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An Innovative Approach to Biological Monitoring Using Wildlife

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1. Introduction

Biological monitoring using wildlife is a useful and important method that helps us to understand the degree of contamination in the environment. The book "Our Stolen Future" (Colborn et al., 1996) has become an influential bestseller worldwide; the authors of this book have pointed out issues relevant to the monitoring of the state of environmental pollution using wildlife. However, there are also many criticisms of the content of this book. For example, the designation of the control areas as non–contaminated is very difficult in the studies that use wildlife (Krimsky, 2000). In studies that use wildlife, there is a lack of epidemiological information on age, sex, movement range and detailed feeding habits. For example, the content of cadmium (Cd) in animals increases with age (Sakurai, 1997), even when the animals live in non-polluted areas. This is because Cd has a long biological half-life in animals (Friberg et al., 1974). Thus, knowledge of the age of targeted animals is necessary for accurate monitoring. However, obtaining an estimate of age in wildlife is very difficult. Carnivorous animals have been used frequently for biological monitoring (Harding et al., 1998; Helander, et al., 2009; Kenntner, et al., 2007; Meador et al., 1999) because it is well known that various contaminants are bioaccumulated in carnivorous animals as they move up the food chain. However, detailed information on feeding habits is sometimes difficult to obtain. According to bird guides, the greater scaup (*Aythya marila*) is classified as a carnivorous bird. However, its rate of intake of animal food changes between 45 and 97 % depending on the environment (Kaneda, 1996). In such a case, is it correct to categorize the scaup among carnivorous birds?

Despite the lack of epidemiological information, we have been investigating the degree of contamination of wild birds with inorganic elements such as Cd (Mochizuki et al., 2002a, 2011d; Ueda et al., 1998), chromium (Cr) (Mochizuki et al., 2002c), molybdenum (Mo) (Mochizuki et al., 2002c), thallium (Tl) (Mochizuki et al., 2005) and vanadium (V) (Mochizuki et al., 1998, 1999). However, there is also problem in the use of statistical procedures in studies that use wildlife because the distribution of the data is very wide. Normally distributed data are sometimes not obtained from samples of wildlife (Mochizuki et al., 2010b; Ueda et al., 2009a). The effects of toxic elements have also been investigated under experimental conditions using cultured bacteria (Kadoi et al., 2009), cells (Mochizuki et al., 2011b), and various experimental animals (Mochizuki et al., 2000). However, biological monitoring is important for the assessment of risk to human health.

Recently, we developed a solvent for use in biological monitoring using wildlife. This method was established using the significant regression lines obtained from the Cd content of kidney and that of liver (Mochizuki et al., 2008). Given that the data from animals were cited in various studies in which no particular contamination was described, we considered that these lines were indicative of normal metabolism in animals. This theory was supported by some evidence obtained from polluted animals, including humans (Mochizuki et al., 2008; Ueda et al., 2009a). Thus, the degree of contamination of humans (Mochizuki et al., 2008; Ueda et al., 2009a), experimental animals (Mochizuki et al., 2008; Ueda et al., 2009b), domestic animals (Ueda et al., 2011) and wild birds (Mochizuki et al., 2011a,c,d; Ueda et al., 2009a) has been analyzed using those indexes. Further, we developed a similar index for lead(Pb); the basis of this study was presented at an International Conference (Mochizuki et al., 2009), and the modified index has also been submitted to a journal for publication.

However, contamination with multiple elements is also an important problem in environmental science. Recently, we investigated the concentration of various elements in the urine of cats (Mochizuki et al., 2010c). In that study, a significant correlation was obtained among multiple elements in urine obtained from healthy cats, although a similar correlation was not observed in urine obtained from cats with urinary tract disease. A loss of balance and equilibrium among multiple elements had occurred in the urine of the diseased cats. This result suggested that similar indexes involving Cd and Pb can be obtained using measurement of multiple elements.

The new technique for biological monitoring is introduced in the first part of this chapter. Subsequently, we will attempt to establish an index to increase our understanding of the degree of contamination with multiple elements using multivariate analysis.

