Laubier (1966: 263); Day (1967: 192); Pleijel & Dales (1992: 158).

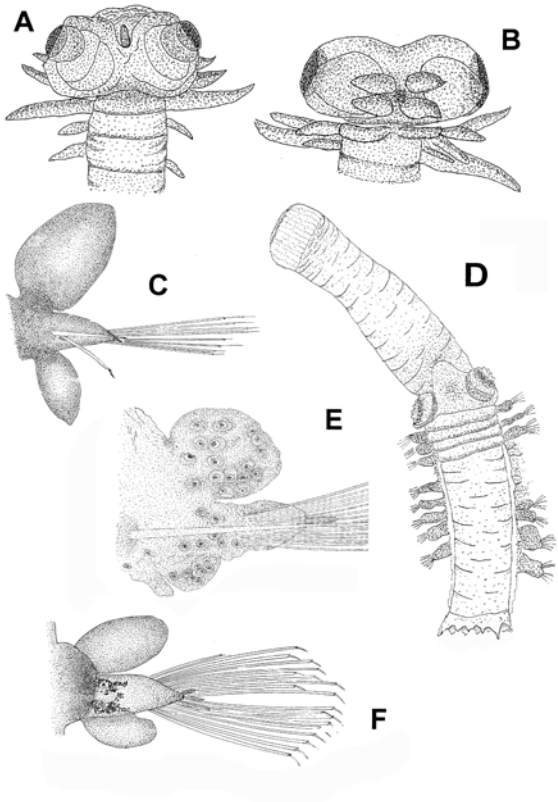

Fig. 5. – *Rhynchonereella petersii* (Langerhans, 1880). A, anterior part of body, dorsal view; B, anterior part of body, ventral view; C, parapod from middle part of body. *Rhynchonereella angelini* (Kinberg, 1866). D, anterior part of body, dorsal view; E, parapod of anterior part of body, specimen in epitoke; F, parapod from anterior part of body, non-reproducing specimen.

Alciopa cari Hering (1892: 753); Ehlers (1912: 17).

Vanadis heterochaeta Viguier (1886: 405, with figure).

Vanadis setosa Greeff (1885: 449, with figure).

Corynocephalus magnachaetus Treadwell (1943: 37).

Callizona petersii Monro (1939: 107).

Material examined: CI-A (1), 11-02-91, 22: 05, 19°13'05" N, 87°23'04" W, ECO-CHZ-02500; CII-6 (1), 05-03-91, 22: 05, 20°57'08" N, 86°26'05" W, ECO-CHZ-02501; CIII-17 (3), 09-05-91, 23: 01, 19°04'00" N, 86°51'00" W, ECO-CHZ-02502; CIII-18 (3), 09-05-91, 20: 25, 18°54'04" N, 87°11'03" W, ECO-CHZ-02503; CIV-17 (1), 07-08-91, 19: 05, 19°04'00" N, 86°51'00" W, ECO-CHZ-02504.

Diagnosis: Body slender, yellowish. Caribbean specimens incomplete, total length: 1–2mm, width: 0.2–0.5mm, 14 parapodia. Dorsal and ventral antennae robust, ventral pair slightly longer than dorsal; median antenna digitiform (Figs 5A; B). Pharynx not observable in the Caribbean specimens; short, cylindrical, with 10–12 marginal rounded or conical papillae in species. Five pairs of tentacular cirri. First pair of tentacular cirri reaching beyond outer margin of eyes, shorter than second; third

pair slender, small; fourth pair robust, longest; fifth pair digitiform, smallest (Fig. 5B). Cordiform dorsal cirri on anterior parapodia, foliaceous on median parapodia. Ventral cirri small, foliaceous, almost as long as chaetigerous lobes, the latter bearing short cirriform appendages. Chaetae of two kinds, compound capillary and compound acicular: former slender, smooth; latter slender, distally serrated. Anterior parapodia with 1–2 acicular chaetae; compound capillary chaetae mainly on median parapodia (Fig. 5C). Segmental glands unpigmented.

Type locality: Madeira, Eastern Atlantic

Distribution: Tropical and subtropical Atlantic, Indian, and Pacific. First record from the Caribbean Sea.

Rhynchonereella angelini (Kinberg, 1866)
Greeff, 1876
(Figs 5D–F)

Krohnia angelini Kinberg (1866: 242).

Rhynchonereella angelini Greeff (1876: 57,with figure); Støp-Bowitz (1948: 34); Fraser (1955: 12); Dales (1955: 439); Hartman (1956: 277); Dales (1957: 113); Imajima (1961: 8, with figure); Tebble (1960: 192; 1962: 400; 1968: 33); Imajima & Hartman (1964: 72); Day (1967: 189); Hartman (1968: 313, with figure); Clark (1970: 42); Dales & Peter (1972: 69); Pleijel & Dales (1992: 156).

Callizona grubey Greeff (1876: 72, with figure); Levinsen (1885: 333, with figure); Apstein (1900: 18, with figure).

Callizona angelini Apstein (1900: 18, with figure); Reibisch (1905: 4, with figure); Southern (1911: 4); Fauvel (1916: 68; 1923: 215, with figure); Monro (1930: 82; 1936: 118); Wesenberg-Lund (1939: 41); Berkeley & Berkeley (1948: 34; 1957: 575; 1958: 400; 1960: 790).

Rhynchonereella picnocera Chamberlin (1919: 147); Treadwell (1928: 462; 1943: 36).

Rhynchonereella parva Chamberlin (1919: 150).

?*Callizona henseni* Apstein (1900: 20, with figure).

Krohnia angelini Ushakov & Wu (1963).

Material examined: CIII-18 (1 spec., sex undetermined plus 2 fragments), 09-05-91, 20: 25,18°54'04" N, 87°11'03" W, ECO-CHZ-02505; EL 2773 (2), 19-08-90, off Punta Allen, Quintana Roo, 19°47.2' N, 87°24.4' W, ECO-CHZ-02506.

Diagnosis: Both females examined represented by fragments only. These specimens were assigned to *R. angelini* by the characters of the parapodia and the pharynx, as follows. Pharynx fully observable in Caribbean specimens, muscular, long, about eight times longer than the eye diameter; margin with remains of papillae (Fig. 5D). Five tentacular cirri. Parapodia with foliaceous dorsal and ventral cirri, chaetigerous lobes elongated, with long, digitiform cirriform appendages; two kinds of chaetae, compound capillary, compound acicular with small, smooth apices. Anterior parapodia with 14–18 acicular chaetae, plus some capillary chaetae as well (Figs 5E; F). Female specimens with parapodia full of gametes (Figs 5E; F). Specimen also with two kinds of chaetae; 16–18 compound acicular chaetae on anterior parapodia with short, smooth apice, acicular chaetae as long as capillary (Figs 5E; F). Some parapodial fragments bear 1–2 acicular chaetae, thus suggesting a decrease in the number of chaetae posteriorly.

Type locality: North Pacific

Distribution: Atlantic, Pacific. First record from the western Caribbean Sea. Hitherto, it was recorded from the eastern Caribbean by Treadwell (1943) from specimens collected during the last cruise of the "Carnegie".

Distribution and abundance: Alciopid polychaetes were absent from the zooplankton samples collected during the Caribe V cruise (November, 1991). Alciopids of the three genera studied herein had low densities in the remaining four cruises; their numerical abundance was highest during May (mean density: 41 ind./1000m^3), and lowest in August (18 ind./1000m^3) (see Table 1). The distribution of the alciopid species included the entire surveyed area (see Fig. 6). The commonest species were *Alciopina parasitica*, *A. paumotanus*, and *Rhynchonereella petersii*. These were collected during three Caribe cruises in varying densities (Table 1). These species accounted for nearly 85% of the alciopids of the three genera treated in this work.

TABLE 1

Mean density (ind./1000 m³) and relative abundance (%) of alciopids of the genera *Alciopina* and *Rhynchonerella* collected during the CARIBE cruises I-IV (CAR-I–IV), Mexican Caribbean Sea.

	CAR–I		CAR–II		CAR–III		CAR–IV	
	February		March		May		August	
	Density	%	Density	%	Density	%	Density	%
A. parasitica Claparède & Panceri, 1867	5.2	44	5.6	33.3	7.3	10	–	–
A. paumotanus (Chamberlin, 1919)	7.8	33	5.6	33.3	14.7	20	–	–
Rhynconerella. gracilis Costa, 1862	–	–	–	–	–		6.2	33.3
R. moebii (Apstein, 1893)	9.1	11	–	–	–		6.0	33.3
R. petersi (Langerhans, 1880)	7.1	11	4.2	33.3	14.7	60	6.0	33.3
R. angelini (Kinberg, 1866)	–	–	–	–	4.4	10	–	–
Number of species	4		3		4		3	

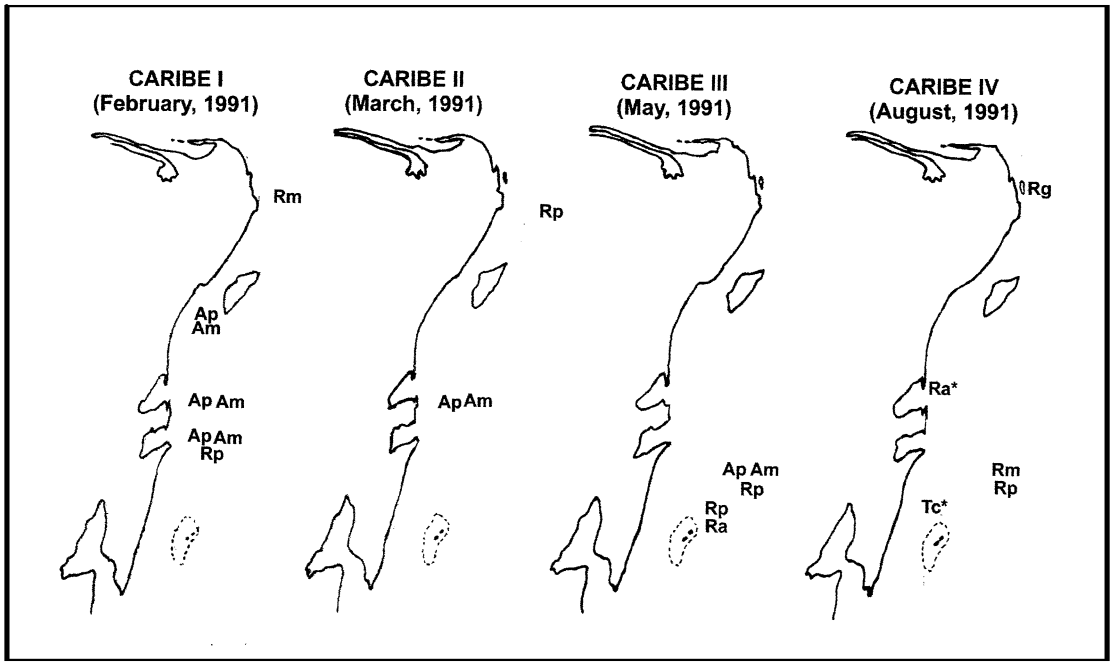

Fig. 6. – Local distribution of the species recorded in the western Caribbean Sea during this survey. *Alciopina parasitica* (Ap); *Alciopina paumotanus* (Am); *Torrea candida* (Tc); *Rhynchonereella gracilis* (Rg); *Rhynchonereella moebii* (Rm); *Rhynchonereella petersii* (Rp); *Rhynchonereella angelini* (Ra). (*) species collected during expedition of the "Edwin Link".

Key to the species of *Alciopina*, *Torrea*, and *Rhynchonereella* recorded in the Caribbean Sea

I. – Chaetigerous lobes without cirriform appendages

A. Simple capillary chaetae

Two kinds of chaetae, capillary and acicular, 4 or 5 pairs of tentacular cirri, parapodial cirri foliaceous, dorsal cirri larger than ventral cirri, body relatively short . . . . .
Alciopina

1) Median antenna protuberant. . . . . .
Alciopina parasitica

2) Median antenna inconspicuous . . . . . .
Alciopina paumotanus

B. Compound capillary chaetae

Three or four pairs of tentacular cirri, only compound capillary chaetae,

pharynx elongated, with pair of lateral horn-like processes . . . . . .
Torrea

Median antenna represented by rounded protuberance, trilobulate papillae between lateral horn-like processes .
Torrea candida

II. – Chaetigerous lobes with cirriform appendage

Dorsal tentacular cirri of third segment normal, anterior parapodia well-developed, all capillary chaetae compound, acicular chaetae simple or compound. . . . . .
Rhynchonereella

A. Simple acicular chaetae

1) Segments 4-6 with 1 or 2 acicular chaetae and some capillary chaetae. Dorsal cirri of first four parapodia shorter than on succeeding parapodia . . . . . .
R. gracilis

2) Segments 4-5 with 2-7 acicular chaetae. Dorsal and ventral cirri of first four parapodia larger than on succeeding parapodia . . . . . .
R. moebii

B. Compound acicular chaetae

1) Acicular chaetae with serrated margin, body small
R. petersii

2) Acicular chaetae smooth, small, body elongated, chaetigerous lobes with long cirriform appendages, tentacular cirri of third segment subequal to second . . . . . .
R. angelini

DISCUSSION

The distributions of most of most known species of the Alciopidae have been described as widespread or cosmopolitan; records of many nominal species are from different latitudes of the Atlantic, Indian, and Pacific oceans (Dales, 1957; Dales & Peter, 1972; Støp-Bowitz, 1981, 1996; Pleijel & Dales, 1992). However, the apparent cosmopolitanism of these and other holoplanktic forms has not been fully demonstrated; many local or regional records are based on a small number of descriptive works without original illustrations or complete

descriptions of the local specimens. Further, some species of the Alciopidae have a complex history of synonymies (Støp-Bowitz, 1992) that implies a certain degree of intraspecific variability; these differences have not been taxonomically evaluated.

Most of the alciopid species examined in this survey were described during the XIX century, and, as far as we could determine, type material of most of these species is not extant. Only for *R. angelini* were we able to locate a single type specimen, deposited in the Swedish Museum of Natural History, Stockholm (cat. T-5555). It is probable that the lack of type specimens affects most of the family, which should undergo a revisionary process including: 1) designation of neotypes based on specimens from the original localities and 2) detailed taxonomic and comparative analyses of the records and of extant specimens deposited in different collections. Some of these nominal species could be taxonomic complexes comprising undescribed taxa. In this work we provide basic elements (descriptions, detailed illustrations) to aid this kind of analysis in reference to the Alciopidae of the northwestern tropical Atlantic region.

The surveyed area receives the influence of the Caribbean Current, which flows westwards from the Lesser Antilles and eventually reaches the Yucatan Channel. The surface zooplankton fauna recorded in this study has a strong affinity to that of the rest of the Caribbean Basin, as stated previously for other planktic taxa (Gasca et al., 1996; Gasca & Shih, 2003); hence, it is speculated that the alciopid species found in this survey are also distributed in the entire Caribbean Basin.

Hitherto, the only previous survey on the pelagic polychaetes from the western Caribbean was that by Jiménez-Cueto & Suárez-Morales (1999), on the family Tomopteridae. They recorded up to 15% of the tomopterid species known worldwide and 17% of those recorded in the tropical northwestern Atlantic (Dales & Peter, 1972; Salazar-Vallejo, 1992; 1996). The corresponding figures for the alciopid fauna reported herein are more representative. The three genera considered here contain a total of 9 species (Støp-Bowitz, 1996), of which 7 (77%) were present in our samples from the western Caribbean. Figures for each genus examined herein are as follows: *Alciopina* (2 species known: 2 in the Caribbean), *Torrea* (2: 1), *Rhynchonereella* (5: 4). It remains clear that these genera are not particularly diverse, but they are all well represented in the surface zooplankton of the Caribbean Sea. To the best of our knowledge, no other survey in the Atlantic Ocean has reported more than 5 species of these three genera (see Støp-Bowitz, 1948; Orensanz & Ramírez, 1973; Núñez et al., 1993).

The number of specimens collected in this survey (24) falls within the range known for this relatively rare group of holoplanktic polychaetes. Núñez et al. (1993) collected only fragments of 4 individuals of one of these alciopid genera in the Tropical Eastern Atlantic. Støp-Bowitz (1948) found between 1 and 9 specimens and 36 individuals of *R. angelini* in the North Atlantic. Also, Orensanz and Ramírez (1973) recorded only 5 species of these three genera from samples collected during seven oceanographic cruises in the southwestern Atlantic; the number of specimens in each of those campaignes ranged between 1 and 3, and only one species was represented by more than 10.

We found differences in the abundance of these polychaetes with respect to night and day samples; up to 70% of the specimens were collected during the night. This information suggests that even within the uppermost layer (0–50m), these species show a well-defined circadian vertical migration, moving closer to the surface at night. As noted by Núñez et al. (1993), alciopids differ from tomopterids in this respect; the latter group migrates extensively along the water column and contains many deep-living species. It is suggested that this was a factor related to the lower representation of tomopterids in the area (see Jiménez-Cueto & Suárez-Morales, 1999) with respect to the Alciopidae, even though these two surveys were based on the same samples. Our data from the western Caribbean confirm that alciopids are upper epipelagic forms with a limited range of vertical migration.

Only two of these species have been recorded from the Caribbean Basin before: *A. paumotanus* (as *Corynocephalus paumotanus*) and *R. angelinii* (as *R. picnocera*), both from the eastern sector (Treadwell, 1943). Hence, five of the seven species of Alciopidae are newly recorded from the Caribbean Basin, although some (i.e. *A. parasitica*, *T. candida*, *R. angelini*, *R. moebii*, *R. petersii*) have been reported previously from adjacent areas of the tropical northwestern Atlantic such as the Bahamas and the Gulf of Mexico (see Rice, 1987; Salazar-Vallejo, 1992; 1996; Long & Zottoli, 1997). These species have not been previously recorded from Mexican waters of the Atlantic Ocean.

