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Concentration and bioaccumulation of organochlorine pesticide residues in herons and their prey in wetlands of Thermaikos Gulf, Macedonia, Greece

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Abstract

Concentrations of the principal organochlorine insecticides were determined in eggs and freshly dead chicks of the Squacco heron (*Ardeola ralloides*), Little Egret (*Egretta garzetta*) and Night Heron (*Nycticorax nycticorax*), as well as in frogs (*Rana* sp.), the main heron prey. Material was collected from the wetlands of the Thermaikos Gulf (Macedonia, northern Greece) in 1992 and 1993. Residues of the organochlorine pesticides α-BHC, β-BHC, lindane, 4,4′-DDD, 4,4′-DDE, heptachlor and dieldrin were found in the eggs, chicks and prey of the herons. α-BHC, β-BHC, and lindane had highest concentration in the Night Heron and lowest in the Little Egret. In all samples examined, the bioconcentration factors (BCF) of these compounds had very high values. BCF of pollutants for the eggs of the Squacco Heron were at lower levels than those of its chicks. BCF for frogs were in almost all cases lower than those for the other samples. Biomagnification factor (BMF) for 4,4′-DDE and β-BHC had the highest values of all other compounds (except in the Night Heron). BMF for the eggs of the Squacco Heron were greater than for its chicks. Variation in the pesticide contents in the different heron species is attributed to different feeding habits; the exception being the occurrence of dieldrin in eggs only and 4,4′-DDE as a remnant of past spraying. Amounts of pesticides detected in this study are too low to affect eggshell thickness in the Squacco Heron or have other effects on the wildlife of the area.

Keywords: Wetlands; Thermaikos Gulf; Greece; Herons; Bioaccumulation; Organochlorine pesticides

1. Introduction

Organochlorine insecticides are widespread environmental contaminants in the global ecosystem [1–4]. Because of their properties, long-term studies on their residue levels are essential in order to understand the extent of environmental contamination in the past and to predict future trends. The temporal trend data may help to prevent further contamination of toxic chemicals and their biological impacts in the earliest stages [4]. In Greece, organochlorine insecticides were widely used in agricultural during the 1950s and 1960s, a period of a rapid development, but were banned in 1972.

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The value of waterbirds as bioindicators of wide-spread marine pollution by lipophilic compounds has been recognized for a long time [2, 4–10]. The degree of variation between samples is favourably comparable with other groups of marine organisms [7]. Relating data from biological indicators to the environment is, however, a complex procedure. Information must be available on the birds' diets and the seasonal range of population sampled. Such information must then be assessed in the light of knowledge of the metabolism and chemodynamics of individual chemicals.

Waterbird tissues and eggs have been analysed for organochlorines from some areas in the Mediterranean region [11–16]. The only study that reported from Greece concerned measurements of trace elements and chlorinated hydrocarbons in eggs of *Pelecanus crispus* in NW Greece [17].

The aims of this study are to assess the level of concentration and bioaccumulation of organochlorine pesticides in eggs, chicks and in the main prey of selected waterbirds nesting in wetlands of the Thermaikos Gulf.

2. Material and methods

2.1. Study area

The wetland complex of Thermaikos Gulf constitute one of the most important and diverse ecosystems for wildlife in Greece, extending over 68.7 km^2 and is protected by the Ramsar Convention (1971) (Fig. 1). This area includes a rich avifauna. About 80 bird species, which are protected by the EEC Directive 79/409, characterized as endangered in Europe, occur in these areas [18]. In spring, Little Egret (*Egretta garzetta*), Night Heron (*Nycticorax nycticorax*) and Squacco Heron (*Ardeola ralloides*) breed in the region in great numbers These heron species breed on trees and bushes at the estuary of Axios rivers, about 1 km from the sea coast (Fig. 1).

The prey of these heron species includes fish such as Sticklebacks (*Gasterosteus aculeatus*), *Aphanius fasciatus*, insects such as Odonata and Orthoptera, and crustaceans; but the most important prey are frogs (*Rana* species (Kazantzidis, Papakostas and Goutner, unpublished data).

Important agricultural areas of Northern Greece drain into the Thermaikos Gulf through the rivers Axios, Loudias and Aliakmon, which also discharge heavy metals [19] and transfer pesticide residues which are toxic and hazardous for various biological organisms.