2. Introduction of CSRL and CEPE

In this section, we explained about Cd standard regression line (CSRL) and Cd equal probability ellipse (CEPE). We selected previous publications that reported the content of Cd in samples of 46 mammals and 55 birds, and we used 101 data points from 27 reports in which the Cd contents were represented as arithmetic means. The 101 data points were plotted on a graph with the Cd content in the liver on the abscissa and the Cd content in the kidney on the ordinate. A significant correlation was obtained, as follows: $Y=0.902X - 1.334$, $Y=\log(y)$, $X=\log(x)$, $R^2=0.944$, $p<0.01$. The regression line obtained after logarithmic transformation was $\log(Y)=0.900\log(X)-0.580$ ($R^2=0.944$, $p<0.01$)(1). When the outliers among the 101 data points were tested by equal probability ellipse, seven data points were identified as outliers as shown in Fig.2. After elimination of these seven points, the regression line obtained was: $\log(Y)= 0.941\log(X)-0.649$, ($R^2=0.965$, $p<0.01$)(2). There were no significant differences between the two lines (1&2) (Ueda et al., 2009a). In mention above, regression line obtained from 101 points and the equal probability ellipse were used as the Cd standard regression line, CSRL, and the Cd equal probability ellipse, CEPE, respectively.

The data from experimental animals to which Cd had been administered were distinct from the CSRL, as shown in Fig. 1.

Similarly, the data from humans who lived in a polluted area and from patients with Itai-itai disease were located outside the CEPE, as shown in Fig. 2. Although the values from humans who lived in non-polluted areas were high, the data were located within the CEPE, as shown in the figure (Fig.2). Detailed information on the references used (Mochizuki et al., 2008), the procedure for calculation of the indexes (Ueda et al., 2009a), and the data from humans and rushes monkeys (Mochizuki et al., 2008) have been described in our previous reports.

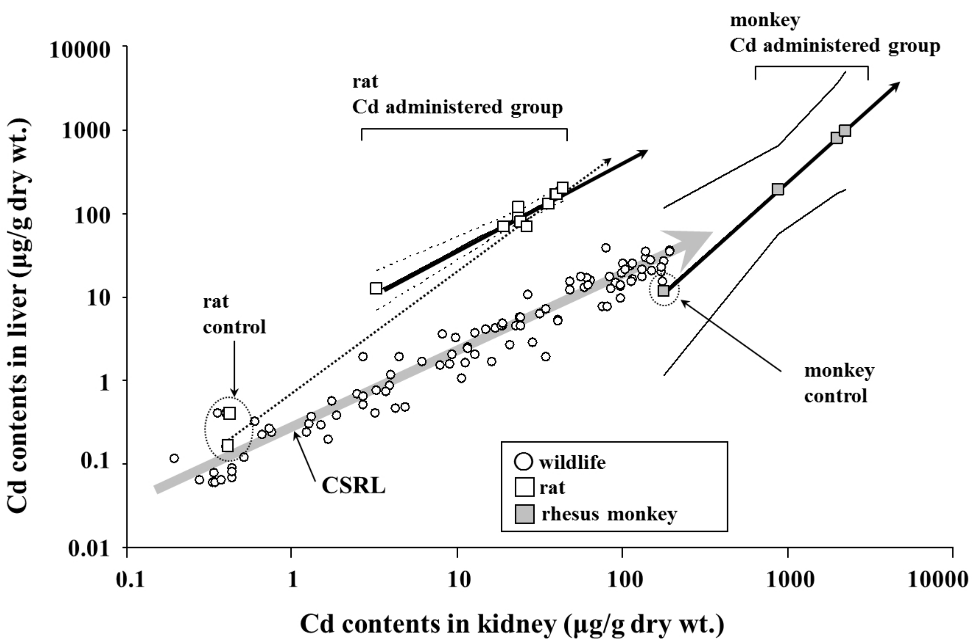

Fig. 1. Comparison of the data from laboratory animals. Original figure from Mochizuki et al. (2008) as modified by Ueda et al. (2009a).

