Lead (Pb) and Copper (Cu) Concentration in the Eggshells of Audouin’s Gulls (Larus audouinii) in Turkey

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Abstract: Audouin’s gull (Larus audouinii) eggshells from 2 archipelagos (Karaburun-İzmir and Aydınçık-Mersin) in Turkey were collected between 2002 and 2003, and analyzed for lead (Pb) and copper (Cu) content. According to the results, mean Cu level of the eggshells was 1.855 ppm for the Aydınçık colony and 10.202 ppm for the Karaburun colony. Mean Pb level of the eggshells was 0.954 ppm for the Aydınçık colony and 4.601 ppm for the Karaburun colony. The detected metal levels did not seem to have any toxic effects on the Audouin’s gull populations. It is suggested that eggshells from abandoned nests of Audouin’s gulls may be useful as bio-indicators for monitoring heavy metal contamination.

Key Words: Audouin’s gull, eggshells, Cu, Pb, Turkey

Introduction

The number of Audouin’s gulls (Larus audouinii) in the Mediterranean is estimated to be 18,600 pairs. Approximately 70% of the Audouin’s gull population breeds at the Ebro Delta in Spain. The remaining part of the population is distributed among the Chafarinas islands (Spain) and Aegean islands. The Hellenic Ornithology Association reported 20 colonies consisting of 530 pairs on the Greek islands and islets in the study they conducted in 1997 (Oro, 1998). Additionally, there were 4 colonies reported in Algiers, 1 colony on Cyprus, 2 colonies in France, 10 colonies in Italy, 4 colonies in Morocco, and 1 colony in Turkey. The most northern breeding site of the bird is Gorgona Island, Tuscany, Italy (Int. Act. Plan for A., 1996).

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watchers. There were 5 adult pairs and 1 young gull recorded; however, a detailed investigation of the pairs, which bred after this observation at the end of April (Çağlayan, 2003), were not made. With the presence of the Audouin’s gull, Aydıncık Island was declared an important bird site (IBS) (Kılıç and Eken, 2004). Moreover, Aydıncık and Karaburun islands were declared natural sites of first degree importance. The first detailed investigation of Audouin’s gulls (Larus audouinii) in Turkey was carried out between 2001 and 2003. According to this there were 17 pairs observed on Aydıncık Island in 2001 and 8 pairs in 2002, and there were 18 pairs of Audouin’s gulls (Larus audouinii) recorded on Karaburun Island.

It was observed that these small colonies chose Aydıncık and Karaburun islands as their breeding grounds; however they left the islands during certain years due to excessive fishing, animal grazing, egg collection, and competition with herring gulls (Çağlayan, 2003). Previously, no studies have been conducted in Turkey on the deposition of contaminants in their breeding grounds and habitats, or on the passage of these contaminants to their eggs.

Studies related to metal contamination in the Audouin’s gull (Goutner et al., 2000) were carried out in the western Mediterranean Basin, mainly in Italy (Leonzio et al., 1989) and at the Ebro Delta and on the Chafarinas islands of Spain (Morera et al., 1997). There are no studies related to metal levels in the colonies of Turkey, which is the most eastern breeding ground of the population.

The aim of the present study was to report reference values for continuous monitoring in the future and to compare colonies under stress from different pollutants. It is hoped that this study will be a suitable reference for the determination of regional contamination and realization of a protection plan for this top predator species.

Material and Methods

Collection Sites and Sampling

In this study, 2 known archipelagos in Turkey (Aydıncık and Karaburun) with Audouin’s gull colonies were selected for eggshell collection. Aydıncık consists of 1 big and 1 small islet about 2 km off the southern Mediterranean coast of Turkey, 5 km southeast of Aydincık Township in Mersin Province, located at lat 36º08’N, long 33º20’ E. Karaburun Island is within the boundaries of Karaburun Township in İzmir Province, on Turkey’s Aegean coast. It is approximately 1.5 km from the port of Karaburun. Its southern slope is covered with dense bushes. There are steep rocks coming from the sea on the northern and northwestern parts of the Island.

The eggshells were collected from deserted nests on both islands at the end of the breeding period. There were 8 eggshells collected from Aydıncık Island and 10 eggshells from Karaburun Island, corresponding to different nests in order to avoid pseudoreplication. Collected eggshells were labeled according to nest number, placed in chemically cleaned glass jars, and transported to the laboratory.

Chemical Analysis

Square wave stripping voltammetry (SWASV) is much more suitable to our purposes than linear wave stripping voltammetry due to its much shorter deposition time and lower detection limit (Somer and Ülkü, 2004); therefore, SWASV (Locatelli and Torsi, 2004) was used to measure Cu and Pb in the eggshells of Audouin’s gulls. Analyses were carried out by the standard addition method using the increase in the magnitude of the stripping peak.