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Food habits of the hollowsnout grenadier, *Caelorinchus caelorhincus* (Risso, 1810), in the Aegean Sea, Turkey

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ABSTRACT. Stomach contents of 148 hollowsnout grenadier, *Caelorinchus caelorhincus* (RISSO, 1810), were examined. Crustaceans were found to be most important prey group in the diet. Polychaetes constituted the second most important prey group. Chaetognathans were only occasionally eaten.

KEY WORDS : *Caelorinchus caelorhincus*, food habits, diet, Aegean Sea.

INTRODUCTION

The hollowsnout grenadier, *Caelorinchus caelorhincus* (Risso, 1810), is a benthopelagic species that lives at depths between 200 and 500m, but has been captured in waters as shallow as 90m and as deep as 850m. (COHEN et al., 1990). However, FROESE & PAULY (2006) gave a depth range of 1250m as the vertical distribution of *C. caelorhincus***.** The species displays a "bigger-deeper" phenomenon (POLLONI et al., 1979) with smaller individuals distributed in shallower waters (<400m) and larger individuals in deeper (>500m) (MADURELL et al., 2004). This may indicate ontogenetic migrations of the species toward deep waters (MORANTA et al., 1998; LABROPOULOU & PAPAOCONSTANTINOU, 2000; MADURELL et al., 2004). The hollowsnout grenadier has a wide distribution from the Mediterranean northward to southern Norway and across to the Shetlands, the Faroes, off southern Iceland and south-eastern Greenland (WHITEHEAD et al., 1984; COHEN et al., 1990). The hollowsnout grenadier is also known from the Mediterranean coast of Turkey (BILECENOGLU et al., 2002).

The community structure (MORANTA et al., 1998; LABROPOULOU & PAPAOCONSTANTINOU, 2000; MADURELL et al., 2004), and age and growth (MASSUTI et al., 1995; D'ONGHIA et al., 2000; FILIZ et al., 2006) of this species were studied by various researchers in the Mediterranean. Length-weight relationships for this species are given by DIAZ et al. (2000), BORGES et al. (2003), MOREY et al. (2003), FILIZ & BILGE (2004), and FILIZ et al. (2006).

In the Aegean Sea, the three Macrourids (*C. caelorhynchus*, *Hymenocephalus italicus* and *Nezumia sclerorhynchus*) are often caught by commercial trawlers targeting deep-water shrimps, *Parapaneus longirostris* (Lucas, 1846) and *Plesionika heterocarpus* (Costa, 1871).

This paper provides the first information on the food habits of *C. caelorhynchus*, one of the most abundant bycatches (no commercial value) in the shrimp trawl fishery in Sigacik Bay; the eastern Aegean Sea, Turkey.

MATERIALS AND METHODS

We sampled 148 (ranging from 113 to 123mm total length) hollowsnout grenadiers on board a 23m commercial fishing vessel (F/V Hapuloglu; 550HP) on 22 March 2003 in Sigacik Bay, Aegean Sea (Fig. 1). A conventional bottom trawl net of 24mm cod-end mesh size was used and three hauls in same day were carried out from dawn to dusk and haul durations ranged from 1 to 3h. The vessel speed was maintained at 2.2-2.5 knots. Dept range of fishing ground was 145-296m (Table 1). The stomachs were individually preserved in 4% buffered formalin for 24 hours, stored in 70% ethanol in marked containers, and analyzed over some months.

Prey items in each stomach were identified to group level, measured, counted and weighed on an electronic balance (precision 0.0001g). Since the copepods were the principal prey group, we paid much more attention to this group and they were identified to the lowest possible taxonomic level.

Diet composition was evaluated using three measures described by HYSLOP (1980): the numerical index (%N); the gravimetric index (%W), and frequency of occurrence (%F). Based on CORTES' (1997) suggestion, the index of relative importance (IRI) was calculated and expressed as a percentage (%IRI).

Subsequently, food items were grouped into categories of preference using the method proposed by MORATO et al. (1998). The categories were defined as follows:

$IRI \geq 30*(0.15*\sum\%O)$. . . main important prey (MIP)

$30*(0.15*\sum\%O) > IRI > 10*(0.05*\sum\%O)$ . . . . . secondary prey (SP)

$IRI \leq 10*(0.05*\sum\%O)$. . . . . occasional prey (OP)

This formula was used for the fist time by Morato in 1995 during a study on feeding habits of *Serranus atricauda* (Personal com. with Morato), but the details of this formula were not given (MORATO et al., 1998). The most commonly used index is the one proposed by HUREAU (1970): Q=(%Nx%W). HUREAU (1970) classified prey as *Preferential* (if Q>=200), Secondary (if 20<Q<200) and Accidental (Q<20). Based on these limits (let take Q>200

as an example) we can calculate the minimum value each variable may have to be classified as preferential: [SORT(200)=14.14]. So, we have assumed that in order for a prey to be classified as preferential, it has to reach at least 15% for each of the variables. Transposing this to the IRI, where IRI=(%N+%W)x%O, we have that for a prey to be classified as preferential it should have 15% of the total %N+15% of the total %Wx15% of the total %O. We know that %N and %W sum 100%, but %O may sum more than 100%. Thus, the formula can be expressed as: (0.15*100+0.15*100)*0.15*%O. The lower limit was calculated assuming 5%: [(0.05*100+0.05*100) * (0.05*∑%O) or 10*(0.05*∑%O)].

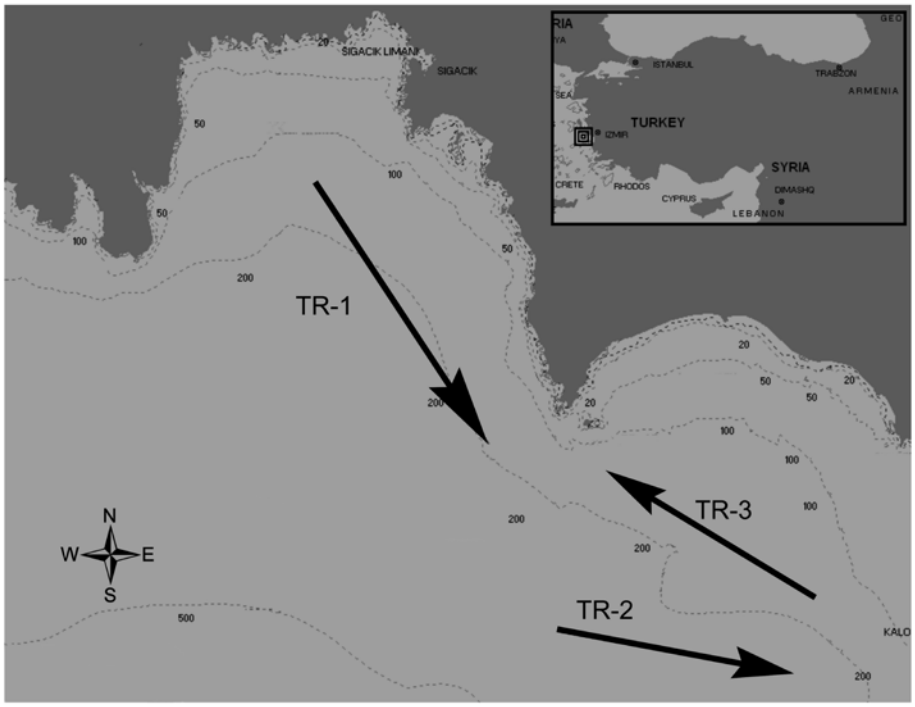

Fig. 1. – Map showing the location where sampling was carried out.

TABLE 1

Sampling locality and depths of specimens collected of *C. caelorinchus fr*om the Aegean Sea, Turkey.

Trawl No	Coordinates (trawl start)	Coordinates (trawl end)	Depth (m)
TR-1	37°88'290 N 26°42'980 E	38°00'760 N 26°49'270 E	150-180
TR-2	37°55'280 N 26°51'990 E	37°54'110 N 27°00'430 E	296-296
TR-3	37°56'286 N 27°01'215 E	37°59'754 N 26°54'075 E	145-165

RESULTS AND DISCUSSION

Of the 148 hollowsnout grenadier stomachs examined, 146 had food (98.6%) and 2 were empty (1.4%). Crustaceans were found to be most important prey group (MIP; IRI≥1196) in the diet. Polychaetes constituted the secondary prey group (SP; 1196>IRI>133), whereas chaetognathans were an occasional prey group (OP; IRI≤133). Crustaceans (especially copepods and decapods) constituted of 98.42% of the diet. Polychaetes and chaetognathans comprised 1.51% and 0.07% of the diet, respectively (Table 2).

Several studies of the diet of this species have been carried out in the north-west Atlantic (Langton & Bowman, 1980), north-east Atlantic (Mauchline & Gordon, 1984) and in the Mediterranean (Macpherson, 1979; 1981; Madurell & Cartes, 2006). Macpherson (1979) examined stomach contents of 160 specimens ranging from 5.0cm to 39.0cm TL and reported that the diet of *C. caelorhincus* consisted of polychaetes (%W=74.2) and benthic crustaceans (%W=25.8) for fish between 10.0-19.0cm TL. Macpherson (1981) also recorded both polychaetes (62.7%) and benthic crustaceans (37.3%) in the stomachs of this species. Langton & Bowman (1980) studied 11 specimens (mean fork length=19.3cm) and found that diet constituted of detritus (36.6%), polychaetes (35.8%) and crustaceans (27.6%). Finally, Madurell & Cartes (2006) examined 877 specimens (between 2.5 and 8.5cm; pre-anal length) for diet composition and stated that polychaetes (58.12% IRI) were the dominant prey for this species, followed by amphipods (19.13% IRI) and copepods (14.42% IRI). Macrourids are characteristically described as generalist feeders, with widely diversified diets (Macpherson, 1979; Mauchline & Gordon, 1984). This probably constitutes an adaptive advantage in the deep-water environments of low productivity inhabited by macrourids (Madurell & Cartes, 2006).

TABLE 2

Percent number (%N), percent weight (%W), frequency of occurrence (%F), index of relative importance (IRI) and percent index of relative importance (%IRI) calculated for each prey item found in the hollowsnout grenadier *C. caelorhincus.*

Prey Items	%N	%W	%F	IRI	%IRI
Polychaeta (larvae)	1.93	7.31	26.03	240.49	1.51
Crustacea*	97.22	92.36	95.89	15651.94	98.42
Copepoda°(pelagic)	74.71	45.54	89.04	10706.94	67.32
Calanoida	11.70	3.01	53.85	791.87	7.22
Nannocalanus minor	0.21	0.31	1.54	0.80	0.01
Calanus gracilis	0.31	0.52	1.54	1.28	0.01
Clausocalanus arcuicornis	0.10	3.36	1.54	5.33	0.05
Clausocalanus sp.	0.21	0.47	3.08	2.10	0.02
Temora stylifera	1.24	0.72	9.23	18.11	0.17
Scolecithrix bradyi	0.31	1.13	1.54	2.22	0.02
Aetideus armatus	80.54	27.11	92.31	9937.02	90.58
Pleuromamma abdominalis	0.21	4.04	3.08	13.06	0.12
Pleuromamma gracilis	0.10	0.20	1.54	0.47	0.00
Lucicutia flavicornis	0.10	0.31	1.54	0.64	0.01
Candacia aethiopica	0.10	1.29	1.54	2.14	0.02
Candacia armata	0.93	1.26	12.31	26.95	0.25
Candacia bispinosa	0.10	0.76	1.54	1.32	0.01
Candacia simplex	0.41	0.56	6.15	5.97	0.05
Candacia sp.	0.21	0.37	3.08	1.79	0.02
Acartia clausi	0.10	0.61	1.54	1.10	0.01
Acartia sp.	0.10	52.47	1.54	80.88	0.74
Cyclopoida	0.21	0.38	3.08	1.82	0.02
Oncaea media	0.10	0.59	1.54	1.07	0.01
Corycaeus typicus	2.69	0.53	23.08	74.31	0.68
Mysidacea	1.93	2.80	23.29	110.19	0.69
Amphipoda	0.23	2.05	4.11	9.36	0.06
Isopoda					
Gnathia vorax	0.54	1.08	9.59	15.50	0.10
Euphausiacea	0.54	9.28	2.75	26.92	0.17
Decapoda	18.87	30.95	95.89	4777.25	30.04
Brachyura (megalopa stage)	0.39	0.67	5.38	5.78	0.04
Chaetognatha	0.85	0.34	9.59	11.38	0.07
Sagitta spp.					

* The values calculated for all prey groups of Crustaceans and Copepods.

In contrast to our findings, the general impression of the previous studies is that hollowsnout grenadier predominantly feeds on polychaetes. In our study, however, copepoda and decapoda are the most dominant prey groups in the diet of this species. In our stomach contents analyses, pelagic copepoda, euphausiacea and chaetognatha of the holoplanktonic groups and brachyura (the megalopa stage) and polychaetes (the larval stage) of the meroplanktonic groups were found. Some benthic organisms including Amphipoda, mysidacea, isopoda and decapoda were also encountered in the stomachs of the species in our study. The pelagic groups were, however, found to be more dominant than the benthic groups in the diet of the species. Consequently, early juveniles of this species feed more on pelagic and less on benthic prey at our study site.

Aetideus armatus was found to be the dominant species of Copepoda in the diet of *C. caelorhynchus*. According to the results of the deep-sea zooplanktonic studies carried out in the Aegean Sea (Moraitou-Apostolopoulou, 1972), *Aetideus armatus* is more abundant than the other calanoid copepods. While the neritic species of copepoda such as *Temora stylifera, Acartia clause* and *Nannocalanus minor* are limited in number, the oceanic species are highly abundant (Table 2). This finding is consistent with the environment where the species lives.

The hollowsnout grenadier mouth shape has been suggested to have an effect on its feeding behaviour. It has an inferiorly positioned mouth and may forage on slow moving prey with the snout orientated towards the substrate (Madurell & Cartes, 2006). Madurell & Cartes (2006) claimed that hollowsnout grenadier has mostly a benthic diet and probably uses the rostrum to root in the sediment since infaunal organisms like polychaetes were common dietary items. As indicated above, in their study, they determined that polychaetes were the dominant prey for this species, followed by amphipods and copepods, according to the values of IRIs they computed. Although the %F value given by Madurell & Cartes (2006) for copepods was 64.5 (quite a high value in the overall stomach contents of *C. caelorhynchus*), the authors classified this group as being of unidentified habits since we know nothing about whether these are pelagic or benthic copepods. On the other hand, ontogenetic migrations of the species toward deep waters (Moranta et al., 1998; Labropoulou & Papaoconstantinou, 2000; Madurell et al.,

2004) have been well documented, i.e., smaller individuals reside in shallower waters (<400m) and larger individuals in deeper waters (>500m). Consequently, given the low occurrence of benthic organisms and the high occurrence of pelagic organisms in the stomachs of fish in our study may indicate ontogenetically based food preferences of *C. caelorhynchus* in the Aegean Sea.

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Un-paint it black: Avian prey as a component of the diet of nestling Hooded Crows *Corvus cornix*

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ABSTRACT. The Hooded Crow (*Corvus cornix*) is often considered the main nest predator of many bird species, especially waterbirds. Such relationship should be particularly clear during the breeding season in wetlands, when the need to supply their nestlings with the highest quality foods forces predators to intensify their hunting activities. Hence, waterbirds should be their basic prey. We examined the composition of the diet of nestling Hooded Crows in the flooded river valley of the "Ujście Warty" National Park in western Poland, which is a bird refuge of international importance and provides nesting habitat for numerous bird species. Despite the richness of potential avian prey, the dominant components of Hooded Crow nestlings' diet were insects, fish and plants. Contrary to expectations, birds were only supplementary to the diet of nestlings, and thus, we suggest that crows are likely to have only a marginal influence on nest failures of potential avian prey species in regions similar to the studied area.

KEY WORDS : *Corvus cornix*, diet, Hooded Crow, nestlings' food, protected areas

INTRODUCTION

The Hooded Crow *Corvus cornix* is a dietary generalist, consuming a wide spectrum of foods of both animal and vegetable origin (Cramp, 1998). Invertebrate prey includes molluscs (Berrow, 1991; Berrow et al., 1991; Berrow et al., 1992 a; b) as well as crustaceans, insects, arachnids and a number of other groups (Deckert, 1980; Ewins, 1986; Massei & Genov, 1995; Cramp, 1998; Horgan & Berrow, 2004). Moreover, Hooded Crows are known to feed on carrion (Picozzi, 1975, author's observations) and prey on a number of vertebrate taxa (Stahl, 1985; Cramp, 1998; Vorgin & Vorgin, 1998; Najbar, 2001; Krivosheev, 2004). There are individual reports of crows catching birds in flight, e.g. the Common Swift (*Apus apus*) (Camolese et al., 2003) and the European Starling (*Sturnus vulgaris*) (Edholm, 1979; Gagsch, 1980) and studies considering the Hooded Crow as a nest predator and demonstrating its influence on the clutch success of numerous bird species (mostly waterbirds) are prevalent (e.g. Witkowski, 1983; Cadiou, 1999; Grant et al., 1999; Green & Yurlov, 1999; Volponi, 1999; Opermanis et al., 2001). For this reason, the idea that birds constitute a basic food item of the Hooded Crow is a predominant one. However, most relevant data provide only indirect information, derived from nest visits on selected bird species. Additionally, such visits seriously increase the risk of nest predation (Major, 1990; Tryjanowski & Kuźniak, 1999). Other data concerning hunting behaviours of the Hooded Crow result from experiments with the use of artificial nests (Göransson et al., 1975; Loman & Göransson, 1978; Sonerud & Fjeld, 1985; Fjeld & Sonerud, 1988), and this approach is also known to be laden with potential biases (e.g. Pärt & Wretenberg, 2002; Zanette, 2002). Except for one study from the Danube Delta (Kiss et al., 1977) using destructive methods (analysis of stomach contents of dead birds), there are no direct, representative data related to the diet of the Hooded Crow on wetlands, the primary biotope of this species (Tomiałojć & Stawarczyk, 2003). Moreover, there are no studies using non-destructive methods to determine the diet of nestling crows. Such data would be indispensable for determining actual relationships between this predator and its potential prey during the breeding season.