A new development in the research area of biological monitoring has been introduced in this section. In the next section we describe the pilot study for establishment of a similar index using multiple elements.

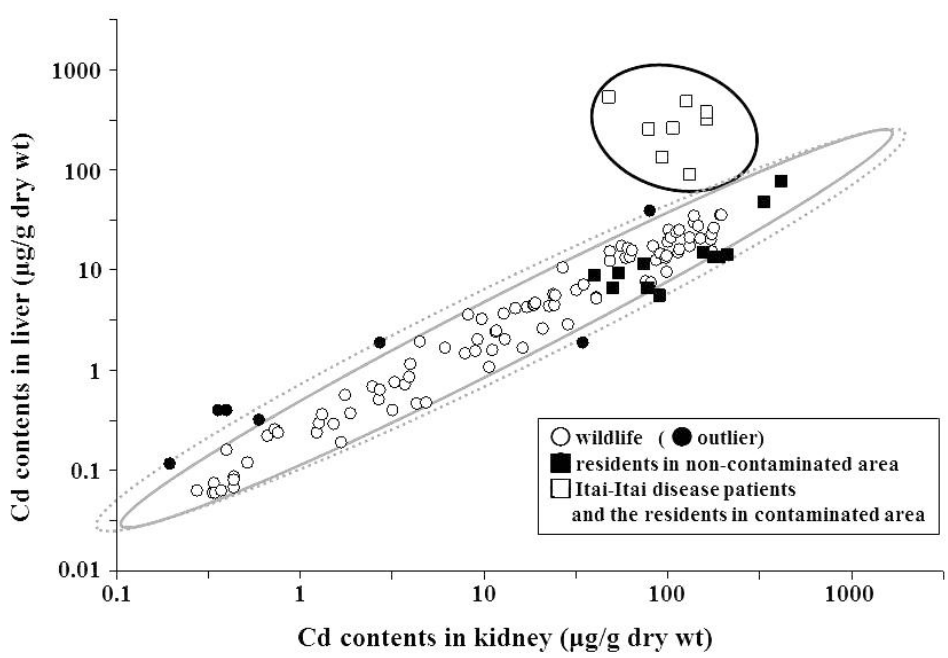

Fig. 2. Comparison of human data. Original figure from Ueda et al. (2009a). Dot-line; equal probability ellipse by 101 data points, solid line; equal probability ellipse by 94 data points.

3. A new index for investigation of contamination by multiple elements

3.1 Materials and methods

3.1.1 The wild birds used in the present study

A total of 127 wild birds, including Anatidae (n=65), seabirds (n=17), common cormorants (*Phalacrocorax carbo*, n=30), Ardeidae (n=10) and others (n=5) was used in the present study. The categories of birds included the following species: the Anatidae included spotbill duck (n=19, *Anas poecilorhyncha*), wigeon (n=15, *Anas penelope*), pintail (n=11, *Anas acuta*), mallard (n=7, *Anas platyrhynchos*), common teal (n=6, *Anas crecca*), gadwall (n=2, *Anas strepera*), common shoveler (n=2, *Anas clypeata*), wood duck (n=1, *Aix sponsa*), garganey (n=1, *Anas querquedula*) and tundra swan (n=1, *Cygnus columbianus*). The seabirds included greater scaup (n=6, *Aythya marila*), tufted duck (n=6, *Aythya fuligula*), Eurasian pochard (n=3, *Aythya ferina*), common scoter (n=1, *Melanitta nigra*) and great crested grebe (n=1, *Podiceps cristatus*). The Ardeidae included black-crowned night heron (n=3, *Nycticorax nycticorax*), little egret (n=3, *Egretta garzetta*), intermediate egret (n=2, *Egretta intermedia*), cattle egret (n=1, Bubulcus ibis) and great egret (n=1, *Egretta alba*). The others included eastern turtle dove (n=1, Streptopelia orientalis), common kestrel, (n=1, *Falco tinnunculus*), sparrowhawk (n=1, *Accipiter nisus*), peregrine falcon (n=1, *Falco peregrinus*) and Eurasian woodcock (n=1, *Scolopax rusticola*). As shown in Fig. 3, the birds were collected from various areas in Japan.