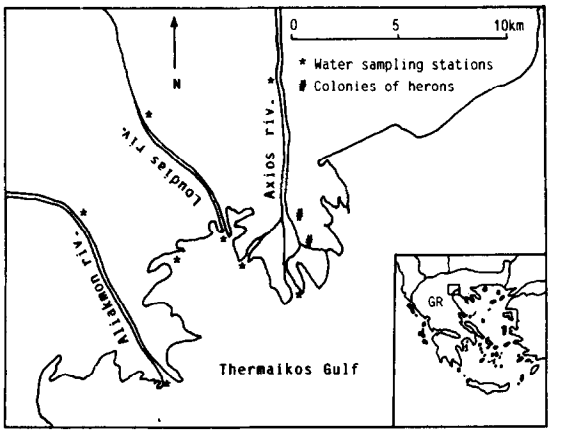

Fig. 1. Map of the region of Thermaikos Gulf including the study area.

2.2. Sampling procedures

In the first half of May 1992 and 1993, eggs of Little Egret and Squacco heron were collected in the Axios heronry. These eggs had rolled out of their nests due to the birds' activities or due to windy weather conditions. During May and June of 1992 and 1993, freshly dead nestlings of Night Heron and Squacco Heron were also collected from the same heronry. These nestling fell out of their nests and died because of injuries or were dead in the nest. Frog samples were collected from foraging habitats of the study heron species in the Axios and Aliakmon river deltas by a hand net. After collection, the bird eggs were brought to the laboratory, frozen, freeze-dried, broken up and homogenised. The residual water content was determined by the freeze-dried material (24 h at 105°C). Dead birds and frogs were kept frozen, at −20°C, in aluminium foil until laboratory analysis.

2.3. Analytical procedures

The contents of every egg and bird tissues were analysed for a series of organochlorine contaminants including: 2,4′-DDT, 2,4′-DDD, 2,4′-

DDE, 4,4′-DDT, 4,4′-DDD, 4,4′-DDE, α-BHC, β-BHC, lindane, heptachlor, heptachlor epoxide, aldrin, dieldrin and endrin.

2.3.1. Egg analysis. The analytical method described by Crivelli et al. in 1989 [17] was used for the egg extraction with slight modifications after verification in our conditions. Whole eggs were homogenized in a blender and an aliquot of 10–15 g was homogenized again with 50 g sodium sulfate. This mixture was Soxhlet extracted for 12 h with 250 ml *n*-hexane (pesticide grade). The extract was evaporated in a rotary evaporator to 15 ml and a portion of 10 ml, subjected to sulphuric acid clean-up [20,21] followed by Florisil chromatography (50–100 mesh from Merck), after overnight drying at 200°C, was eluted with 100 ml of *n*-hexane. The purified sample was evaporated in a rotary evaporator to ~10 ml and in a N_2 stream at 35°C to ~0.5 ml for the organochlorine compounds analysed by gas chromatography.

2.3.2. Bird and frog tissue analysis. The analytical method for frog and bird samples described by Martin and Hartman in 1985 [22] was used after slight modifications. About 20 g of frozen bird or frog samples were homogenized in a blender with 100 g anhydrous sodium sulfate. The mixture was then transferred to a Waring blender and 125 ml petroleum ether were added. The mixture was blended at a medium speed for ~2 min [22]. Petroleum ether was decanted through a 100 mm powder funnel with solvent-washed glass wool into a 500 ml Erlenmeyer flask. Then, 125 ml portion of petroleum ether was added twice to the blender cup, blended again for 2 min and decanted through the same powder funnel. The total extract was concentrated to ~5 ml and 15 ml of *n*-hexane were added. The solution was evaporated in a N_2 stream at 35°C to ~10 ml. The extract was subjected to sulphuric acid clean-up [20] and the purified sample was analysed for organochlorine residues by gas chromatograph. Results are expressed in μg/kg on a whole frog or bird chick basis.

2.3.3. Water and sediment analysis. Eight sampling stations were established in the estuaries of rivers Axios, Loudias and Aliakmon in the period between January 1992 and November 1993 for the collection of water samples. The analytical procedures for water have fully been described by Albanis et al. [23]. Water analyses data were used in bioaccumulation estimations of organochlorine residues in studied organisms.