In this paper we present the results of a study on the composition of the diet of nestling Hooded Crows. The research was carried out in the lowland of a permanently flooded river estuary, abundant in potential avian prey species of this predator. Therefore, we paid special attention to the contribution of birds in the diet of this species. Furthermore, we demonstrated the qualitative and quantitative contribution of all food components.

MATERIALS AND METHODS

The study was carried out in the "Ujście Warty" National Park (52°36'N, 14°47'E) in Western Poland, on the Warta River at its estuary into the Odra River. This area is under protection of the RAMSAR convention. Moreover, this area is a wildfowl refuge of international importance (Grimmet & Jones, 1989), where approximately 160 bird species breed (Bartoszewicz et al., 2000). Exemplary mean breeding pair densities of potential avian prey for crows in the study area are: Common Coot (*Fulica atra*), 50.7 pairs/km^2 (SE=20.0), Black-Headed Gull (*Larus ridibundus*), 85.1±14.7, Mallard

(*Anas platyrhynchos*), 4.6±0.9, and Garganey (*Anas querquedula*), 0.9±0.4 (unpublished data from years 1995-1999 and 2003 from yearly monitoring reports, see also BARTOSZEWICZ et al., 2000). Breeding density of Hooded Crows in the study area was 2.9±0.2 pairs/km^2 (ZDUNIAK, 2006).

The study site (16km^2) is located in the western, periodically flooded part of the Park. It is covered by a mosaic of herbaceous vegetation, dominated by the Reed-Canary Grass (*Phalaris arundinacea*) and arborescent vegetation, consisting exclusively of mature willows (*Salix* sp.) and willow shrubs. Additionally, in this area there are shallow lakes, old river-beds, ditches and dikes. The characteristic feature of the Park is its highly changeable and unpredictable water-table (for details about the study area see: CHMIEL et al., 2000; CHOIŃSKI, 2000).

During the breeding season (April-June) of 2003, 82 samples of food were taken from 38 nestlings in 15 nests. Samples were taken with the use of tartar emetic (for details see ZDUNIAK, 2005). Briefly, this involved the oral administration of a 1.5% solution of antimony potassium tartrate through a flexible plastic tube attached to a syringe. After administration of emetic, birds were placed on a foil in a warm and quiet place and observed for the vomiting reflexes.

Samples were collected 4 times from 8 nestlings (3 nests), 3 times from 8 nestlings (3 nests), twice from 4 nestlings (2 nests) and once from 18 nestlings (7 nests).

Food samples were analysed under a binocular scope and their contents were divided into the following categories: a) plants; b) insects (chitin, and in some cases whole parts of the body containing soft tissues); c) fish (fish bones, scales, parts of the cranium); d) amphibians (bones); e) birds (feathers, bones); f) bird eggs (eggshells, predominantly of the Common Coot *Fulica atra*); g) molluscs (parts of shells); h) crayfish (parts of limbs and carapace); i) undetermined material (partly digested soft tissues and fine fragments of bones and shells); j) others (leeches, mammal bones, stones). The material was defined according to appropriate guides (ADOLPH, 1927; GĄSOWSKA, 1962; FERENS, 1967; PUCEK, 1984), dried and weighed with digital scales to an accuracy of 0.001g.

We considered food samples from the same nest on different days as independent observations. This approach to the data analysis results from the fact that in the study area the access to different sources of crows' food is changing during the period of nestling feeding, which in turn is connected with changeable water conditions. Hence, we assumed that the composition of the diet of nestling Hooded Crows in one nest varies from day to day. Also in the study of BERROW et al. (1992a) the diet of Hooded Crows changed during the breeding season. Consequently, the diet of nestlings from the same nest during one nest visit was treated as one collective sample.

All calculations were conducted using STATISTICA for Windows package (StatSoft Inc., 2005). Data are presented as means with 95% confidence limits (95% CL).

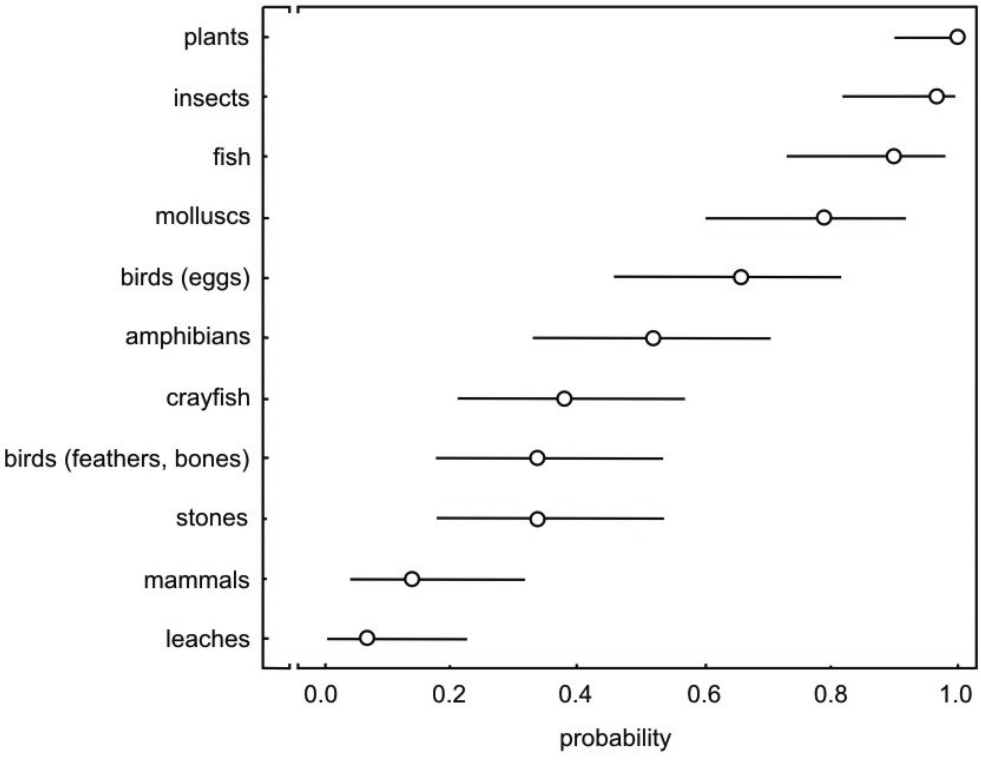

Fig. 1. – Probability of finding a particular diet component of nestling's food in a sample (n=29). Means are given with 95% CL.

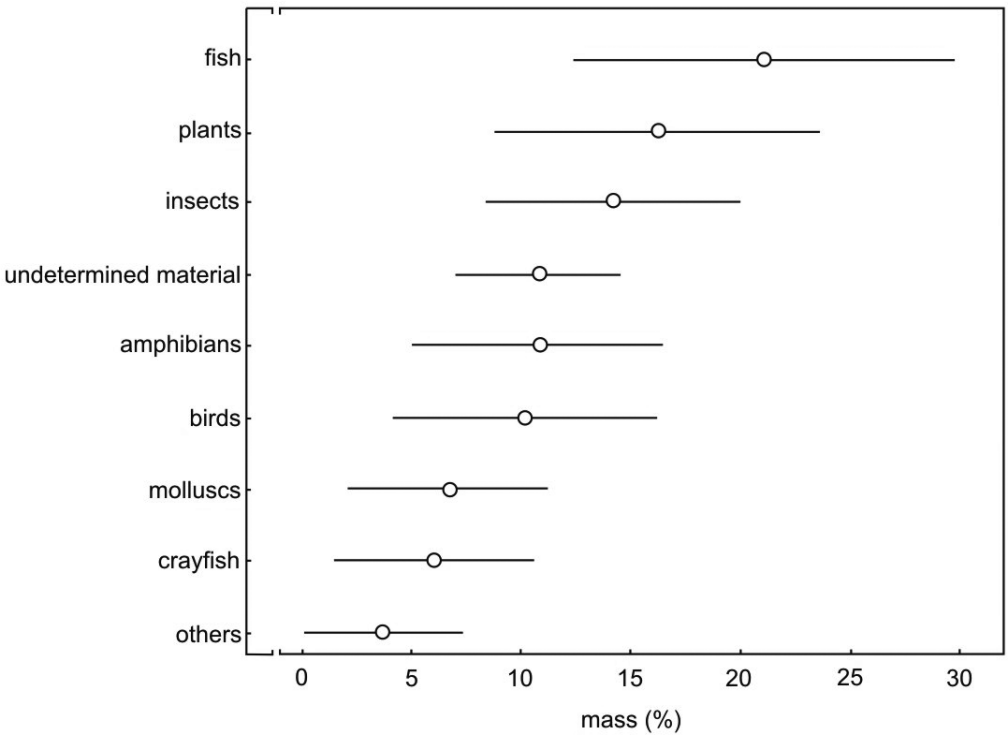

Fig. 2. – Contribution of components of nestling's diet in the dry mass of the samples (n=29). Means are given with 95% CL.

RESULTS

The greatest frequency of occurrence of particular diet component in a sample of nestling's food (n=29) was noted for plants (p=1.00, CL: 0.90-1.00) and insects (0.97, 0.82-1.00), followed by fish (0.90, 0.73-0.98) and molluscs (0.79, 0.60-0.92). Remaining components were far rarer (Fig. 1). Among these, eggshells were found with a frequency of 0.66 (0.46-0.82) and other avian remains (bones and feathers, mainly of Mallard chicks) with a frequency of 0.34 (0.18-0.54).

The contribution of particular components in individual dry mass samples (n=29) was very diverse (Friedman's ANOVA rang test, χ^2=41.54, df=8, p<0.001). Greatest mean contributions were noted for fish remains ($\bar{x}$=21.1%, CL: 12.3%-29.9%), plants ($\bar{x}$=16.3%, 8.8%-23.8%) and insects ($\bar{x}$=14.3%, 8.4%-20.1%). Other components had a lower contribution to the diet (Fig. 2), and all avian remains (eggshells, feathers, bones) constituted on average 10.2% (4.2%-16.3%) of the dry mass samples.

DISCUSSION

The results presented indicate that the diet of nestling Hooded Crows in flooded wetland habitats is very diverse. Even though the study area provides nesting habitat for numerous avian species that represent potential prey of the Hooded Crow (mainly Common Coot and Mallard; ZDUNIAK, 2006), bird remains found in the samples had a relatively low frequency of occurrence. The fact that birds are only a supplement in the crow nestlings' diet is also confirmed by the low contribution of avian remains in the dry mass of the samples.

Results obtained by other authors in areas less abundant in potential avian prey species also indicate a low contribution of bird remains in the Hooded Crow's diet. At sites located on the sea shore in Ireland (BERROW et al., 1992a) insects along with crustaceans, snails and bivalves were most common in pellets and stomachs of adult crows throughout the year**.** The frequency of avian remains in pellets was up to 6% representing less than 5% of the total dry mass of pellets. In contrast, avian remains were not found in the stomachs of any birds sampled at this time. During the breeding season, the volume of insects in the pellets varied between 10% and 20%, while remains of all vertebrates constituted between 7% and 20% of the samples by volume. Unfortunately, the authors do not give any details about the contribution of avian prey to the diet of crows.

According to PICOZZI (1975) insects were very frequent in the stomachs of adult crows during the breeding season (ranging from 18% to 80% of stomachs analysed). The percentage of samples with eggshells and feathers ranged from 4% to 10% and from 0% to 4% in the breeding season. In the agricultural landscape of Germany (DECKERT, 1980) insects was the main food of the Hooded Crow, whereas remains of birds or eggs constituted 0%-7% of stomach and pellet contents. The dominance of insects in the diet of the Hooded Crow was also reported by HORGAN & BERROW (2004), and HOUSTON (1977) considers

insects as a good source of protein for nestling crows. Results obtained in the Danube Delta (KISS et al., 1977), the only study conducted on a large river estuary rich in potential avian prey species, also confirm the low significance of birds as food for the Hooded Crow. Avian remains were found in 15.6% of the analysed stomachs, and major components of the diet were plants followed by fish, insects and small mammals. On the other hand TENOVUO (1963), reports that a blend of bird's eggs and insects constituted 80% of the volume of stomach contents in nestling Hooded Crows at sites located on the sea shore in Finland.

Our study showed that despite the richness of potential avian prey, birds are only supplementary to the Hooded Crow nestlings' diet during the critical breeding season. A previous study based on egg shells eaten by the Hooded Crow (ZDUNIAK, 2006) showed that, in the same study area, this nest predator is opportunistic and concentrates on the most abundant and most commonly available avian prey species. Therefore, our findings indicate that the influence of crows on the nests failures of avian prey species in regions similar to the study area should be considered marginal.

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Allometry of external morphology and sexual dimorphism in the red porgy *(Pagrus pagrus)*

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ABSTRACT. Body weight (W), nine morphometric variables and total length (TL) were estimated for each sex in the protogynous red porgy, (*Pagrus pagrus)* reared in captivity. The power function $Y=aX^b$ was used to evaluate growth-related changes in morphology. The first derivative, $dY/dTL=abTL^{b-1}$, where Y is each morphometric character, was used to study the growth pattern of each character relative to growth in length (TL). The length-weight relationship showed isometric growth and no inter-sex variability. The analysis of the morphometric variables demonstrated the existence of a substantial degree of differences between the sexes. Males showed an isometric growth pattern (I) for head height and an accelerated (+A) or retarded (-A) growth pattern for the pre-orbital and post-orbital distance, respectively. However, females showed a retarded (-A) growth rate of the head height and an isometric growth pattern (I) for pre-orbital and post-orbital distances. Thus, the male red porgy appears to have a taller head compared to females. Additionally, the head in males is wider and the orbits are positioned more posteriorly in the head. The adaptive significance of these differences remains currently unclear but should be studied in relation to protogynous hermaphroditism and the specific features of male biology in the red porgy.

KEY WORDS : *Pagrus*, porgy, morphometry, allometry, sexual dimorphism

INTRODUCTION

The red porgy (*Pagrus pagrus*, L., 1758) is a marine fish of great economic importance in both the Mediterranean Sea and the Atlantic Ocean (Vassilopoulou & Papaconstantinou, 1992; Harris & McGovern, 1997). Due to its wide geographical distribution, high market demand and good growth rates (Pajuelo & Lorenzo, 1996; Maragoudaki et al., 1999; Fostier et al., 2000) there is a strong interest in breeding this species commercially (Kolios et al., 1997; Bodington, 2000). Thus, it is considered as a new candidate species for the diversification efforts of the Mediterranean aquaculture.

The red porgy is a protogynous hermaphroditic species. In captivity, it reaches sexual maturity at the age of 3-4 years (Kokokiris et al., 1999; 2001). Depending on latitude, spawning occurs from February to mid-June (Pajuelo & Lorenzo, 1996; Kokokiris et al., 2001). Although some immature individuals develop testicular tissue and function as males throughout their life (primary males), the majority of males derive from the sex change of adult females after sexual maturation (Kokokiris et al., 1999). Adult females cannot be distinguished from males in view of their external morphology. Although there is an interest for external morphometric characters to distinguish sexes, (mainly for aquacultural purposes), the allometry of its external morphology has not been studied so far. The present study examines the allometric growth pattern of a number of morphometric characters searching for any dimorphism useful to distinguish sexes.

MATERIALS AND METHODS

Fish were caught by bottom trawls at Heraklion Bay (Crete, Greece) and were acclimatized to rearing conditions in outdoor tanks ($10m^3$) under natural photoperiod and water temperature (National Center of Marine Research). Fish were fed with commercial pellets (sea bream pellets, Biomar), provided by self-feeders (Kokokiris et al., 1999; 2001).

Sampling was carried out five times throughout the year (April, May, July, August and September). Two hundred ninety five (295) individuals were examined for morphometric analysis (0.09-2.5Kg, total body weight, 17.5-49cm, total length). Fish were killed with an overdose of 2-phenoxyethanol. Total length (TL) and nine other morphometric variables were measured with a caliper to the nearest 0.1mm. Abbreviations of the morphometric variables measured and their indications on the fish body are given in Table 1 and Fig. 1.

After measuring, the fish were dissected and the gonads removed. A tissue sample from the middle of the right gonad was fixed in Bouin solution, prepared for analysis using routine histological techniques (sections of 4-6μm stained with Harris haematoxylin and eosin) and observed under light microscopy for sex identification (Kokokiris et al., 1999; 2001).