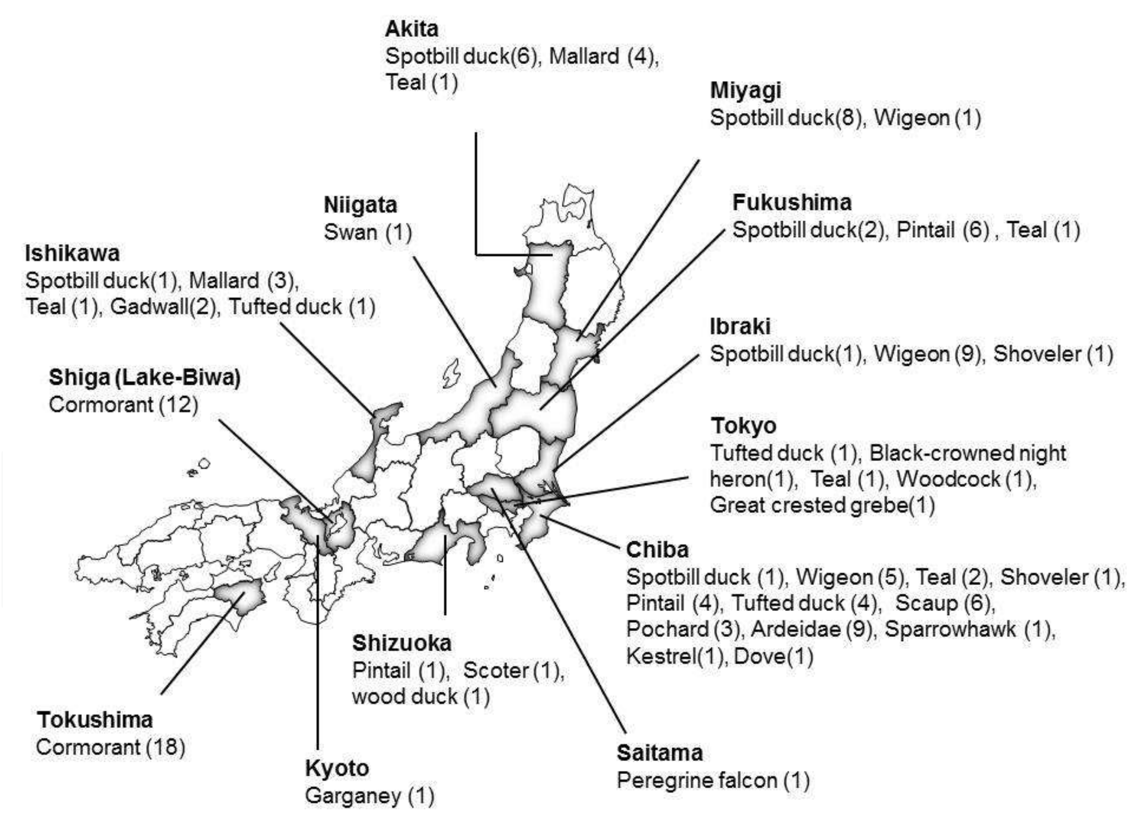

Fig. 3. The wild birds and collection areas used in this study. Number in brackets indicated the number of samples.

Most of the wild birds were collected as part of another National Investigation conducted by the Environment Agency in Japan (the present; Ministry of the Environment in Japan) in

1995. Other birds, which were protected in the Gyoutoku bird observatory in Chiba Prefecture, were transported to our laboratory after death.