2.3.4. Quantification. The purified final samples were analysed by a Varian 3300 gas chromatograph equiped with a Ni63 electron capture detector (ECD) and two glass columns (2 m long, 0.3 mm I.D.) packed with 1.5% OV-17 + 1.95% OV-210 on chromosorb Q (100–120 mesh) and 4% SE-30 + 6% SP-2401 on Supelcoport (100–200 mesh). Confirmation and the determination of organochlorine pesticides were made using a Shimadzu gas chromatograph equipped with ECD and a capillary column DB-1, 30 m long, I.D. 0.32 mm. Pure reference standard solutions (from Supelco) were used for instrument calibration, recovery, quantification and confirmation. Recoveries of pesticides from fortified chicken eggs and tissues, and frog tissues ranged from 90–104%, 84–112% and 77–109%, respectively.

Table 1 gives the retention times obtained for the 13 selected organochlorine compounds by GC-ECD using the packed columns 1.5% OV-17 + 1.95% OV-210 and 4% SE-30 + 6% SP-2401, as well as the capillary column DB-1. Fig. 2 shows the simultaneous determination of typical chromatograms, obtained from water samples spiked with 13 compounds at 0.2–25 μg/l and from Squacco heron eggs.

2.4. Measurement of eggs and eggshell thickness

Maximum length and breadth of 20 Squacco Heron eggs were measured by slide callipers to the nearest 0.01 mm. Their weight and volume were measured to the nearest 0.01 g and 0.1 ml. Eggshell thickness was measured by a micrometer graduated in units of 0.05 mm. Measurements of all parameters did not differ significantly between 1992 ($n = 12$) and 1993 ($n = 8$) (t-test, $P > 0.05$ in all cases), so data were pooled.

3. Results

Residues for the pesticides β-BHC, β-BHC, lindane, 4,4′-DDD, 4,4′-DDE, heptachlor and dieldrin found in the eggs, chicks and prey of the

Table 1
Analysed organochlorine compounds and their retention times in three columns

No.	Compounds	t_r (min)		
		1.5% OV-17 + 1.95% OV-210	4% SE-30 + 6% SP-2401	DB-1
1	α-BHC	2.56	1.89	4.79
2	β-BHC (lindane)	3.22	2.87	5.08
3	β-BHC	3.76	3.45	5.51
4	Heptachlor	4.09	3.76	8.47
5	Aldrin	4.71	4.25	10.35
6	Heptachlor epoxide	5.67	4.89	12.57
7	4,4′-DDE	7.13	6.82	18.39
8	Dieldrin	10.20	9.48	18.39
9	2,4′-DDD	10.92	9.87	18.84
10	Endrin	11.97	11.43	20.16
11	2,4′-DDT	13.25	12.49	23.14
12	4,4′-DDD	14.25	13.53	24.42
13	4,4′-DDT	15.67	14.27	30.46

herons are shown in Table 2. Other organochlorine pesticides such as 2,4′-DDD, 2,4′-DDE, 2,4′-DDT, 4,4′-DDT, heptachlor epoxide, aldrin, and endrin were not detected at all. In the tissues of the three heron species examined, α-BHC, β-BHC and lindane were commonly found. All three compounds had highest concentration in the Night Heron and lowest in the Little Egret, whose tissues also contained the lowest number of pollutants (Table 2). Of the other pesticides, heptachlor was only detected in Squacco Heron, 4,4′-DDD in Night Heron and 4,4′-DDE in the tissues of both species' chicks.

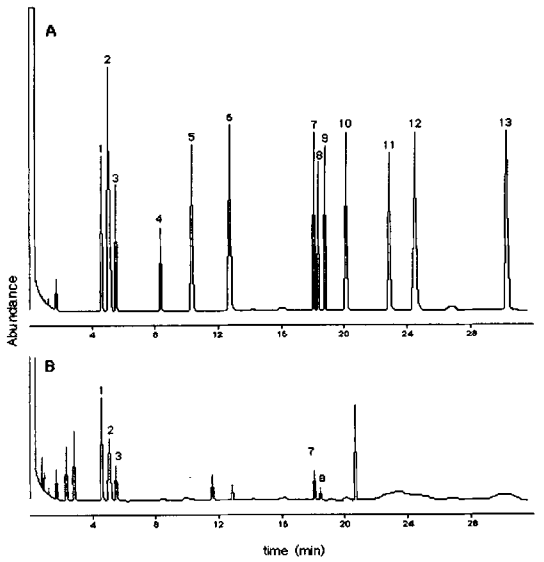

Fig. 2. GC-ECD chromatogrammes for (A) ~0.2–25 μg/l of 14 selected organochlorine compounds in spiked water and (B) Squacco heron egg sample under the same conditions at a DB-1 column 30 m long, containing 5% methylsilicone, at 200°C. For peak numbers, see Table 2.