The structure of the sampled population according to the length of females and males is presented in Fig. 2. The TL frequency distribution and mean TL values differed significantly between females and males (t-test, $P<0.05$). In order to meet the assumptions necessary to carry out the comparisons of the morphometric variables between sexes (same TL frequency distribution and same size

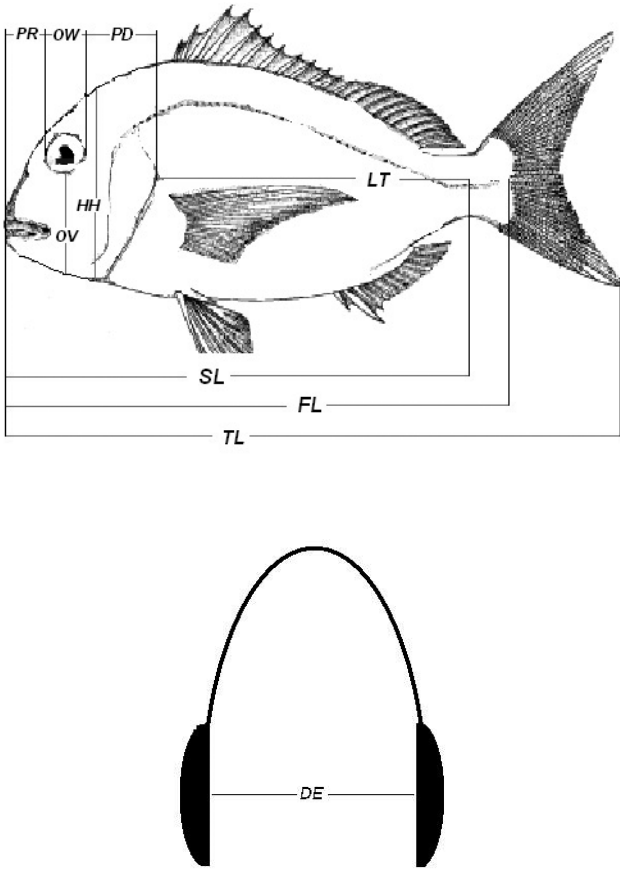

Fig. 1. – Identification of the different morphometric characters on the fish body.

TABLE 1

Morphometric measurements made on *P. pagrus*. Abbreviations and names of variables are those used in text.

Term	Description
Head width	Upper distance between the tip of the orbits
Fork length	Distance from the tip of the nose to the middle part of the caudal fin
Head height	Distance from the lower end to the upper end of the head
Trunk length	Distance from the tip of the posterior margin of the opercula to the end of the vertebral column
Vertical position of the orbit	Distance from the tip of orbit to the lower end of the jaw
Orbit width	Diameter of the orbit
Post-orbital distance	Distance from the end of the orbit to the posterior margin of the opercula.
Pre-orbital distance	Distance from the tip of the nose to the front end of the orbit
Standard length	Distance from the tip of the nose to the end of the vertebral column
Total length	Distance from the tip of the nose to the longest caudal fin ray

DE: Head length, FL: Fork length, HH: Head height, LT: Trunk length, OV: Vertical position of the orbit, OW: Orbit width, PD: Post-orbital distance, PR: Pre-orbital distance, SL: Standard length, TL: Total length.

range), individuals were taken randomly from each 5cm TL group of females and males, within the TL size range from 25 to 50cm (MINOS et al., 1995). TL values were tested for normality and homogeneity of variances and then frequency distributions and mean values were compared between sexes by means of Kolmogorov-Smirnov and t-test, respectively (ZAR, 1999). This procedure was repeated until TL mean values were similar (t-test, $P>0.05$) between sexes (Fig. 3, MINOS et al., 1995; TIDU et al., 2004). Finally, one hundred seven females (107) and fourty eight males (48) were used for the morphometric analysis.

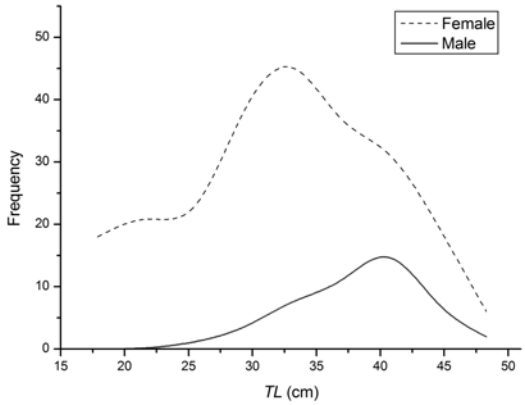

Fig. 2. – Total length frequency distributions of male and female individuals of *P. pagrus*.

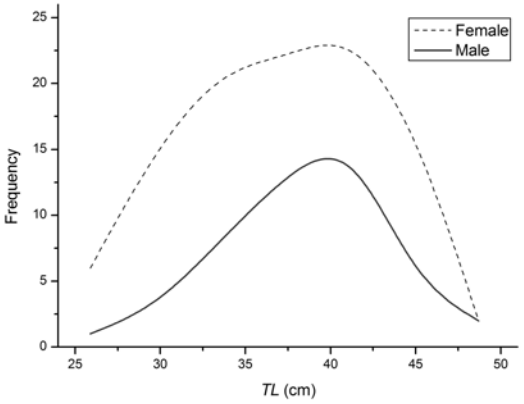

Fig. 3. – Total length frequency distributions of male and female individuals of *P. pagrus* from the selected specimen per total length class size.

The allometric growth (differential increase) of each variable relative to TL was calculated from the function $Y=aTL^b$ using the first derivative with respect to the total length $dY/dTL=axbxTL^{b-1}$ where a and b are constants and Y is the morphometric variable (MINOS et al., 1995). Data were $\log_{10}$ transformed and least-square regression analysis was applied to calculate the parameters of the allometric equation of each variable versus TL. The significance of the slope was tested by means of a t-test (ZAR, 1999). The morphometric variables were then divided into three categories: positive allometry (+A),

when the slope (b, allometry coefficient) was significantly higher than 1 and the variable increased relatively to TL; negative allometry (-A), when the slope was significantly lower than 1 and the variable decreased relative to TL; and isometry (I) when the slope showed a non significant difference from 1, indicating direct proportionality between the variable and TL.

The relationship between the length and the weight was estimated using also the equation $W=aTL^b$, where W is the body weight in grams (g). The W was plotted against TL and a least-square regression analysis was applied to calculate the coefficients a and b. The significance of the slope was tested by comparing the slope with the expected slope of 3.0 under isometric growth using a t-test (ZAR, 1999).

To examine differences in morphometric dimensions between females and males, the regression slopes of each variable versus TL were tested by means of t-tests (MINOS et al., 1995; BRACCINI & CHIARAMONTE, 2002).

RESULTS

Weight versus total length

Regression analysis showed that the slope (b) was not significantly different from 3.0 (t-test, P>0.05), in both females and males, indicating an isometric growth pattern for both sexes ($b_{Females}$=2.958, b_{Males}=2.989, Table 4).

Morphometric variables versus total Length (TL)

The parameters of the equation of each morphometric variable versus total length (TL) of females and males are presented in Table 2. In females, three dimensions (trunk length, vertical position of the orbit, standard length) revealed a positive allometric relationship (+A, t-test, P<0.05) and three (head width, orbit width, head height) had a negative allometric relationship (-A, t-test, P<0.05) (Table 2). Three dimensions (fork length, pre-orbital distance, post-orbital distance) had an isometric relationship (I) with TL (P>0.05).

In males, two dimensions (trunk length, pre-orbital distance) had a +A relationship and two dimensions (orbit width, post-orbital distance) a –A relationship (t-test, P<0.05). Five dimensions (head width, fork length, head height, vertical position of the orbit, standard length) increased isometrically with TL (P>0.05, Table 2).

Regression analysis (comparison of slopes between sexes) revealed that six morphometric characters, (standard length, fork length, trunk length, post-orbital distance, pre-orbital distance and head height) were significantly different between females and males (t-test, P<0.05, Table 3). The non-dimorphic measurements were head height, orbit width and vertical position of the orbit. Especially, the dimensions measured on the head (head height, pre-orbital distance, post-orbital distance) had not only different growth patterns between sexes, but head height and pre-orbital distance had a higher rate of change during growth in males and only post-orbital distance in females. Body dimensions including trunk length and standard length had a higher rate of change in females but the fork length dimension had a higher growth rate in males (Table 3).

TABLE 2

Ontogenetic changes in morphometric measurements (see Table 1) for female and male red porgy. Values given are from the equation $Y=aTL^b$. a, b, parameters of the equation; r^2, coefficient of determination; SE_b, standard error of b; SE_e, standard error of estimation; * as P< 0.05. Slope patterns are: +A, positive allometry; –A, negative allometry; I, isometry.

Dimension	a	b	r^2	SE_b	SE_e	Slope (b)
Females						
DE	0.114	0.911	0.96	0.021	0.031	**-A ***
FL	0.940	0.976	0.96	0.021	0.033	**I**
HH	0.372	0.903	0.95	0.020	0.033	**-A***
T	0.354	1.119	0.98	0.014	0.023	**+A ***
OV	0.089	1.068	0.95	0.025	0.041	**+A ***
OW	0.335	0.543	0.86	0.013	0.052	**-A ***
PD	0.098	0.974	0.92	0.028	0.045	**I**
SL	0.727	1.028	1.00	0.007	0.012	**+A ***
PR	0.103	1.015	0.94	0.024	0.039	
Males						
DE	0.099	0.948	0.93	0.044	0.027	**I**
FL	0.733	1.042	0.95	0.034	0.031	**I**
HH	0.204	1.068	0.82	0.073	0.066	**I**
LT	0.409	1.077	0.98	0.024	0.022	**+A ***
OV	0.114	1.001	0.92	0.042	0.038	**I**
OW	0.301	0.573	0.68	0.058	0.052	**-A ***
PD	0.355	0.622	0.66	0.066	0.060	**-A ***
PR	0.065	1.140	0.88	0.062	0.056	**+A ***
SL	0.835	0.989	0.99	0.015	0.013	**I**

TABLE 3

Regression slopes and t-test analysis of sexual differences in morphometric measurements (see Table 1). P, statistical difference (α=0.05).

Dimension	Females	Males	P
	Equation	Equation	
DE	$Y=0.114\ TL^{0.911}$	$Y=0.099\ TL^{0.948}$	>0.05
FL	$Y=0.940\ TL^{0.976}$	$Y=0.733\ TL^{1.042}$	<0.05
HH	$Y=0.372\ TL^{0.903}$	$Y=0.204\ TL^{1.068}$	<0.05
LT	$Y=0.354\ TL^{1.119}$	$Y=0.409\ TL^{1.077}$	<0.05
OV	$Y=0.089\ TL^{1.068}$	$Y=0.114\ TL^{1.001}$	>0.05
OW	$Y=0.335\ TL^{0.543}$	$Y=0.301\ TL^{0.573}$	>0.05
PD	$Y=0.098\ TL^{0.974}$	$Y=0.355\ TL^{0.622}$	<0.05
PR	$Y=0.103\ TL^{1.015}$	$Y=0.065\ TL^{1.140}$	<0.05
SL	$Y=0.727\ TL^{1.028}$	$Y=0.835\ TL^{0.989}$	<0.05

DE: Head length, FL: Fork length, HH: Head height, LT: Trunk length, OV: Vertical position of the orbit, OW: Orbit width, PD: Post-orbital distance, PR: Pre-orbital distance, SL: Standard length, TL: Total length.

TABLE 4

Parameters of the length-weight relationship ($W=aL^b$) between body weight (g) and total length (cm) of *P. pagrus* at various regions in Mediterranean and adjusted areas or rearing conditions. n, sample size; min and max, minimum and maximum length (cm); a and b, parameters of the equation; SE_b, standard error of b; r^2, coefficient of determination; M, male; F, female; A, all individuals, * Length measured was Fork Length; P: statistical significance.

Location	Author	Sex	n	Length		A	b	SE_b	r^2	P
				Ìin	Ìax					
Canary Islands, Eastern Atlantic	Pajuelo & Lorentzo (1996)	F	758	16.8	56.2	0.0132	3.032	0.026	0.98	>0.05
		M	230	22.8	57.2	0.0133	3.043	0.018	0.98	<0.05
		A	1858	4.7	57.2	0.0179	2.958	0.023	0.99	>0.05
South-West coast of Portugal.	Concalves et al. (1997)	A	23	13.5	36.2	$3.5x10^{-5}$	2.866	0.077	0.98	>0.05
Eastern Adriatic (Croatian waters)	Dulcic & Kraljevic (1996)	A				$5.3x10^{-6}$	3.343	0.127	0.98	<0.05
Western Mediterranean	Morey et al. (2003)	A	127	4.8	33.4	0.0282	2.800	0.248	0.95	>0.05
Aegean Sea, Eastern Mediterranean	Moutopoulos & Stergiou (2002)	A	35	13.0	51.7	0.0152	3.005	0.067	0.98	>0.05
Kastelorizo island, Eastern Mediterranean	Vassilopoulou (1989)	F	95	13.3*	41.8*	$1.9x10^{-5}$	3.020		0.98	
		M	23	13.3*	41.8*	$3.4x10^{-5}$	2.920		0.99	
		A	142	13.3*	41.8*	$2.4x10^{-5}$	2.980		0.98	
Kastelorizo island, Eastern Mediterranean	Vassilopoulou & Papaconstantinou (1992)	F		10*	46*	$3.3x10^{-5}$	2.928		0.98	
		M		10*	46*	$4x10^{-5}$	2.897		0.99	
Crete island, Eastern Mediterranean	Machias et al. (1998)	A	1817	11*	37*	0.020	3.105		0.99	
Crete island, Eastern Mediterranean, Reared populations	Machias et al. (1998)	A	1142	8.5*	39.5*	0.016	3.205		0.99	
Crete island, Eastern Mediterranean, Reared populations	Present study	F	107	25.5	48.4	0.0239	2.958	0.049	0.97	>0.05
		M	48	25.8	49	0.0206	2.989	0.135	0.93	>0.05

DISCUSSION

The relationship of body weight versus length showed an isometric growth pattern for both female and male red porgy. Similarly to this study, an isometric growth pattern has been also reported for the wild populations in various geographic areas where this species has been studied (see Table 4).

The analysis of morphometric variables demonstrated a substantial degree of differences between the sexes concerning either the growth pattern or the rate of change of some cranial variables. Males showed an isometric (I) growth pattern for head height (HH) and an accelerated (+A) or retarded (-A) growth pattern for the pre-orbital (PR) and post-orbital (PD) distance respectively. However, females showed a retarded (-A) growth rate of the head height (HH) and an isometric growth pattern for the pre-orbital (PR) and post-orbital (PD) distances (length of the head, I). These results indicate a sexual dimorphism of the skull. Males tend to have a taller head, and a longer pre-orbital area than females. Significant changes in the morphology of the head have also been reported in the red snapper, *Chrysophrys auratus* and the redbanded porgy, *Pagrus auriga*. In the red snapper, as individuals age, a large hump grows on their forehead, apparent in both sexes but more prominently so in males. The formation of the hump is due to the enlargement of particular areas of supraoccipital and frontal bones of the skull (hyperostosis, Gauldie & Czochanska, 1990; Smith-Vaniz et al., 1995) but the cause of its presence is still unclear. It has recently been suggested that it possibly has a genetic basis (Smith-Vaniz et al., 1995). Also, in males of this species, the snout may become fleshy and pronounced, sometimes with another distinct hump or bump. Similarly, the adults of the redbanded porgy (*Pagrus auriga)* have a slight hump above eyes and the snout becomes more bulbous (Fisheries Global Information System, 2004).

Due to protogyny of the red porgy, male individuals derive from females after a sex change (Kokokiris et al., 1999). The dimorphic characters in *P. pagrus* may reflect the adaptation of males and females to different social or/and reproductive roles rather than different niche utilization as both sexes were grown under the same artificial environment (rearing conditions). They are possibly related to protogynous hermaphroditism and to behavioral or/and social (demographic) changes of the social system, which induce the sex change process in hermaphroditic species (Shapiro, 1992). However, further research is required in order to identify any relationship between the sex change of females and the sexual dimorphism in this species.

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Geographic variation of the Perny's Long-nosed squirrels (*Dremomys pernyi*) (Milne-Edwards, 1867) (Rodentia: Sciuridae) from southwestern China based on cranial morphometric variables

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ABSTRACT. A sample of 114 specimens of *Dremomys pernyi* was investigated, 73 of which had intact skulls and were subjected to multivariate, coefficient of difference (C. D.), and cluster analyses. Results indicate that 4 subspecies (groups) of *Dremomys pernyi* inhabit southwestern China: *D. p. pernyi* in the center and western Sichuan province and central and northwestern Yunnan province ; *D. p. flavior* in the areas ranging from the southwestern Guangxi province, southwestern Guizhou province to the southern Yunnan province (east of Honghe River) ; *D. p. howelli* in the western Yunnan province (west of Nujiang River) ; and one new group (*D. pernyi* Wuliangshan group) in the Wuliangshan areas (between the Honghe River and the Lancangjiang River), Jingdong, Yunnan province. The results also probe into the relationships between subspecies differentiation of *D. pernyi* and the geographic structure and evolution in southwestern China.

KEY WORDS : Geographic variation, Subspecies, Numerical analysis, *Dremomys pernyi*, Morphometry

INTRODUCTION

Perny's Long-nosed squirrel *Dremomys pernyi* (Milne-Edwards, 1867) mainly lives in southern China (including Tibet and Taiwan), but also occurs in northern Vietnam, northern Myanmar, and parts of northeastern India.