3.1.2 Analytical procedure

Samples of kidney were removed from the birds, and about 200 mg of each sample was put into a Pyrex tube (Corning, USA), and dried in an oven at 70℃ to determine the dry weight of the sample. The appropriate volume of HNO_3 : $HClO_4$ (1:1, Wako Pure Chemical, Ltd., Japan) was added to the dried samples, and the samples were digested at 180℃. The contents of various elements in the kidneys of the birds were analyzed using inductively coupled plasma emission spectrometry (ICP-AES, FTP08, Spectro A.I., Germany). The eight target elements were: Cd, Cr, copper (Cu), lithium (Li), Mo, titanium (Ti), Tl and V. The standard additional method was employed for the analysis. The detailed methods of sample preparation and the analytical procedure have been described previously (Mochizuki et al., 2002b).

3.1.3 Statistical methods

The statistical analyses used in the present study were carried out using computer software such as Lotus 2001 (Lotus Development), Excel 2003 (Microsoft Corporation), and JUMP (SAS Institute, Japan) to obtain the regression line, the confidence intervals, and the logarithmic transformation. Factor analysis was carried out using Excel add-in software (Esumi, Japan).

3.2 Factor analysis

3.2.1 Establishment of an index for multiple elements

The contents of the eight elements measured in the kidney are shown in Table 1. The data were recalculated using factor analysis. The multiple variables used in the present study (contents of elements) were merged using factor analysis, a form of multivariate analysis. Thus, a higher factor score was thought to indicate more serious contamination by multiple elements. We obtained three significant factors, as shown in Table 2. No tendency for contamination was observed when the mean values of each category were compared. Thus, it is thought that the comparison using only mean values makes it difficult to understand the degree of contamination by multiple elements.

	Anatidae	**Seabird**	**Cormorant**	**Ardeidae**	**Others**
n	65	17	30	10	5
Cd	8.33±1.48	8.36±4.00	1.97±0.50	4.38±0.98	9.17±6.73
Cr	2.67±0.55	1.69±0.32	1.65±0.37	3.86±0.93	0.33±0.200
Cu	24.21±2.87	40.99±6.78	12.85±0.86	21.12±2.77	19.04±4.19
Li	1.85±0.52	1.24±0.36	1.62±0.43	3.04±0.92	0.32±0.23
Mo	6.88±1.04	3.80±0.94	4.62±0.43	5.35±1.39	12.57±8.98
Ti	2.07±0.56	0.80±0.34	1.32±0.34	2.94±0.88	0.83±0.83
Tl	9.17±2.14	2.87±0.49	3.33±0.49	5.07±1.45	1.97±1.85
V	2.50±0.60	1.10±0.32	2.35±0.48	3.04±0.92	1.75±1.16

Table 1. The contents of the elements in kidneys from birds of each category. The results are represented as mean contents (μg/g dry wt.), and the standard error of the mean.

	n	Factor 1	Factor 2	Factor 3
		Cr, Li, Ti	Mo	Cu
Anatidae	65	0.101±0.155	0.111 ±0.099	-0.026±0.099
Seabird	17	-0.261±0.078	-0.126±0.122	0.656±0.222
Cormorant	30	-0.133±0.103	-0.230±0.063	-0.259±0.019
Ardeidae	10	0.483±0.264	-0.067±0.159	-0.115±0.063
Others	5	-0.600±0.087	0.494±0.848	-0.112±0.206

Table 2. The mean factor score and standard error of the mean for each category of birds.