Eggshells were not cleaned before metal analysis. Samples (0.2 g) of each eggshell batch were placed in beakers and dried at 100 °C until they reached constant weight. The egg samples were digested in nitric acid and then 1.5 ml of ultra pure HNO₃ (Merck) was added to each sample. The temperature was gradually increased until the sample was completely dissolved (without complete dryness) and 10 ml of de-ionized water was added to each of them. All the reagents were of analytical grade and all solutions were prepared with de-ionized water.

The heavy metals in the dissolved samples were first determined using a Perkin-Elmer Optima 4300 DV ICP-OES spectrophotometer; however, metal content was too low to give meaningful results; therefore the determinations were carried out with the SWASV method using a CHI 660 B electrochemical analyzer equipped with a BAS cell stand. The working and auxiliary electrodes included a hanging mercury drop electrode (HMDE) and Pt wire, and the potentials were measured against a Ag/AgCl (3 M KCl) reference electrode. To the electrochemical cell, 1.5 ml of the dissolved sample was
transferred using perchloric acid (70%, Merck) as a supporting electrolyte and to acidify the working medium. Then, 0.1 M of stock solutions prepared from Cu (NO₃)₂ (Merck) and Pb (NO₃)₂ (Merck) were used for the preparation of standard addition solutions of 10⁻³, 10⁻⁴, and 10⁻⁵ M by daily dilution. The increase in the magnitude of the peak after standard additions of the stock solutions was recorded to determine the heavy metal content. Two blank solutions were prepared using equivalent amounts of acid, and Pb and Cu were determined in these solutions and extracted to calculate the actual amounts present in the samples. The detection limit was 0.1 ppm for each metal.

**Statistical Analysis**

Pb and Cu concentrations in the eggshells collected from the Aydıncık and Karaburun colonies were compared. Descriptive statistics are shown in the Table. Since the sample size was very small, and Cu and Pb data were not normally distributed, we used nonparametric Kruskal-Wallis statistical procedures. Correlations were analyzed between Pb and Cu values for the 2 islands using Spearman’s rank correlation coefficients. Statistical analyses were performed using SPSS v.13.0.

**Results**

Pb and Cu concentrations in the eggshells belonging to the Aydıncık and Karaburun colonies are shown in Table, with mean, median, standard deviation, and variance values. There was a significant correlation between Pb and Cu in the Karaburun colony (r = 0.671, P < 0.05). On the other hand, there was a strong correlation between the 2 metals in the Aydıncık colony (r = 0.972, P < 0.001). Pb concentration of 5 of the 8 samples collected from the Aydıncık colony ranged between 0.59 and 2.62 ppm. Pb content in 9 of the 10 samples collected from the Karaburun colony ranged between 0.98 and 18.42 ppm. Mean eggshell Pb concentration in the Karaburun colony (4.601 ± 5.804 ppm) was much higher than in the Aydıncık colony (0.954 ± 1.005 ppm). The difference in Pb levels was statistically significant (H = 3.04; P > 0.05).

Cu in 5 of the 8 Aydıncık samples ranged from 0.44 to 5.86 ppm, while Cu in 9 of the 10 Karaburun samples ranged between 0.56 and 49.953 ppm. Mean Cu level in the Karaburun eggshells (10.202 ± 16.040 ppm) was much higher than that of the Aydıncık eggshells (1.855 ± 2.570 ppm). Cu content in the Aydıncık samples was

<table>
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<th>Eggshell number</th>
<th>Pb</th>
<th>Cu</th>
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<tr>
<td></td>
<td>Karaburun</td>
<td>Aydıncık</td>
</tr>
<tr>
<td>1</td>
<td>8.89</td>
<td>1.25</td>
</tr>
<tr>
<td>2</td>
<td>18.42</td>
<td>2.62</td>
</tr>
<tr>
<td>3</td>
<td>1.34</td>
<td>0.59</td>
</tr>
<tr>
<td>4</td>
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<tr>
<td>5</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>6</td>
<td>2.42</td>
<td>nd</td>
</tr>
<tr>
<td>7</td>
<td>1.17</td>
<td>2.11</td>
</tr>
<tr>
<td>8</td>
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<tr>
<td>9</td>
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<tr>
<td>10</td>
<td>8.89</td>
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<tr>
<th></th>
<th>n</th>
<th>Mean</th>
<th>Median</th>
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<th>Max</th>
<th>Std Dev</th>
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<tbody>
<tr>
<td>Pb</td>
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<td>4.601</td>
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<tr>
<td>Cu</td>
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<td>10.202</td>
<td>2.11</td>
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<td>49.95</td>
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</table>
homogenous; however, Cu content in the Karaburun samples was heterogeneous, with high variance (Table). Although Cu content in the eggshells collected from Karaburun seemed to be significantly higher than that of the eggshells collected from Aydıncık, the difference in Cu levels was not statistically significant ($H = 2.17; P > 0.05$).