With regard to subspecies differentiation, ALLEN (1940) listed 6 subspecies: *D. p. pernyi*, *D. p. flavior* (Allen, 1912), *D. p. howelli* (Thomas, 1922), *D. p. senex* (Allen, 1912), *D. p. modestus* (Thomas, 1916), and *D. p. calidior* (Thomas, 1916) ; ELLERMAN (1940) added 5 more subspecies: *D. p. griselda* (Thomas, 1916), *D. p. chintalis* (Thomas, 1916), *D. p. lichiensis* (Thomas, 1922), *D. p. mentosus* (Thomas, 1922), and *D. p. imus* (Thomas, 1922) ; ELLERMAN & MORRISON-SCOTT (1950) recognized only 3 subspecies: *D. p. pernyi*, *D. p. imus*, and *D. p. owstoni* (Thomas, 1908) ; MOORE & TATE (1965) indicated that there were 6 subspecies, comparable with the former results. They argued that *D. p. griselda*, *D. p. lichiensis*, and *D. rufigenis lentus* (A.B. Howell, 1927) were synonyms of *D. p. pernyi*, *D. p. mentosus* and *D. p. imus* were synonyms of *D. p. howelli*, *D. p. modestus* was a synonym of *D. p. senex*, *D. p. chintalis* was a synonym of *D. p. calidior*, and agreed on *D. p. owstoni* and *D. p. flavior* as being valid subspecies ; CORBET & HILL (1992) only listed all of subspecies names but provided no further discussion.

Clearly, there has been disagreement as to the subspecies differentiation of *D. pernyi*, and also the geographic variation of the species has been studied insufficiently. Therefore, based on the analysis of cranial morphometric variables, we attempted a more detailed analysis of the geographic variation of *D. pernyi* in southwestern China. Additionally, we discuss the relationships between geographic variation in *D. pernyi* and the geological changes in this area.

MATERIALS AND METHODS

Data collection

This study was conducted at Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ, CAS) (Kunming, China), and based on the mammal collections of the Museum of Vertebrates of KIZ, CAS, and the Institute of Zoology, Chinese Academy of Sciences (IOZ, CAS) (Beijing, China). Numbers and collection localities of specimens that were examined in the study are listed in the Appendix.

A series of 114 specimens were studied, 73 of which had intact skulls and were suitable for quantitative analyses. Specimens that were used in the study were all adults. Four external measurements ; head and body length (HB), tail length (TL), hind foot length (HFL) and ear length (EL) were recorded from original labels attached to skins. Since these measurements may show considerable inter-observer variation, they were not included in our analyses. Skull variables were recorded as described previously by MUSSER (1979), MUSSER & HEANEY (1992). All 15 cranial measurements were taken with a digital caliper to its greatest accuracy (0.01mm): greatest length of skull (GLS) ; condylobasal length (CBL) ; basal length (BL) ; rostral length (ROL) ; upper tooth row (UTR) ; the first upper molar length (FUML) ; palatal length (PL), the distance from the anterior edge of the premaxillary to the posterior edge of the palatine ; postpalatal length (PPL), the distance from the posterior edge of the palatal to the anterior edge of the foramen magnum ; interorbital breadth (IOB) ; zygomatic breadth (ZOB) ; length of diastema (LDS) ; breadth of incisive foramen (BIF) ; length of incisive foramen (LIF) ; lower tooth row (LTR) ; height of Mandibular (HM).

Data analysis

Based on the 15 variables described above, principal component analyses (PCA) were conducted to highlight differences in skull shape between the samples. This technique combines the variables to show the maximum variation between individuals without assuming prior grouping based on putative subspecies identification. The coefficient of difference between groups (C. D.; MAYR, 1969) was calculated using the following equation: C. D.=$(M_b-M_a)/(SD_a+SD_b)$; where M_b is the mean of population b, M_a is the mean of population a, SD_a is the standard deviation of population a, and SD_b is the standard deviation of population b.

Squared Euclidean distances between the group centroids were calculated using Mahalanobis distances and were subjected to cluster analysis by Hierarchical Cluster methods. Statistical analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA).

Because no holotypes could be examined in this study, the different subspecies were identified based on their collection sites. The type specimen of *D. p. pernyi* was from Moupin, Sichuan, China, collected by Perny and *D. p. pernyi* mainly occurs in western Sichuan; the type specimen of *D. p. howelli* was from S. W. of Tengyueh, extreme western Yunnan, China, collected June 4, 1912, by E. B. Howell; the type specimen of *D. p. flavior* was from Mongtz, southeastern Yunnan, China, collected in 1911 by H. Orii (MOORE & TATE, 1965).

In order to establish if there is sexual dimorphism in *D. pernyi*, an analysis on both sexes was first performed.

RESULTS

Test on sexual dimorphism in *D. pernyi*

68 out of 73 samples had information concerning their sex, and they were classified into a male (38 specimens) and a female group (30 specimens). Tests of Equality of Group Means were performed, and the results indicated that none of skull variables showed significant differences between males and females (Table 1). In other words, there is no sexual dimorphism in these 15 cranial dimensions in *D. pernyi*.

TABLE 1

Tests of Equality of Group Means for males and females. Variable codes are given in the text

Variables	Wilks' Lambda	F	df1	df2	Sig.
GLS	1.000	0.016	1	66	0.898
CBL	0.999	0.076	1	66	0.898
BL	0.999	0.068	1	66	0.795
ROL	1.000	0.027	1	66	0.871
UTR	0.998	0.105	1	66	0.747
FUML	0.990	0.648	1	66	0.424
PL	0.996	0.234	1	66	0.630
PPL	0.999	0.084	1	66	0.898
IOB	0.999	0.096	1	66	0.757
ZOB	1.000	0.021	1	66	0.887
LDS	1.000	0.023	1	66	0.879
BIF	0.987	0.883	1	66	0.351
LIF	0.998	0.102	1	66	0.751
LTR	0.994	0.400	1	66	0.529
HM	1.000	0.007	1	66	0.933

Sig. level<0.05

Multivariate analysis

As noted above, 73 individuals with intact skulls were available that could be assessed by principal component analyses (PCA). Eigenvalues for the first three principal components were 11.17, 1.06 and 0.94 respectively, accounting for 87.79% of the total variance. Most characteristics had high positive loadings on the first principal component, suggesting that this component (74.45% of the total variance) represents size variation in the sample, with specimens increasing in size from left to right. The second principal component (7.09% of variance) is strongly correlated with BIF and LIF (factor loadings>0.50). The third principal component (6.26% of variance) is correlated primarily with FUML and PL (factor loadings>0.50) (Table 2). Table 2 indicates that PPL is the only variable, which is negatively correlated with the third PC, and it suggests that Wuliangshan specimens are the smallest and the *D. howelli's* are the largest in absolute dimension (Table 4).

TABLE 2

Factor loadings and percentage of variance explained for principal component analysis. Variable codes are given in the text.

Variables	PC1	PC2	PC3
GLS	**0.866**	0.347	0.273
CBL	**0.851**	0.434	0.227
BL	**0.857**	0.425	0.211
ROL	**0.841**	0.341	0.227
UTR	**0.851**	0.327	0.375
FUML	0.280	0.147	**0.850**
PL	**0.722**	0.330	0.549
PPL	**0.738**	0.388	-0.360
IOB	**0.822**	0.137	0.168
ZOB	**0.900**	0.230	0.170
LDS	**0.839**	0.390	0.224
BIF	0.324	**0.831**	0.003
LIF	0.233	**0.816**	0.315
LTR	**0.832**	0.271	0.396
HM	**0.858**	0.157	0.237
Eigenvalues	11.17	1.06	0.94
Variance explained (%)	74.45	7.09	6.26

Fig. 1 (A, B) shows a plot of the samples of *D. pernyi* on principal components (Fig. 1. A: PC1 versus PC2, Fig. 1. B: PC1 versus PC3).

Inspection of Fig. 1 suggests that the samples are separated into 3 different groups. According to previous results of subspecies differentiation (MOORE & TATE, 1965) and taking into consideration the collecting localities, we find that the samples (*D. p. howelli*) originating from west of the Nujiang River (including Longling, Tengchong, Lushui, and Gongshan) form a clearly separated group. The samples (*D. p. pernyi*) of the western Sichuan province (including Yajiang, Muli, Daofu, and Daocheng) and Yunnan province (including Kunming, Anning, Binchuan, Weixi, and Deqin areas) overlap the

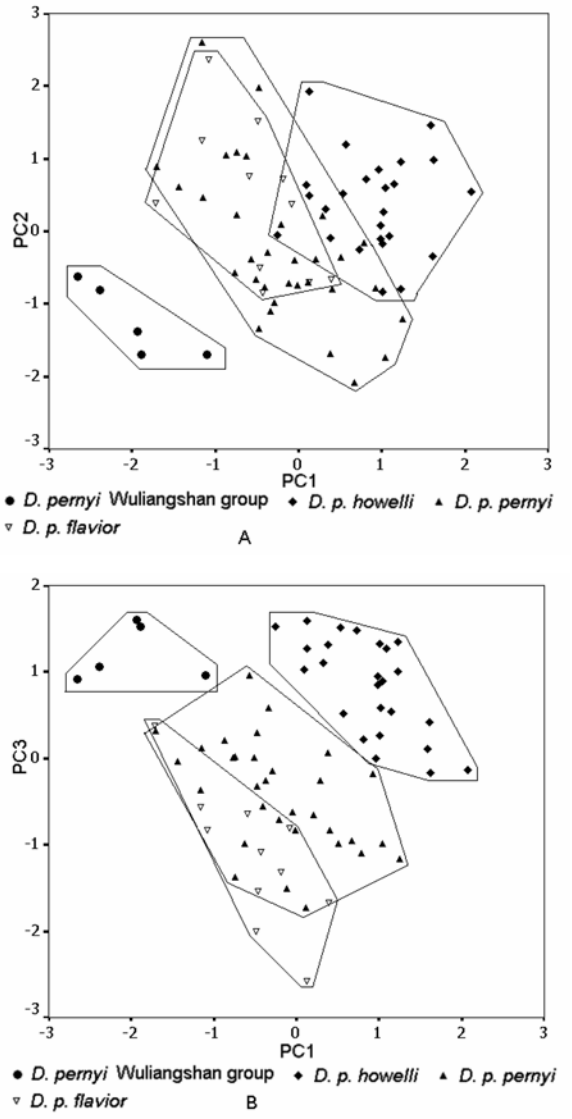

Fig. 1. – Plots of the *D. pernyi* subspecies in multivariate space.

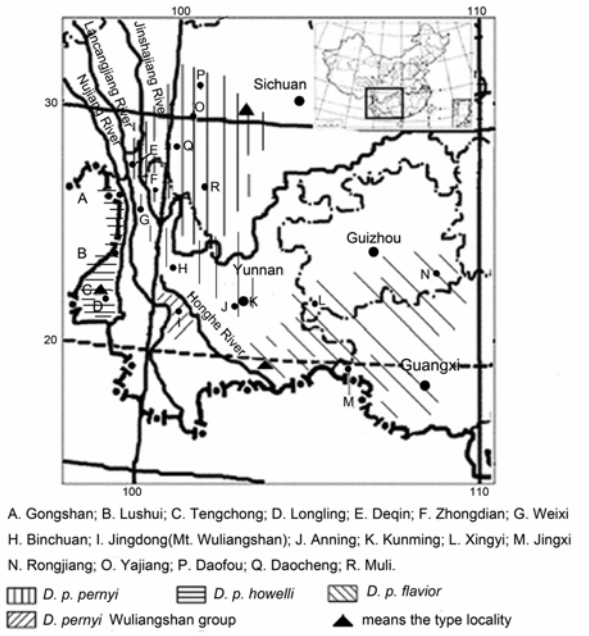

Fig. 2. – The geographic distribution of samples examined in this study.

samples (*D. p. flavior*) of the southwestern Guangxi province (Jingxi area) and the southwestern and southern Guizhou province (Xingyi and Rongjiang areas). These two subspecies also form one group. The most interesting result in this study is that the samples of Mt. Wuliangshan (Jingdong, Yunnan province) are unmistakably separate from the other three subspecies and form a clearly distinct group. Fig. 2 shows the geographic distribution of samples in this study.

Coefficient of Difference (C. D.)

According to the MYAR's theory on subspecies differentiation, the C. D. on the conventional level of subspecific differtiation should be equal to or larger than 1.28. The results of comparison on the C. D. in the skull measurement between *D. pernyi* Wuliangshan group and the other three adjacent subspecies of *D. pernyi* respectively indicate (Table 3): (1) C. D.s of all variables are larger than 1.28 between *D. pernyi* Wuliangshan group and *D. p. howelli*; (2) with the exception of C. D.s for FUML, PL, BIF, LIF, LTR, and HM, the remaining nine C. D.s are larger than 1.28 between *D. pernyi* Wuliangshan group and *D. p. pernyi*; (3) C. D.s of GLS, CBL, BL, UTR, PPL, ZOB, and LDS are larger than 1.28 between *D. pernyi* Wuliangshan group and *D. p. flavior*. Based on these comparisons, it is clear that the samples from the Mt. Wulianshan area markedly differ from the adjacent known subspecies of *D. pernyi*, strongly suggesting that this group should be recognised as a new group within *D. pernyi*.

TABLE 3

Comparison of Coefficient of Difference (C. D.) between *D. pernyi* Wuliangshan group and other three adjacent subspecies of *D. pernyi* respectively: 1: *D. p. howelli*, 2: *D. p. pernyi*, 3: *D. p. flavior*, 4: *D. pernyi* Wuliangshan group. Variable codes are given in the text.

Variable	1-4	2-4	3-4
GLS	**3.13**	**1.54**	**1.56**
CBL	**2.98**	**1.74**	**1.55**
BL	**2.81**	**1.55**	**1.52**
ROL	**3.30**	**1.37**	1.26
UTR	**3.66**	**1.53**	**1.31**
FUML	**1.67**	0.18	1.06
PL	**2.61**	0.38	0.32
PPL	**2.42**	**2.62**	**2.14**
IOB	**2.68**	**1.36**	0.83
ZOB	**3.89**	**2.18**	**2.03**
LDS	**3.14**	**1.48**	**1.79**
BIF	**1.43**	0.68	0.93
LIF	**2.33**	0.77	0.98
LTR	**2.71**	0.86	0.82
HM	**2.40**	1.09	0.86

Cluster Analyses

According to the results of Multivariate analysis and Coefficient of Difference, the samples of Mt. Wuliangshan should be classified as a distinct new group. Cluster analysis of Squared Euclidean distances between the different group centroids calculated using the Mahalanobis distances produced a dendrogram of four groups (Fig. 3).

Fig. 3 shows that *D. p. pernyi* and *D. p. flavior* cluster closely together and form a monophyletic group. This group then clusters with the *D. pernyi* Wuliangshan group to form a group that is clearly separated from *D. p. howelli*.

External and cranial measurements of the four groups of *D. pernyi* are given in Table 4.

TABLE 4

External and cranial measurements of four groups of *D. pernyi*: (Mean ± std. Deviation) / range. Variable codes are given in the text.

	HB	TL	HFL	EL	GLS	CBL	BL	ROL	UTR	FUML
D. p. howelli	188.16±10.63	142.36±15.76	44.40±2.89	20.48±2.12	53.66±1.21	47.34±1.25	44.13±1.21	13.25±0.51	24.87±0.58	2.02±0.08
N=25	174.00-210.00	95.00-165.00	36.00-50.00	17.00-24.00	51.15-56.57	44.28-49.73	40.84-46.27	12.29-14.12	23.71-25.81	1.87-2.17
D. p. pernyi	179.09±12.26	144.44±11.35	42.28±3.30	19.75±3.21	49.68±1.15	43.68±0.98	40.81±1.05	11.87±0.62	22.61±0.56	1.85±0.10
N=32	151.00-200.00	120.00-165.00	35.00-48.00	14.00-25.00	47.47-52.39	41.52-45.97	38.57-42.90	10.77-12.97	21.28-23.62	1.61-2.04
D. p. flavior	175.91±10.10	132.00±14.67	42.73±2.72	20.09±3.67	49.37±0.92	43.56±1.18	40.70±1.03	11.72±0.58	22.27±0.48	1.70±0.15
N=11	162.00-190.00	95.00-150.00	39.00-47.00	10.00-24.00	47.55-50.48	41.83-45.53	39.29-42.18	10.83-12.71	21.56-22.95	1.37-1.88
D. pernyi	156.60±12.22	136.40±4.98	42.00±1.23	20.60±0.55	46.02±1.23	39.66±1.33	37.25±1.24	10.64±0.28	21.03±0.47	1.87±0.01
Wuliangshan group, N=5	137.00-170.00	132.00-145.00	41.00-44.00	20.00-21.00	45.22-48.18	38.63-41.74	36.12-39.15	10.31-10.93	20.52-21.64	1.85-1.89
Total	180.18±13.74	141.30±13.75	43.05±3.10	20.11±2.82	50.75±2.56	44.64±2.46	41.68±2.26	12.24±0.97	23.22±1.37	1.88±0.15
N=73	137.00-210.00	95.00-165.00	35.00-50.00	10.00-25.00	45.22-56.57	38.63-49.73	36.12-46.27	10.31-14.12	20.52-25.81	1.37-2.17

(continue)

	PL	PPL	IOB	ZOB	LDS	BIF	LIF	LTR	HM
D. p. howelli	27.05±0.54	17.49±0.72	14.34±0.55	27.42±0.78	13.46±0.50	1.82±0.16	3.48±0.29	22.69±0.51	6.24±0.24
N=25	25.85-28.10	15.59-19.21	13.09-15.56	25.96-28.74	12.47-14.60	1.53-2.15	3.01-4.32	22.00-23.69	5.84-6.88
D. p. pernyi	23.96±0.71	17.23±0.52	13.44±0.66	25.64±0.81	11.95±0.49	1.59±0.21	2.98±0.43	20.61±0.61	5.63±0.22
N=32	22.61-25.54	16.09-18.13	11.88-14.62	24.20-27.99	11.00-12.94	1.17-2.02	2.28-4.00	19.51-21.74	5.15-6.20
D. p. flavior	23.82±0.60	17.18±0.74	12.80±0.46	25.02±0.64	12.06±0.40	1.70±0.22	3.05±0.39	20.44±0.46	5.52±0.21
N=11	22.73-24.98	15.90-18.63	12.17-13.63	23.71-25.66	11.24-12.56	1.27-2.02	2.48-3.80	19.76-21.02	5.16-5.91
D. pernyi	23.35±0.88	14.35±0.58	12.22±0.24	22.99±0.36	10.63±0.40	1.32±0.19	2.57±0.10	19.52±0.66	5.16±0.21
Wuliangshan group, N=5	22.34-24.17	13.98-15.38	12.07-12.64	22.71-23.60	10.24-11.20	1.16-1.54	2.51-2.75	18.46-20.03	4.79-5.30
Total	24.96±1.66	17.11±0.98	13.57±0.87	25.98±1.44	12.39±0.97	1.67±0.24	3.14±0.45	21.22±1.23	5.79±0.41
N=73	22.34-28.10	13.98-19.21	11.88-15.56	22.71-28.74	10.24-14.60	1.16-2.15	2.28-4.32	18.46-23.69	4.79-6.88

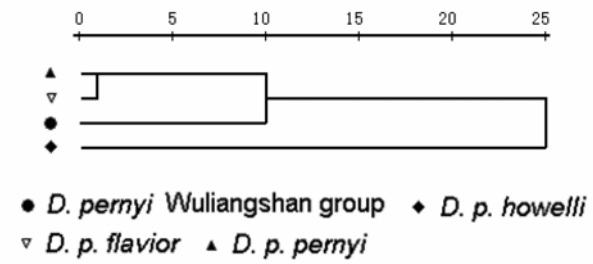

Fig. 3. – Dendrogram from UPGMA cluster analysis of Mahalanobis distances among the four subspecies groups recognized here.