Four of the detected pollutants, namely α-BHC, β-BHC, lindane and 4,4′-DDE were commonly found in eggs and chicks of the Squacco Heron, but were in greater concentrations in the latter. Heptachlor was detected in the chicks, but not in the eggs and, inversely, dieldrin was found in eggs, but not in chicks of the Squacco Heron.

All pollutants found in heron chicks were also detected in frogs (Table 2), their main prey, but at lower concentrations, except β-BHC which, on average, was a little higher than that found in the Little Egret.

Of the organochlorine pesticides, three (α-BHC, lindane and 4,4′-DDE) were also found in water, but their concentrations were very low compared with those found in herons and frogs.

The available data allow the calculation of bioconcentration factors (BCF: concentration in organism/concentration in water) values only for the compounds β-BHC, lindane and 4,4′-DDE (Fig. 3). These values were relatively low in frogs

Table 2
Mean and range of organochlorine concentrations in herons and frogs (μg/kg dry weight) and water (μg/l) at Thermaikos Gulf

Compound		Squacco Heron		Little Egret nestlings (n = 8)	Night Heron nestlings (n = 9)	Frogs (n = 10)	Water (n = 144)
		Eggs (n = 20)	Nestlings (n = 11)				
α-BHC	x ± S.D.	6.45 ± 3.84	7.58 ± 3.96	2.23 ± 1.48	9.72 ± 4.34	2.45 ± 1.24	0.002
	Range	(nd–16.15)	(nd–26.83)	(nd–4.24)	(nd–26.8)	(nd–3.60)	(nd–0.01)
β-BHC	x ± S.D.	6.34 ± 4.81	8.22 ± 4.58	7.90 ± 3.21	12.24 ± 5.84	0.56 ± 0.46	—
	Range	(nd–17.09)	(nd–18.94)	(0.21–12.5)	(nd–21.1)	(nd–1.19)	(nd)
Lindane	x ± S.D.	2.23 ± 1.87	10.34 ± 6.62	5.56 ± 3.27	20.2 ± 11.17	3.64 ± 4.43	0.023
	Range	(nd–6.68)	(nd–16.7)	(0.56–8.54)	(nd–31.4)	(nd–30.9)	(nd–0.21)
Heptachlor	x ± S.D.	—	3.52 ± 1.71	—	—	1.46 ± 0.76	—
	Range	(nd)	(nd–6.12)	(nd)	(nd)	(nd–3.82)	(nd)
4,4′-DDD	x ± S.D.	—	—	—	0.67 ± 0.32	0.49 ± 0.23	—
	Range	(nd)	(nd)	(nd)	(nd–2.00)	(nd–1.35)	(nd)
4,4′-DDE	x ± S.D.	3.80 ± 2.18	6.46 ± 3.72	—	1.46 ± 0.46	0.29 ± 0.17	0.006
	Range	(nd–6.52)	(nd–12.65)	(nd)	(nd–6.51)	(nd–0.64)	(nd–0.03)
Dieldrin	x ± S.D.	0.15 ± 0.06	—	—	—	—	—
	Range	(nd–0.24)	(nd)	(nd)	(n.d)	(nd)	(nd)

x, mean; S.D., standard deviation; nd, not detectable.

compared with those found for eggs and heron tissues, indicating an accumulation of 1225, 158 and 48 times in relation to water. The respective BCF values of eggs and heron tissues were higher (except in the case of lindane in the eggs of the Squacco Heron and of β-BHC in the Little Egret having similar value to frogs) suggesting a considerable bioconcentration. β-BHC, lindane and 4,4′-DDE accumulated 3225, 97 and 633 times, respectively, in the eggs and 3790, 449 and 1077 times in tissues of of the Squacco Heron; and 4860, 878 and 243 times in tissues of the Night Heron. Accumulation of β-BHC was 1115 times and lindane was 242 times in the Little Egret. Such estimations were not