**Discussion**

Due to the limited number of eggs found in the nests, the studies were conducted with broken eggshells rather than egg contents in order to avoid inducing any adverse effect on the breeding activities of the birds. Materials used to determine metal accumulation in birds are internal organs and egg content (Stronkhorst et al., 1993), eggshells (Burger, 1994; Morera et al., 1997; Ayas, 2007), feathers (Burger, 1993; Goutner et al., 2001), and feces (Dauwe et al., 2000).

It was estimated that the number of gulls in the studied colonies would increase in the future, provided that anthropogenic factors, such as fishing and grazing, are appropriately controlled. The limited number of collected samples represents a problem that hampered statistical analysis. The metal values found in eggshells were highly variable, with high intra group variances (Table). For instance, Pb levels observed in the Karaburun Island eggshells were very different from each other and significantly higher than those in the Aydıncık samples; however, the difference in Pb levels in the eggshells obtained from the 2 islands were not statistically significant due to high variation in the Karaburun values.

The fact that some of the Karaburun samples had Cu and Pb levels higher than those of the Aydıncık samples was probably due to the close proximity of Karaburun to the industrial regions and high pollution levels of İzmir Bay. Previous studies reported that the rivers and streams flowing into the Aegean Sea are the main sources of heavy metal pollution in İzmir Bay (TÇSV, 1991; Tekin, 1992; Balcı and Turkoglu, 1993).

It is known that heavy metals in the environment can be accumulated by gulls and pass into their eggs (Furness, 1993). Arıç and Şen (1999) determined that heavy metals are deposited in sediments and in pelagic fish, which are the main food source of gulls. Burger (1994) found that egg laying was one of the methods of removing heavy metals from the organs of herring gulls (Larus argentatus). Scheuhammer (1987) stated that the passage of heavy metals to gull eggs could only take place when there is an excessive accumulation of heavy metals in the organs of the adult birds.

Goutner et al. (2000) explained that the high level of mercury accumulation observed in the feathers of Audouin’s gulls was due to the elevated concentration of mercury in the Aegean Sea, resulting from the high pollution load of the rivers and streams flowing into it. Aydıncık is very far from any industrial region, and the rivers flowing near it pass through agricultural areas rather than industrial. The largest source of water close to Aydıncık Island is the Göksu River. Previous studies revealed that the pollution load of this river consists mainly of agricultural contaminants, such as organochlorine pesticides and fungicides containing mercury, zinc, and manganese (Ayas and Kolankaya, 1996; Ayas et al., 1997).

The Cu level in seabirds is general fairly small (0.15-1.8 ppm/fresh-weight) (Walsh, 1990; Stronkhorst et al., 1993). Morera et al. (1997) reported that the level of Cu in egg contents and eggshells of Audouin’s gulls was 2.58 and 2.14 ppm, respectively. Leonzio et al. (1989) found that the eggs of Audouin’s gulls were heavily contaminated with mercury and chloride-containing hydrocarbons, and that the level of Pb was > 0.1 ppm; however, in the present study the mean Pb level in Audouin’s gull eggshells from the eastern Mediterranean were much higher (from Karaburun: 4.601 ppm; from Aydıncık: 0.954 ppm).

The metal content of eggshells can provide a good idea about the metal content of egg contents. Morera et al. (1997) found that the Cu level in egg contents and eggshells were very close to each other (2.58 and 2.14 ppm, respectively). In some studies the content of certain metals in egg contents were 2-35 times higher than the corresponding concentrations in eggshells (Mora, 2003); therefore, it can be concluded that the Cu and Pb levels in egg contents of the Audouin’s gull colonies on Karaburun and Aydıncık islands must be at least equal to or higher than the respective concentrations determined in their eggshells.

As seen from the Table, the levels of Cu and Pb in each egg sample were quite different. This difference is more apparent in the Karaburun samples. The main reason for this difference is the restricted number of samples; however, this may also be due to other
environmental and physiological factors, such as environmental pollution load, age of the egg laying female, and inter- and intra-clutch variability (Becker, 1989; Sanpera et al., 1997).

In seabirds, Becker (1992) found inter-clutch differences in Hg concentration, both in Larus argentatus and Sterna hirundo, as well as decreasing Hg levels according to the laying sequence (a-, b-, c- eggs). Additionally, Morera et al. (1997) detected a significant female effect on Hg concentrations in Larus audouinii. Morera et al. (1997) found that concentrations of elements such as Zn, Cu (which was also determined in our study), Mn, and Hg were 80%-99% greater in the egg contents of Audouin’s gulls than in eggshells (Larus audouinii).

In conclusion, the realization of population and pollution studies related to Audouin’s gull will be useful to future planning and programs on behalf of its protection. Moreover, it is suggested that Audouin’s gull can be an appropriate bio-indicator organism for monitoring environmental pollution.

References


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