DISCUSSION

Subspecies differentiation of *D. pernyi* in southwestern China

In earlier studies (Allen, 1940; Ellerman, 1940; Ellerman & Morrison-Scott, 1950; Moore & Tate, 1965; Corbet & Hill, 1992), 3 subspecies of *D. pernyi* were recognised in southwestern China: *D. p. howelli*, *D. p. pernyi*, and *D. p. flavior*. On the basis of 15 cranial measurements, the results of multivariate analysis (Fig. 1) confirmed these three subspecies. However, in the present study, one new group was revealed: the *D. pernyi* Wuliangshan group. The group was first suggested by the results of the principal component analyses, and subsequently corroborated by differences in the coefficient of difference (C.D.). The results of cluster analyses (Fig. 3) indicate that *D. p. pernyi* and *D. p. flavior* cluster closely together, and indicates the high degree of similarity in the 15 cranial measurements between the two groups. Moreover, this suggests that these two groups might be closely related. On the other hand, *D. p. howelli* is the most distinct group. As the area of distribution of the *D. pernyi* Wuliangshan group is separated from other subspecies by rivers, it has likely adapted to local environmental characteristics.

Finally, the distribution area of the different groups (Fig. 2) indicates that each of them has its own area of distribution, suggesting that they are geographically different subspecies (groups). We therefore suggest that the samples of Mt. Wuliangshan should be assigned to a separate subspecies of *D. pernyi*, but, more studies, especially molecular data analysis should be performed to confirm this.

Relationships between subspecies differentiation of *D. pernyi* and geographical evolution in southwestern China

The study indicates that there are at least 4 subspecies (groups) of *D. pernyi* in southwestern China. The area covered by these subspecies (southeastern Tibet, western Sichuan province, southwestern, western, and northwestern Yunnan province, southwestern Guangxi province, and southwestern Guizhou province) is not only recognized by the highest species diversity in China, but is also well known to include some of the most abundant biodiversity areas in the world (CHEN, 2002). In his book, SUN (1996) indicates that many complex geological changes took place in these areas during the Cenozoic, especially from the end of the Tertiary to the early stage of the Pleistocene. The collision of the Indian and Eurasian plates resulted in the drastic uplifting of Tibet-Qinghai plateau and rapid river cutting in these areas. Subsequently, high mountains and deep gorges may have acted as barriers to gene flow and may have promoted elevated rates of speciation. Our results also suggest that rivers function as barriers in subspecies differentiation: the Nujiang River acts as a barrier to separate *D. p. howelli* from other subspecies of *D. pernyi.* The *D. pernyi* Wuliangshan group, on the other hand, is distinctly separated from *D. p. flavior* by the Honghe River.

ZENG (1991) indicated that the (pre-)historic Jinshajiang River would have been a southward flowing river in the earlier Pleistocene, parallel along the Nujiang River and the Lancangjiang River, and continuing southeastward along the Mt. Wuliangshan area. CLARK et al. (2004) also considered that the (pre-)historic Jinshajiang River flowed into the Honghe River in its early times, but later changed its flow. In other words, in the early Pleistocene, the (pre-)historic Jinshajiang River flowed into the Honghe River and may have acted as a barrier to separate the Mt. Wuliangshan area from adjacent eastern and northeastern areas (such as Kunming, Anning, and Binchuan areas). During the later geological times, this river changed its flow and became the current Jinshajiang River.

From a biogeographical point of view, subspecies differentiation of *D. pernyi* reflects the isolating effects of rivers, but, in order to estimate the differentiation time of these subspecies, and the current rivers' resultant effects on the subspecies differentiation, molecular data, especially phylogenies of subspecies of *D. pernyi* are needed.

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APPENDIX

Specimens examined in the study

D. p. howelli N=46

Yunnan Province: Longling (KIZ 620021, 620022, 620027); Tengchong (KIZ 539, 548, 565, 598, 601, 650299, 76315); Lushui (KIZ 74011, 74093, 74095, 74127, 74166, 74179, 74197, 74198, 74199, 74240, 74263, 74282, 74283, 74309, 74308, 74334, 74380, 74382); Gongshan (KIZ 73406, 73423, 73446, 73560, 73639, 73640, 73641, 73645, 73683, 73690, 73720, 73721, 73719, 73816, 73819, 73827, 73833, 73864).

D. p. pernyi N=43

Sichuan Province: Yajiang (KIZ 820272, 820277); Muli (KIZ 641, 611, 650092, 650093, 820891); Daofou (KIZ 820841, 820243, 820244); Daocheng (KIZ 810272, 810350, 810356, 810357).

Yunnan Province: Deqin (KIZ 79540, 79565, 79609, 79683, 79454, 79465, 79526); Zhongdian (KIZ 810066, 810424); Binchuan (KIZ 810642, 810643, 810688, 810694, 810695, 810706, 810707); Weixi (KIZ 810517); Kunming (KIZ 630094, 630100, 640204, 640206, 640560, 640561, 640562, 67036, 67037, 76688); Anning (KIZ 63I-0207, 63I-0272).

D. p. flavior N=20

Guizhou Province: Xingyi (KIZ 631022, 631023, 631028, 631036, 631068, 631069, 631072, 631096, 631097, 631098, 631103, 631104, 631106, 631107, 631128, 631200, 631275); Rongjiang (KIZ 630641).

Guangxi Province: Jingxi (IOZ 21460, 21460A).

D. pernyi Wuliangshan group N=5

Yunnan Province: Jingdong, Wuliangshan (KIZ 640040, 640365, 640366, 640372, 640385).

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Gender specific behavioural patterns of captive alpine musk deer (*Moschus sifanicus*)

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ABSTRACT. Alpine musk deer (*Moschus sifanicus*) have evolved behavioural characteristics which have contributed to their survival and proliferation in a unique environmental niche. Understanding these characteristics, specifically those relating to gender is vital in developing appropriate social and economic management systems for captive farming. This study compared the behavioural patterns of female and male captive musk deer to explore differences in gender. Thirty two adult captive musk deer, 19 males and 13 females, were observed from August 2002 to January 2003, at Xinglongshan Musk Deer Farm (XMDF) Gansu Province, China. The frequencies of 12 behavioural categories were recorded and compared between the two sexes. The results showed (1) that behavioural patterns were similar between female and male deer, (2) that female alpine musk deer can express male specific behaviour, such as tail-pasting and, (3) that gender differences in feeding and ruminating behaviour may be related to energy requirement and resource allocation patterns.

KEY WORDS : Alpine musk deer (*Moschus sifanicus*); Captivity; Behavioural patterns; Gender

INTRODUCTION

Musk deer (*Moschus* spp.) are well known for the production of musk, a highly valued ingredient of perfumes and some Chinese traditional medicines which is secreted only by the adult male (ZHANG, 1979). Musk deer occur in at least 13 countries in South Asia, East Asia, Southeast Asia and eastern Russia, with populations currently in decline as a result of habitat loss and intensive illegal hunting for musk (HOMES, 1999). Alpine Musk Deer (*Moschus sifanicus*), an endemic species to the Qinghai-Xizang Plateau, Western China, is estimated at less than 100,000 individuals (SHENG & OHTAISHI, 1993) and is currently listed as vulnerable under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and protected as a Category I key species in China (SHENG & OHTAISHI, 1993).

Since 1958, captive farming has been conducted to conserve wild populations and sustainably utilize musk deer resources (HOMES, 1999; ZHANG, 1979). The predominantly farmed species include forest musk deer (*Moschus berezovskii*) and alpine musk deer (*Moschus sifanicus*), with captive populations in China of approximately 2000 individuals (HOMES, 1999; PARRY-JONES & WU, 2001). Successful captive breeding and the development of sustainable musk extraction methods from living deer has significant implications for the conservation and sustainable use of the existing wild musk-deer resources (ZHANG, 1979). In spite of husbandry advances, many problems including high mortality, low production of musk, and shortened life span, remain to be solved before sustainable utilization of musk deer resources can be achieved (HOMES, 1999; PARRY-JONES & WU, 2001).

In China, the management and breeding patterns of musk deer farming is based on the assumption that captive musk deer have been domesticated, and hence factors such as nutrition have been overwhelmingly emphasized, while natural behavioural characteristics have not been adequately taken into account. In fact, musk deer are a typical small solitary forest ruminant (SSFR), which are difficult to manage and breed on farms due to their solitary habits, territorial behaviour and excitable nature (GREEN, 1987).

The objective of the present study was to determine behavioural patterns of captive male and female musk deer, exploring these factors over reproductive seasons. Based upon this knowledge, an appropriate musk deer management system could be developed, in addition to providing a comparative data set for wild populations.

MATERIALS AND METHODS

Animals, housing and managing

This study was conducted in Xinglongshan Musk Deer Farm (XMDF), at Xinglongshan National Nature Reserve, Gansu Province in northwest China. Located at 2000-2100m elevation, the reserve has a continental mountain climate with short, cool summers and long, harsh winters. January is the coldest month with average and minimum temperatures of 9ºC and –28ºC respectively. The warmest month is July, averaging 14ºC. Rainfall is mainly in July, August and September, with annual precipitation of 48-62.2mm.

A total of 32 captive adult alpine musk deer were studied at XMDF, including 19 male and 13 female ranging from two to seven years old. All deer were captive born

and were housed at XMDF for at least 2 years prior to this study. Five to seven individuals were housed in an outdoor yard measuring 10m×10m square with seven brick stalls, measuring 2mx2m with a height of 2m.

Five to eight enclosures were lined up in a row, separated by an iron-mesh fence which enabled olfactory and auditory communication between neighbouring inmates, but prevented physical contact. No communication was possible between rows. Animals were fed twice daily, at dawn and dusk, on a diet of fresh leaves (in summer and autumn) or dried leaves (in winter and spring) collected from the natural habitats of wild musk deer, and supplemented by artificial foods (consisting of flour, wheat bran and some vegetables in season). The amount of food provided was held constant and water was provided *ad libitum*.

During the study, males and females were housed separately from March to October. From November to February one male was introduced into each of the female enclosures, as with commercial breeding practices. All animals were individually identified by a numbered plastic ear tag.

Ethogram

On the basis of published behaviour patterns of musk deer (ZHANG, 1979; SHENG & OHTAISHI, 1993; GREEN, 1987), and preliminary observations, captive musk deer behaviour was characterized as follows:

Resting (RE): Animal is lying on the ground, and in inactive and relaxed state. Standing-alert (SA): Animal is still, alert and gazing at stimuli or potential stimuli. Locomotion (LO): Animal is moving without any accompanying behaviours. Feeding/Drinking (FD): Animal is feeding or drinking. Ruminating (RU): Animal expresses typical behavioural series of rumination including regurgitating, chewing and swallowing. Tail-pasting (TP): Animal is rubbing its tail and scent-marking on the surface of the wall or doorframe. Urinating/Defecating (U/D): Animal fully or partially exhibits activities such as earth-scratching, urinating and pellet covering. Environmental sniffing (ES): Animal explores the wall or ground with its nose. Ano-genital sniffing (AS): Animal sniffs the ano-genital region of another musk deer, sometimes with licking. Self-directed behaviour (SD): Animal exhibits activities directed to itself, including self-grooming with mouth, self-scratching and other self-directed behaviours. Affinitive interaction (AI): Direct physical contact between individuals, without obvious conflict, such as mutual grooming, nursing and licking. Agonistic interaction (CI): Obvious aggressive behaviours with or without direct body contact. Miscellaneous behaviour, (MB): All other behaviours.

Data collection and statistical analysis

At XMDF, the main fawning season is from June to July (MENG et al., 2003a), with most mating occurring in late November (MENG et al., 2003b). Therefore observations made from August to October are referred to as the "pre-rut season", and from November to February as the "rut season".

Binoculars (10×42°) were used to observe behaviour and verify animal identification. A focal musk deer was randomly selected from a group. All occurrences of behaviour were recorded during a five minute period. All observations were conducted by the same researcher and took place three days a week for six months (total observation duration is 300 hours). Attempts were made to sample each individual once a week.

Behavioural frequencies, means and standard error (SE) were computed for every observation. Behaviour samples less than 5 minutes in duration were excluded from the data analysis. For statistical analyses, we used the average from all 5min observations as one data point for each individual per month. As female and male musk deer were housed in different enclosures during pre-rut season, and the behaviour of the two genders was not related, the Mann-Whitney U Test was used to test the potential differences between male and females in pre-rut season. During rut season, however, male and female musk deer were enclosed together, and as their behavioural modes were related the Wilcoxon Signed Rank Test was utilized. Statistical analysis was conducted using SPSS11.0 program (SPSS Inc., Chicago, Illinois) with a two tailed significance level of $P=0.05$.

RESULTS

Pre-rut season

The behavioural frequencies of male and female alpine musk deer during pre-rut season are shown in Fig. 1. Females were recorded resting (1.40±0.88) more frequently than males (1.04±0.66) ($P<0.01$). Similarly in females, frequencies of feeding/drinking (1.55±0.25) and ruminating (0.92±0.16) were significantly higher than in males (IN, 0.77±0.19; RU: 0.41±0.17; IN, $P<0.01$; RU: $P<0.01$). There was no significant difference ($P=0.064$) in the frequency of urinating and defecating (U/D) between females (0.09±0.02) and males (0.07±0.03). No tail pasting was observed for female deer during pre-rut season, whilst the male deer did exhibit this behaviour (0.10±0.05 occurrences).

Rut season

The distribution of behavioural patterns of captive musk deer during the rut season is shown in Fig. 2. The male deer rested (0.18±0.06) less frequently than the females (0.31±0.07), however, the difference was not significant ($P=0.44$). Males showed slightly higher frequencies of standing-gazing (3.57±0.65) and moving (2.53±0.53) than females (SG: 2.68±0.39; MO: 1.93±0.36), however no significant differences were found (SG, $P=0.250$; MO, $P=0.302$). Males fed (0.64±0.26) more frequently and ruminated less (0.28±0.18) than females (IN: 0.33±0.09; RU: 0.09±0.03), but only the difference in the former behaviour was significant (IN: $P=0.031<0.05$; RU $P=0.329>0.05$). During rut season, females were observed to exhibit tail-pasting, but its frequency (0.08±0.04) was significantly less than in males (0.43±0.19) ($P=0.002<0.01$). Furthermore, females showed less ano-genital sniffing and environment sniffing

(AS: 0.03±0.02; ES: 0.03±0.02) than males (AS: 1.45±0.40; ES: 0.03±0.02) though the differences were insignificant (AS: P=0.234; ES: P=0.329). Males exhibit agnostic interactions (0.99±0.35) significantly more frequently than females (0.37±0.10; P<0.05), whilst females expressed more affinitive interactions (AI: 0.04±0.03), however, these differences are not significant (P=0.287).