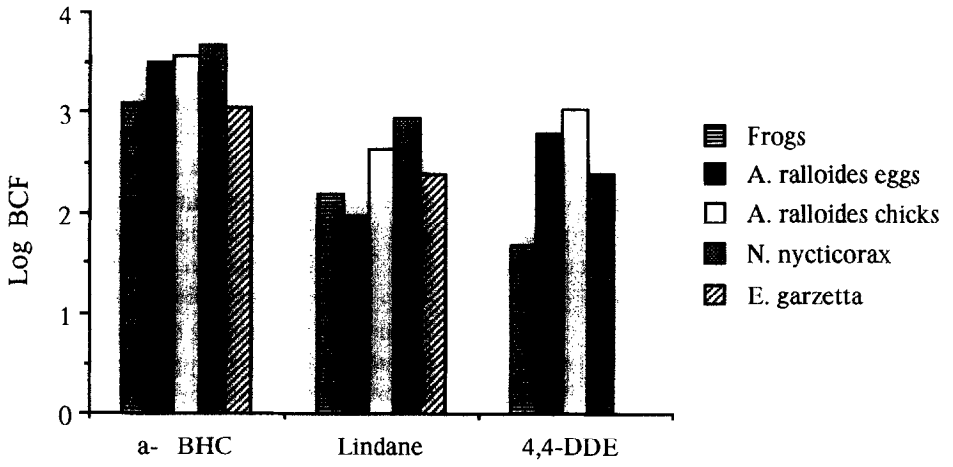

Fig. 3. Comparative mean values of bioconcentration factors (BCF) of organochlorine pesticides in herons and their prey in Thermaikos Gulf wetlands.

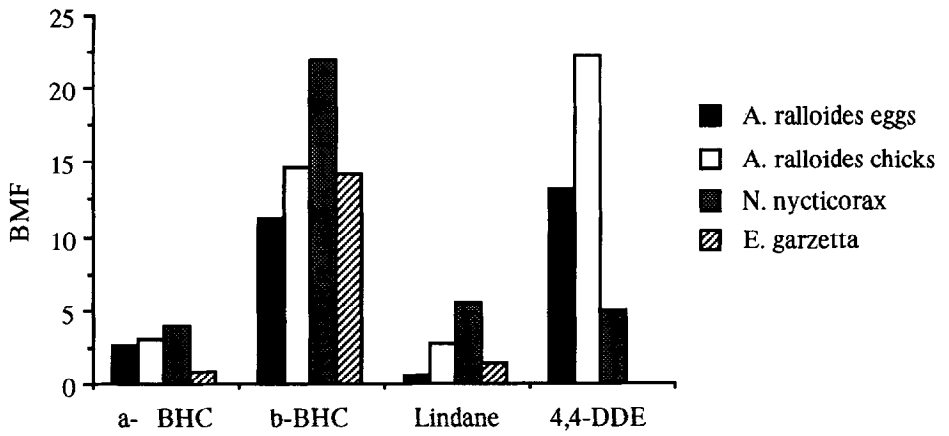

Fig. 4. Comparative mean values of biomagnification factors (BMF) of organochlorine pesticides in herons in relation to their prey at Thermaikos Gulf wetlands.

possible for the other compounds detected in organisms, since these compounds were not detected in water samples in the study area.

The biomagnification factor (BMF = the ratio of organochlorine concentration in the eggs and tissues of herons to organochlorine concentration in frogs) were very much lower than those of BCF. The BMF value for α-BHC was lower than that of β-BHC in all biological material examined (Fig. 4). BMF was a little greater (1.2 times) for the tissues than for the eggs of the Squacco Heron and it was again highest for the Night Heron and lowest for the Little Egret chicks (4.4 times).

The BMF values for β-BHC were a little higher in the chicks than in the eggs of the Squacco Heron; this was again highest for the Night Heron and lowest for the Little Egret chicks (1.5 times). With the exception of the Night Heron, the BMF for 4,4′-DDE and β-BHC had the highest values from all other compounds in the biological material examined. β-BHC, although not detected in water, had a considerable bioaccumulation in most of the organisms examined.