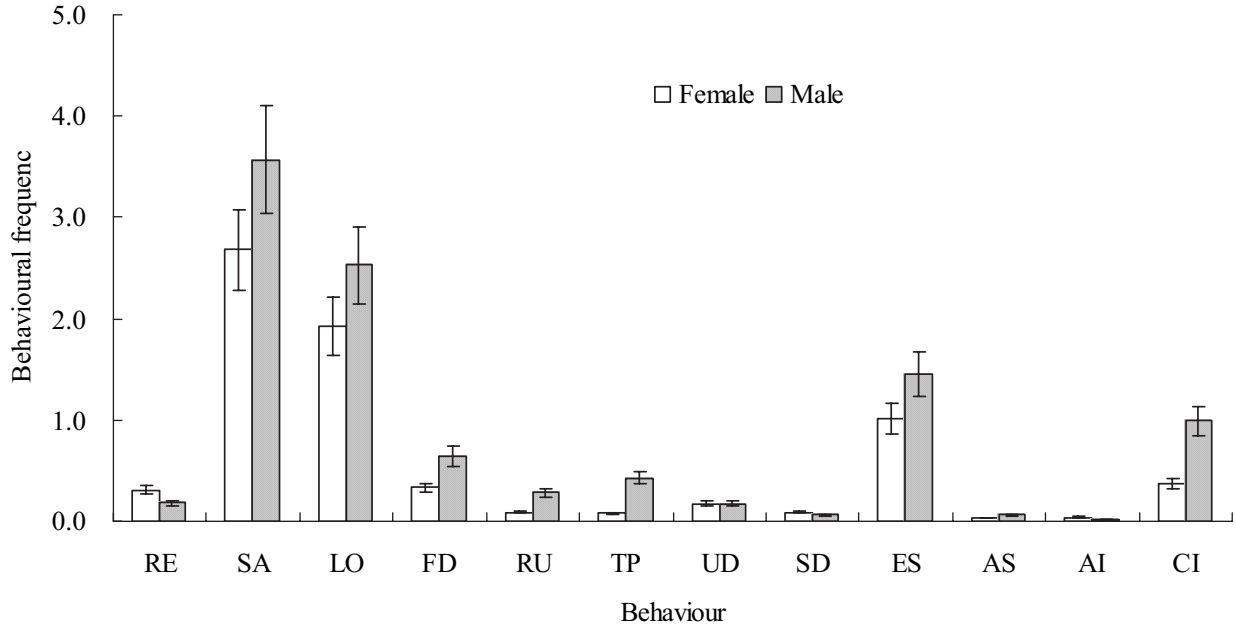

Fig. 1. – Frequency of female and male musk deer behaviour during pre-rut season.
(RE): Resting, (SA): Standing-alert, (LO): Locomotion, (FD): Feeding/Drinking, (RU): Ruminating, (TP): Tail pasting, (UD): Urinating/Defecating, (SD): Self-directed behaviour, (ES): Environmental sniffing, (AS): Ano-genital sniffing, (AI): Affinitive interaction, (CI): Agonistic interaction.

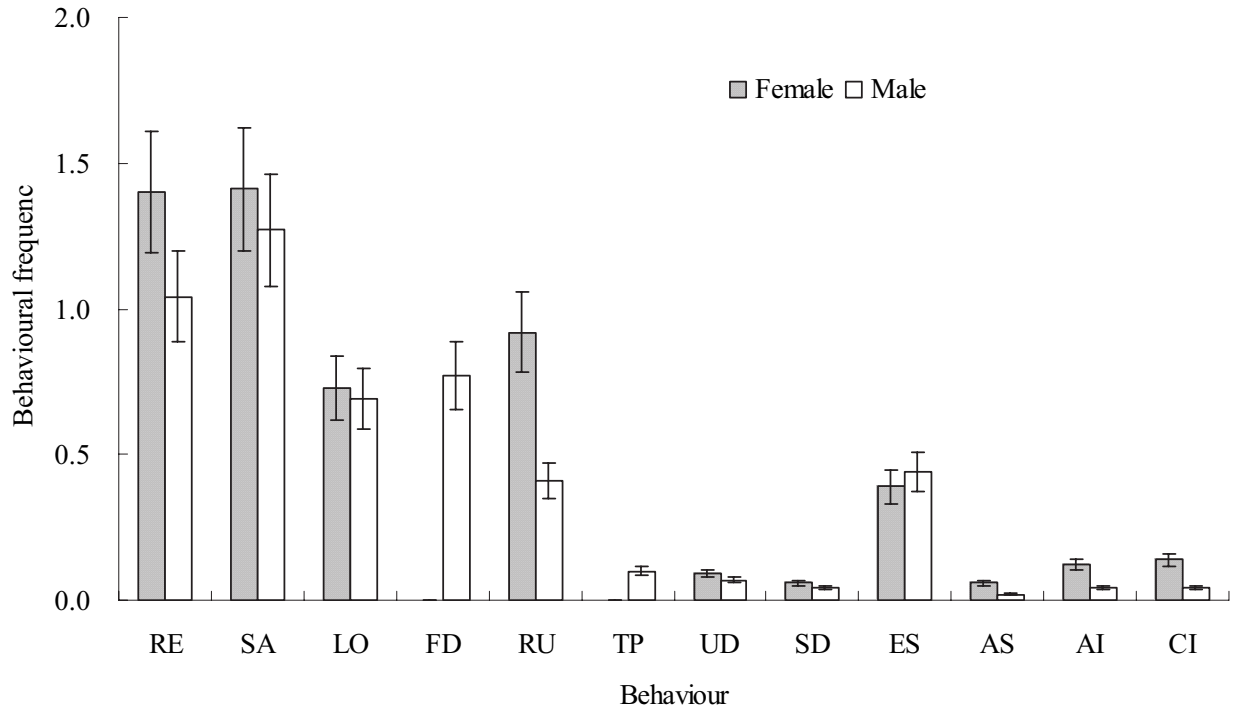

Fig. 2. – Frequency of female and male musk deer behaviour during rut season.
(RE): Resting, (SA): Standing-alert, (LO): Locomotion, (FD): Feeding/Drinking, (RU): Ruminating, (TP): Tail pasting, (UD): Urinating/Defecating, (SD): Self-directed behaviour, (ES): Environmental sniffing, (AS): Ano-genital sniffing, (AI): Affinitive interaction, (CI): Agonistic interaction.

Pooling rut and pre-rut

Pooled behavioural data, ignoring seasonal effects are shown in Table 1. Higher occurrence of resting in female musk deer (0.84±0.43) as compared to males (0.71±0.41) suggest that females rest significantly more than males (P=0.03<0.05).

TABLE 1

Comparison of the behavioural frequency between female and male musk deer

Behaviour patterns	Female (n=36)	Male (n=13)	Sig.
Resting, RE	0.84±0.43	0.71±0.41	*
Standing-alert, SA	2.06±0.24	2.15±0.31	ns
Locomotion, LO	1.35±0.22	1.40±0.25	ns
Feeding/Drinking, FD	0.93±0.15	0.72±0.15	ns
Ruminating, RU	0.49±0.09	0.36±0.13	ns
Tail-pasting, TP	0.04±0.02	0.23±0.08	**
Urinating/Defecating, UD	0.13±0.03	0.10±0.04	ns
Self-directed behaviour, SD	0.08±0.02	0.05±0.02	ns
Environmental sniffing, ES	0.71±0.16	0.83±0.17	ns
Ano-genital sniffing, AS	0.04±0.01	0.03±0.02	ns
Affinitive interaction, AI	0.08±0.03	0.02±0.01	ns
Agonistic interaction, CI	0.25±0.06	0.41±0.15	ns

Note: Data showed as mean ± S.E.; *: significantly different (P<0.05); **: highly significantly different (P<0.01); ns: no significant difference (P>0.05).

DISCUSSION

According to the resource allocation theory, animals allocate resources and energy in reproduction and growth in order to maximize their fitness (SCHUTZ et al., 2001). In captivity, this can be achieved through behavioural variation, to maximize survival in an environment vastly different from which the animal evolved. SCHULTZ et al. (2003) proposed that in domesticated animals the frequency of energetically costly behaviour is expected to decrease, particularly in breeds that are selected to invest a higher proportion of energy into reproductive traits. Whilst captive musk deer were found to have little degree of domestication (MENG et al., 2006), the identical environment in which both male and female were housed and managed at XMDF, may contribute to the generally similar behavioural frequencies recorded in this study. Still, similar behavioural patterns have also been recorded for wild ungulate populations. For example SHI et al. (2003) reported no significant differences in general activity budgets between adult male and female feral goats (*Capra hircus*).

Despite similar patterns of behaviour for males and females, behavioural frequency did vary seasonally. During the pre-rut season, females displayed more feeding, resting and ruminating, as has been reported for other ungulates (*Ovis canadensi*; PELLETIER et al., 2004). Various studies have investigated the role of sexual dimorphism in ungulates in regards to feeding. Based on ungulate species, PELLETIER et al. (2004), proposed that foraging time should be higher in females, proportional to the dimorphism in body size. Whilst our study supports this theory with female musk deer recorded a lower body mass (ZHANG, 1979; MENG et al., 2006), this trend was not consistent during the rut season, as males recorded significantly higher feeding rates.

The sexual differences in time spent foraging and ruminating, however, could partly be due to the lactation. In captive female musk deer lactation occurs during the pre-rut season (August to October) following annual weaning of young in October (MENG et al., 2003a). This process is an energetically costly activity. For example lactating female ibex (*Capra ibex nubiana*) consume approximately 50% more food per kg of body weight than adult males (GROSS et al., 1996). During this time females showed high feeding and ruminating frequencies to compensate for higher energy loss during fawning and lactating.

There is also a potential trade-off between foraging and vigilance (RUCKSTUHL et al., 2003). Vigilance is a behaviour that increases the probability that an animal will detect a given stimulus at a given time (DIMOND & LAZARUS, 1974). When a stimulus occurs, musk deer cease resting and elicit a standing-alert behaviour to avoid predation. Wild animals increase vigilance at the expense of feeding time in response to predation risk or threats from conspecifics. For example, RUCKSTUHL et al. (2003) recorded higher vigilance in lactating female *Ovis canadensis* as compared to males, in response to increased requirements to prevent predation on offspring. In the wild, musk deer are solitary and highly territorial, occupying a home range of 20-30ha (GREEN, 1987). In captivity, however, several musk deer are housed in a relatively small area (100m square), thus the potential threat from conspecifics, in addition to management practices and other human activities are greatly increased. We recorded a higher frequency of elicit stanging-alert behaviour in females musk deer as compared to males, concurring with findings for *Ovis canadensis* (RUCKSTUHL et al., 2003).

Male musk deer also have a number of seasonal reproductive requirements. During rut season, males compete for female mates and often engage in aggressive interactions (ZHANG, 1979; SHENG & OHTAISHI, 1993). Unlike wild conditions, in which male home ranges are mutually exclusive, captive farming directly increases the proximity of competing males, and accordingly the likelihood of agonistic interactions. These behaviours are translated in the higher frequency of agonistic interactions for males during the rut season, along with an increased frequency of feeding. Physiological requirements, such as musk production may also play a major role in behavioural patterns. The secretion of musk by adult males, which occurs annually in June and July (ZHANG, 1979; MENG et al., 2003a; b; ZHOU et al., 2004), is an energetically costly process. During this process male deer become excited, refuse food and cease defecating, as suggested by the significant decrease in feeding during the pre-rut season (August to October).

In solitary animals, such as musk deer, the development of complex olfactory signalling systems is common, with scent marking used as a primary means of communication. GREEN (1987) and SOKOLOV (1984), defined tail-pasting as a male specific scent marking behaviour. Our

results, however, showed captive female alpine musk deer at XMDF exhibit this behavioural pattern during the rut season. Preliminary observations indicated that sexually experienced females were more likely to exhibit this behaviour which in-between mating bouts. In contrast to males, females exhibited tail pasting for a shorter durations, and with less intensity. However, the obvious up-down and left-right movements could be readily identified as tail pasting. In this study, tail-pasting observations were infrequent, however trends follow those reported by SHENG & OHTAISHI (1993), who observed an increase in scent making by male forest musk deer (*Moschus bererovskii*) during the rut season (SHENG & OHTAISHI, 1993). Further investigation of both male and female tail-pasting is needed to determine the underlying mechanism of this behaviour, its specificity to musk deer species, and whether it is a redundant behavioural characteristic of captive individuals.

On the basis of these results, we conclude that captive male and female musk deer generally display similar behavioural patterns. Significant seasonal gender differences were recorded for feeding and ruminating behaviour, which are likely linked to energy and resource allocation patterns. Furthermore we recorded the expression of tail pasting behaviour by female musk deer.

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SHORT NOTES

Aggressive interactions among birds in winter-fruiting plants

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KEY WORDS : frugivorous birds, aggressive interactions, *Euonymus europaeus*, *Viburnum opulus*, Spain

Bird social organization in the nonreproductive season varies from solitary individuals that aggressively defend food resources to single or mixed species flocks whose members do not interact aggressively, while many species show a wide behavioural spectrum depending on the energetic costs and profits that each strategy involves at any given point in time (1-4). Very few field studies have examined the behaviour of birds feeding on fleshy fruits (5-7), particularly aggressive encounters, which are considered to be rare and therefore are poorly documented (8). Although aggressive interactions have been recorded in spring, probably due in part to paternity guarding (9), they are more frequent during winter in temperate and cold latitudes where fruit availability is low and fruit defence advantageous in spatially concentrated and lasting fruit patches (8; 10-16).

I report a study of aggressive interactions among frugivorous birds feeding on spindle *Euonymus europaeus* (Celastraceae) and guelder rose *Viburnum opulus* (Caprifoliaceae) from late autumn through to winter in northwestern Spain. I discuss the possible causes with relation to food availability and energy demand.

The study area covers 1.5km^2 and is located in the Torío river valley (30TTN93 U.T.M. coordinates, 900-1000m a.s.l., León province, NW Spain). It is part of the Supramediterranean bioclimatic stage in the Mediterranean biogeographic region, but very near the Eurosiberian region. The landscape is a mosaic of riparian woodland, hedges, irrigated pastureland, scrub, and Pyrenean oak *Quercus pyrenaica* woods. Cold winters with low overall food availability, fleshy fruit included, characterize the area. Eighteen plant species bear fleshy fruit but only eight do during winter period (spindle, guelder rose, hawthorn *Crataegus monogyna*, dog rose *Rosa canina*, blackthorn *Prunus spinosa*, dogwood *Cornus sanguinea*, ivy *Hedera helix*, and privet *Ligustrum vulgare*), and only four (guelder rose, dog rose, ivy, and privet) are available in late winter (pers. obs.). Seasonal abundance of insects and other invertebrates peaks in summer, progressively decreasing by up to 90% in normal winters (17).

Spindles and guelder roses are shrubs or small trees. The spindle fruit is a pink capsule when ripe and opens up revealing three or four small seeds covered in a fleshy, orange aril; each arillate seed is the unit taken by a feeding bird. The guelder rose fruit is a small red drupe when ripe. Between November 1996 and January 1997 I spent 55.5 hours, distributed over 19 days, directly observing birds feeding on the fruits of four spindle plants. Similarly, between late December 2004 and February 2005 I spent 36 hours, distributed over 12 days, observing birds feeding in six guelder rose plants. Plants were not situated close together thus were not observed simultaneously. Both winters were very cold with mean minimum temperatures below zero and the ground regularly covered in snow, particularly in the period December 2004-February 2005. Each visit by an individual bird was scored as one feeding visit if the bird was seen to eat at least one seed or fruit; however, I could not differentiate individuals as birds were not marked. I noted the number of birds involving in aggressive interactions while they fed in the same plant. In spindle I particularly recorded whether the attacked bird was displaced from the plant or not, and in guelder rose I particularly recorded additional birds that visited the plants and were attacked but did not succeed in feeding. Guelder roses were observed during a short, strictly winter period, so temporal variation in attack rate was not analysed for this plant species.

I recorded 330 feeding visits to spindle. Ten bird species visited spindle, principally blackcaps *Sylvia atricapilla* (40.6% of visits) and robins *Erithacus rubecula* (33.9%). I recorded 450 feeding visits to guelder rose. Five bird species visited guelder rose, principally song thrushes *Turdus philomelos* (48.4%) and robins (42.7%). During the period December 2004-February 2005 the blackcap was virtually absent from the study area, as usually happens in the harshest winters (pers. obs.). The other bird species feeding on spindle and/or guelder rose fruit were the black redstart *Phoenicurus ochruros*, blackbird *Turdus merula*, redwing *T. iliacus*, mistle thrush *T. viscivorus*, long-tailed tit *Aegithalos caudatus*, great tit *Parus major*, blue tit *P. caeruleus*, and bullfinch *Pyrrhula pyrrhula*.

Aggressive interactions always involved just two individuals. In spindle I recorded 19 aggressive interactions, 15 of which (c.80%) resulted in the attacked bird being displaced from the plant. Aggressive interactions in spindle involved just two species, robins and blackcaps. Fourteen of the aggressive interactions were intraspecific (eight robin-robin interactions and six blackcap-blackcap interactions) and five were interspecific, robin attacking blackcap, interactions. Aggressive interactions accounted for 18.7% of feeding visits by robins (21/112) and 12.7% of feeding visits by blackcaps (17/134) to spindle (no significant interspecific differences: $\chi^2_1=1.28$, $p>0.05$). The attack rate increased during the study period (0.02

attacks/10min up to 14 December, 0.11 attacks/10min from 15 December; z=-3.42, p<0.001, test for Poisson rates). Aggressive interactions accounted for 17.7% of feeding visits by robins (34/192) and 0.9% of feeding visits by song thrushes (2/218) to guelder rose (significant interspecific differences: χ^2_1=33.87, p<0.001). All the aggressive interactions were intraspecific. Moreover, 6.3% of robins (13/205) visited guelder rose but did not succeed in feeding because of attacks by other robins.