For lindane, BMF was greater for the chicks than in the eggs of the Squacco Heron; it was highest for the Night Heron and lowest for the Little Egret chicks (3.6 times). For heptachlor, BMF was 2.41 in the chicks of the Squacco Heron. 4,4′-DDD had a low BMF value (0.73) in the Night Heron (where it was only detected), 6.9 times lower than 4,4′-DDE. For 4,4′-DDE, BMF was greater in the chicks than in the eggs of Squacco Heron, and both were higher than that in the Night Heron.

Of the eggshells of the 20 Squacco Heron eggs examined, eight were 0.25 mm thick and ten 0.20 mm (mean 0.22 ± 0.03 mm). The amounts of 4,4-DDE found in these two thickness groups did not differ significantly ($Z = -0.089$, NS, Mann-Whitney U-test).

4. Discussion

Of the organochlorine pesticides commonly found in samples from the three heron species studied, α-BHC and lindane were also found in water and frogs. These pollutants exhibited a considerable bioaccumulation in the material examined and this takes place through the food chain [24]. The differences in the amounts of these pesticides between the species may be mainly due to differences in the feeding habits and foraging habitat of each species, as supported by other studies on heron carcasses and/or eggs [25–27]. During the breeding period in our study area, the Little Egret used freshwater, saltmarshes and ricefields as foraging habitats [28]. The Night and Squacco Heron never used saltmarshes for feeding, only the

other habitats. The lowest amounts of pollutants were detected in the Little Egret chicks, probably because their prey is more variable than the other two species [29]. Additionally, of all these frog-eating species, the Night Heron fed their young with the largest frogs [29]. This could explain highest amounts of the above-mentioned pollutants being found in the chicks of the Night Heron, as it is expected that large frogs should contain higher amounts of pesticides. In this study, the bioaccumulation through the food chain was more evident in the Squacco Heron because eggs were also analysed. Only lindane was not biomagnified in eggs for unknown reasons.

The presence of β-BHC in frogs supports the idea that it passed through the food chain and hence was detected in Squacco Heron eggs and in all heron species' chicks. On the other hand, there was no detectable concentration of β-BHC in water or sediment which may indicate that it was used locally in the study area, probably in rice fields, during the study period.

Detection of both metabolites of DDT, 4,4′-DDD and 4,4′-DDE in frogs and in the Night Heron and absence from the Little Egret chicks may denote differences in the foraging habitats used, as mentioned above. The same may hold true for the Squacco Herons as far as 4,4′-DDD is concerned, but for 4,4′-DDE the case may be different as this compound is very resistant [30,31] and is still detected in the study area, evidently as a remnant from spraying in the past, passing into the food chain.

Detection of heptachlor in frogs and in chicks, but absent in eggs of the Squacco Heron indicates that this pesticide was taken up by the birds through their prey, collected locally. Inversely, as dieldrin was only found in Squacco Heron eggs and in no other type of sample, it could only come from the adult female deposits. Various studies in egrets and herons (including the Night Heron) have supported the idea that similar pollutants originate from contaminated wintering areas of the adult females [32–36]. One Squacco Heron ringed in our study area as a chick in 1983 was recovered in Ghana in the autumn of the same year (G. Papakostas, pers. commun.). Additionally, Cramp and Simmons [37] mention recoveries of Squacco Heron ringed in the neighbouring countries of Bulgaria and the former Yugoslavia. These suggest that birds from our region may winter in central Africa where pesticides banned in Europe for many years are still used extensively.

At present it is difficult to obtain a complete picture about the environmental behaviour of organochlorine pesticides in the marine habitats of the wetlands of Thermaikos Gulf due to the limited amount of information available. The amounts of pesticides detected in all of our samples were very low, much lower than the previously reported examinations of eggs in the Mediterranean and Black Sea areas for other waterbirds (the Night Heron included) during the period 1984–1987 [14,15,38, 39]. The amounts detected in our biological samples are well below those which are known to cause harmful effects to birds and other animals [26,30]. Thus, it seems logical that the DDE contained in the Squacco Heron egg samples had no effect on their eggshell thickness, whereas numerous studies during the periods of 1975, 1978 and 1984 on herons and other birds have shown an inverse correlation of these two parameters [30,40,41].

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