Several plant species lacked fruit during autumn-winter, the availability of fleshy fruit decreased from 31.3 fruits/m^2 in November 1996 to 1.1 fruits/m^2 in January 1997 (18), and some of the longer lasting fruits were not suitable for robins and blackcaps due to their large size (e.g. hips, sloes). Therefore fruit was in limited supply to frugivorous birds during winter. The availability of insects and other invertebrates also decreased noticeably (pers. obs.); however, bird energy demand increased owing to the drop in temperature (3). Low availability of arthropods and cold temperature increases winter fruit removal rate of bird-dispersed plants in temperate zones (19). Winter food shortages were therefore likely to lead to an increase in aggressive encounters as birds concentrated in the few sources of fruit. An increase in population does not explain the increase in aggressive interactions as bird density (robins plus blackcaps) decreased from 29 birds/10ha in November 1996 to 7 birds/10ha in January 1997 (18).

In conclusion, robins, as usual, attacked conspecifics as well as blackcaps within their territories (20; 21) and fed on fruit alone (22; 23). In contrast, blackcaps frequently feed on fruit in small intraspecific aggregates without attacking conspecific individuals or other bird species (8; 22-24); however, in this study they rarely joined other conspecific individuals and attacked them from late December (pers. obs.). In severe winters at temperate latitudes, up to 19% of feedings events involved aggressive encounters among birds feeding on fleshy fruit. Relatively high levels of winter feeding aggressions are likely to influence 1) the identity and number of birds visiting and consuming the fruit of each plant species; 2) the density and spatial distribution of the bird species; and 3) seed dispersal processes (3; 25). Further research is required to determine the generality of the findings and their ecological repercussions.

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New Palaearctic species of Phthiracaroidea (Acari, Oribatida)

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The paper describes three new ptyctimous mites (Acari, Oribatida) belonging to the superfamily Phthiracaroidea. The new species represent three genera and were collected from different and distant areas of the Palaearctic Region, i.e. Canary Islands, Turkey and Ukraine. All types are deposited in Department of Animal Taxonomy and Ecology, A. Mickiewicz University, Poznań, Poland. All measurements are given in micrometers.

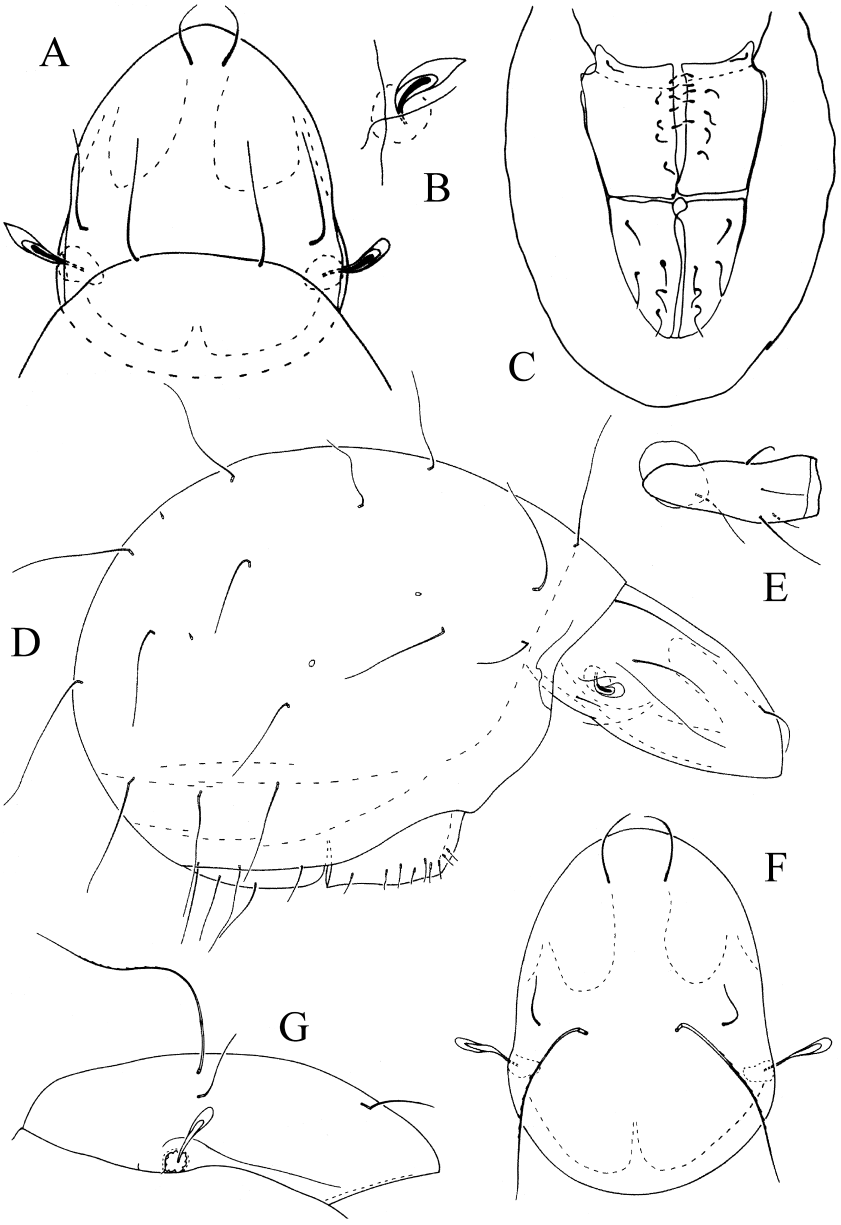

Fig. 1. – A-E. *Phthiracarus schusteri* sp. nov. (holotype): A- prodorsum, dorsal view, B – sensillus, dorsal view, C – ventral side, D – lateral view of body, E – trochanter and femur of leg I, lateral view. F-G. *Austrophthiracarus gomerensis* sp. nov. (holotype): F – prodorsum, dorsal view, G – prodorsum, lateral view

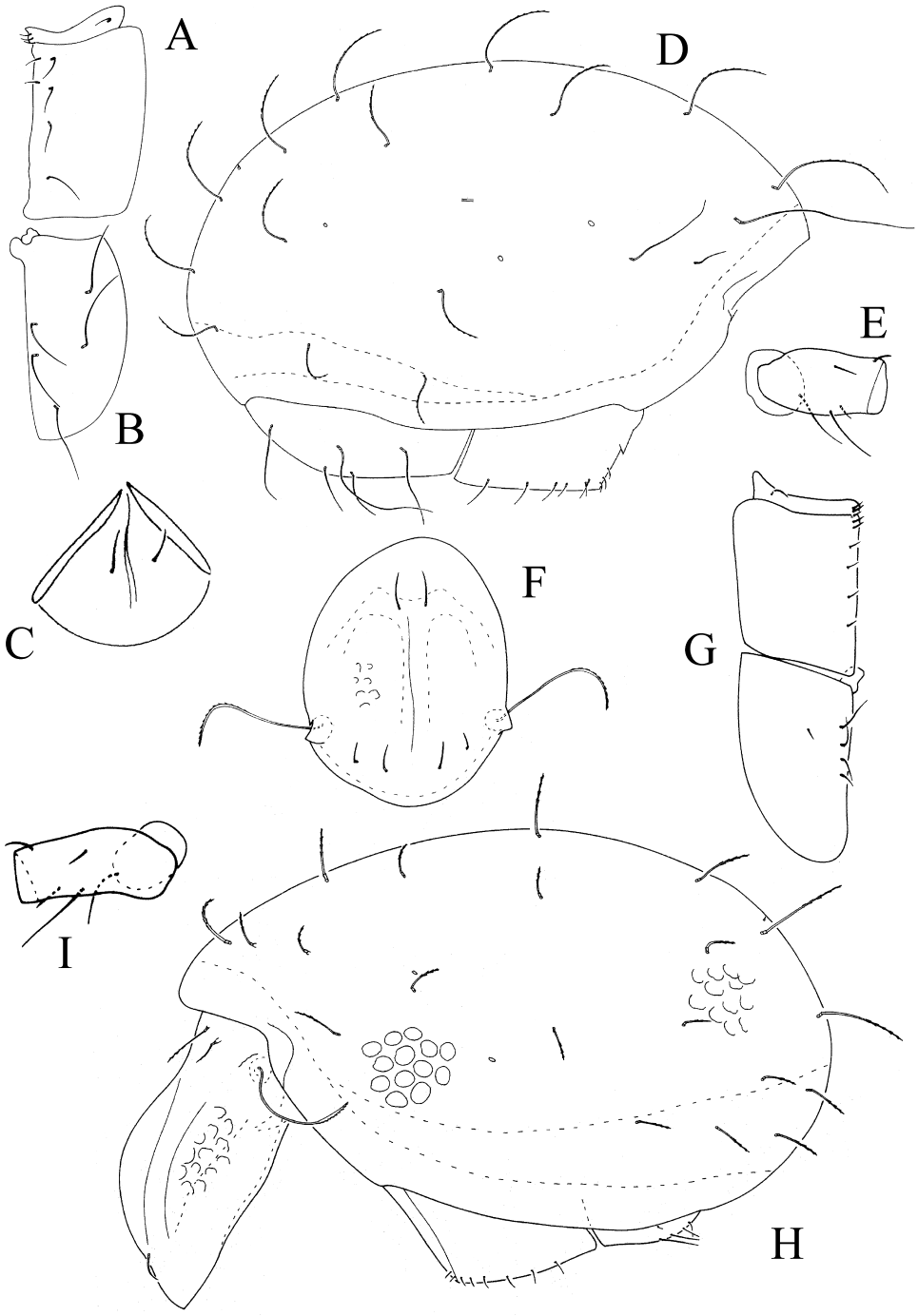

Fig. 2. – A-E. *Austrophthiracarus gomerensis* sp. nov. (holotype): A – left genitoaggenital plate, B – left anoadanal plate, C – mentum of infracapitulum, D – notogaster, lateral view, E – trochanter and femur of leg I, lateral view. F-I. *Atropacarus (Atropacarus) paraserratus* sp. nov. (holotype): F – prodorsum, dorsal view, G – right genitoaggenital and anoadanal plates, H – lateral view of body, I – trochanter and femur of leg I, lateral view.

Phthiracarus schusteri sp. nov.

(Figs 1 A-E)

Description. Measurements of holotype: prodorsum: length 202, width 149, height 83.5, sensillus 25.3, setae: interlamellar 78.4, lamellar 53.1, rostral 43.0, exobothridial 17.7; notogaster: length 352, width 238, height 250, setae: c_1 83.5, h_1 86.0, ps_1 75.9; genitoaggenital plate 88.5x60.7, anoadanal plate 88.5x50.6.

Rather small species. Colour dark yellow brown. Integument densely porous.

Prodorsum with distinct and long lateral carinae. Sigillar fields present, median longer than lateral ones. Sensilli

short, spindle-shaped, pointed distally, smooth. Setae of medium length, attenuate, in>le>ro>ex.

Notogaster with 15 pairs of non vestigial, fairly long setae, setae c_1 shorter than distance c_1-d_1. Setae of row c near the anterior border, setae c_2 slightly more remote than setae c_1 and c_3. Vestigial setae f_1 far anteriad of setae h_1. Two pairs of lyrifissures ia and im present.

Ventral region. Setae h of mentum longer than distance between them. Formula of genital setae: 4+3: 2. Anoadanal plates with well-developed anal and adanal setae. All setae except shorter ad_3 setae similar in length.

Setation of legs complete. Setae d of femora I located in the middle of article.

Types. Holotype. Turkey, RS 576a (detailed list of localities is accessible from the collection of Prof. R. Schuster, University of Graz).

Etymology. The specific epithet is chosen in honour of the owner of the sample, an excellent acarologist – Prof. R. Schuster.

Comparison. The new species is similar to some other Palaearctic species but is distinguishable by the following characters: setae of row c of notogaster located near of anterior border, vestigial setae f_1 placed anteriorly to setae h_1 and adanal setae ad_1 and ad_2 not longer than anal setae. Comparison to similar species: *Phthiracarus opacus* Niedbała, 1986 has vestigial setae f_1 located posteriorly to h_1 setae and adanal setae ad_1 and ad_2 longer than other setae of the plates; *P. assimilis* Niedbała, 1983 has longer setae of body, h setae of infracapitulum shorter than distance between them and adanal setae ad_1 and ad_2 longer than other setae; *P. clavatus* Parry, 1979 has setae of row c of notogaster more remote from border, setae h of infracapitulum not longer than distance between them and adanal setae ad_1 and ad_2 vestigial or shorter than other setae; *P. dominiaki* Niedbała, 1984 has longer setae of body, especially adanal setae, setae of row c remote from anterior border, and vestigial setae f_1 located posteriorly of h_1; *P. peristomaticus* Willmann, 1948 has setae c_1 and c_2 of notogaster remote from anterior border vestigial, setae f_1 located posteriorly of h_1 and longer adanal setae ad_1 and ad_2; *P. similis* Niedbała, 1981 has setae of row c remote from border and adanal setae and setae ad_1 and ad_2 vestigial.

Austrophthiracarus gomerensis sp. nov

(Figs 1 F-G; 2 A-E)

Description. Measurements of holotype: prodorsum: length 278, width 202, height 106, sensillus 45.5, setae: interlamellar 180, lamellar 45.5, rostral 55.7, exobothridial 20.2; notogaster: length 575, width 364, height 353, c_1 131, c_2 172, c_3 35.3, h_1 and ps_1 116; genitoaggenital plate 151x101, anoadanal plate 197x106.

Colour light brown. Surface of body punctate.

Prodorsum with indistinct sigillar fields, median longer than lateral ones. Lateral carinae distinct, long. Sensilli club-like, smooth, rounded distally. Interlamellar setae rigid, erect, bent posteriorly covered sparsely with small spines in distal half. Lamellar and rostral setae simple, smooth, in>ro>le>ex.

Notogaster with 17 pairs non vestigial, rather short (c_1<c_1-d_1) setae, bent anteriorly as interlamellar setae covered sparsely with small spines in distal half. Additional setae in rows h and ps. Only setae c_2, c_3 and cp smooth. Setae c_2 very long, setae c_3 very short. Setae of row c_{1-3} remote from anterior border in equal distance. Vestigial setae f_1 posteriorad of h_1 setae.

Ventral region. Setae h of mentum equal to distance between them. Genitoaggenital plates with 9 pairs of genital setae with arrangement 4+2: 3. Anoadanal plates each with 5 pairs of setae, anal setae shorter than adanal, setae ad_2 the longest, flagelliform, smooth.

Legs. Formulae of setae and solenidia of "complete type". Spiniform setae d of femora I located at the end of article.

Types. Holotype and 15 paratypes. Canary Islands (no MMMCCCVII), La Gomera, 4km S of Hermigua, bushes above road, 3 III 2002, leg. W. Niedbała. One paratype. Canary Islands (no MMMCCCIX), La Gomera, El Convento, banana plantation, 3 III 2002, leg. W. Niedbała.

Etymology. The specific epithet *gomerensis* refers to the locality of this species in Gomera island.

Comparison. The new species may be differentiated from its congeners by the presence of smooth setae c_2, c_3 and cp, unusual length of setae c_2 (very long) and setae c_3 (very short) as well as by the very long adanal setae ad_2.

Remark. The species of this genus have been found mostly in the southern hemisphere. However, the ranges of individual species have reached further to the North, namely to the Palearctic Region especially in its eastern margins, more specifically to the islands of Japan (1) and northern India (2).

Atropacarus (Atropacarus) paraserratus sp. nov.

(Figs 2 F-I)

Description. Measurements of holotype: prodorsum: length 220, width 152, height 86.0, sensillus 81.0, setae: interlamellar 37.9, lamellar 22.8, rostral 30.4; notogaster: length 424, width 263, height 242, setae: c_1 40.5, h_1 63.2, ps_1 58.2, ps_2 32.9; genitoaggenital plate 101x70.8, anoadanal plate 101x63.2, the longest setae of anoadanal plates 17.7.

Small species. Colour grey-brown. Sculpture of body strong, surface covered with deep, regular concavities.

Prodorsum with distinct median ridge. Sigillar fields narrow, joined. Posterior furrows feeble. Lateral carinae absent. Sensilli long, narrow, slightly dilated in distal half, covered with small spines. Setae short, pointed distally covered with small spines; in>ro>le.

Notogaster with 20 pairs of non vestigial, obtuse, thick, rather short (c_1<c_1-d_1) setae, only setae h_1 and ps_1 longer; all setae covered with small spines. Neotrichy in setae of rows c, h and ps. Setae c_{1-3} slightly remote from anterior border. Vestigial setae f_1 located slightly anteriad of h_1. Lyrifissures invisible because strong tegument.

Ventral region. Setae h of mentum shorter than distance between them. Formula of genital setae: 6: 3. Anoadanal plates with very short setae, diminishing posteriorly, setae ad_3 minuscule.

Setation of legs of "complete type". Setae d of femora I spiniform and slightly remote from distal end of article.

Type. Holotype. Ukraine (no U 96-23), „Tovtra Puszcza", Miodobory locality, wood dust from sycamore trunk in oakhornbeam forest, 28 VIII 1996, leg. A. Szeptycki.

Etymology. The prefix *para* is Latin meaning "near" and refers to similarity of the new species to the species *Atropacarus* (*A*.) *serratus* (Feider et Suciu, 1957).

Comparison. The new species is similar to *Atropacarus* (*A*.) *serratus* (Feider et Suciu, 1957) in the longest setae h and ps of notogaster but the notogastral setae are not so inflated and the number is 20, not 19 pairs. The second similar species *A*. (*A*.) *csiszarae* (Balogh et Mahunka, 1979) has also 19 pairs of notogastral setae of similar length and sigillar fields of prodorsum not joined.

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