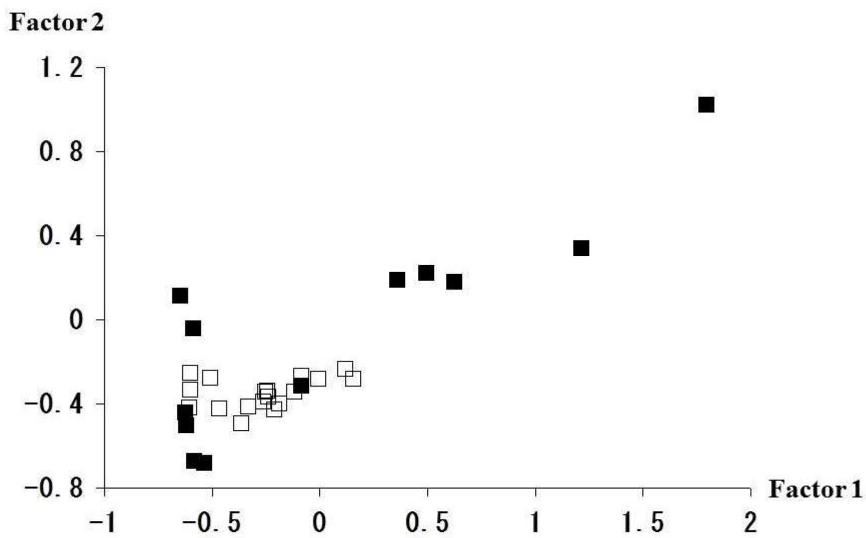

Fig. 4. The relation between the factor score of factor 1 and that of factor 2 for the common cormorant. Filled squares; common cormorants collected in Shiga Prefecture; empty squares; common cormorants collected in Tokushima Prefecture.

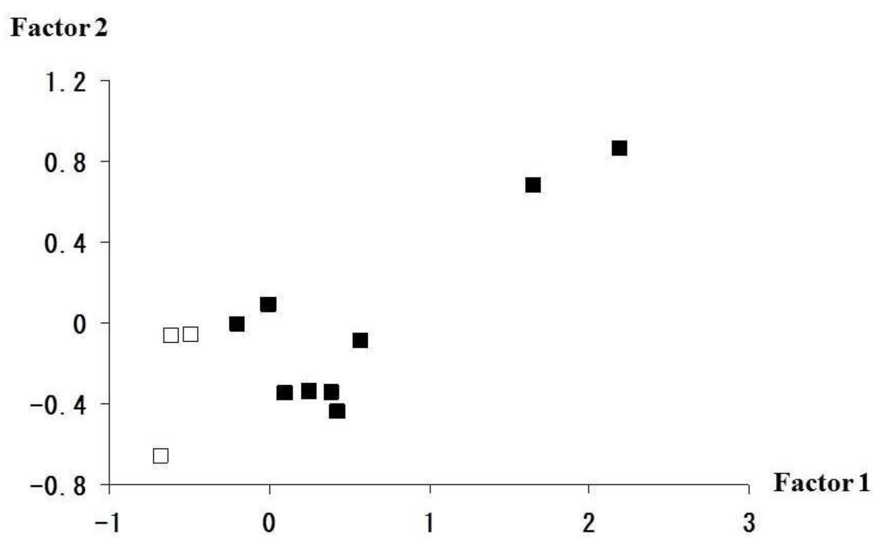

Fig. 5. The relation between the factor score of factor 1 and that of factor 2 for wild birds collected in Chiba Prefecture. Filled squares; Anatidae, empty squares; other birds.

We used previously published data to develop the indexes for Cd and Pb in our previous studies. However, we were unable to use a similar method in this study. Thus, the correlation between the factor score of factor 1 and that of factor 2 was investigated using various methods of classification. The correlation (Y=0.499X – 0.177, R^2=0.656) was found between the factor score of factor 1 (Cr, Li and Ti) and that of factor 2 (Mo) in the results from common cormorants, as shown in Fig. 4. A similar correlation was obtained using the results from wild birds captured in Chiba Prefecture (Fig. 5).

Further, a correlation was also obtained when Figs 4 and 5 were summarized, as shown in Fig. 6. The regression line obtained was: Y=0.474X – 0.199, R^2 =0.698. When the outliers among the data points were checked using the method of the 95% equal probability ellipse, three data points were identified as outliers. It was thought that correlation between two variables indicated normal equilibration in the target animals investigated using multiple elements. As mentioned above, we decided to use the regression line obtained in Fig. 6 and the equal probability ellipse as the multiple elements standard regression line, MSRL, and the multiple elements equal probability ellipse, MEPE, respectively.

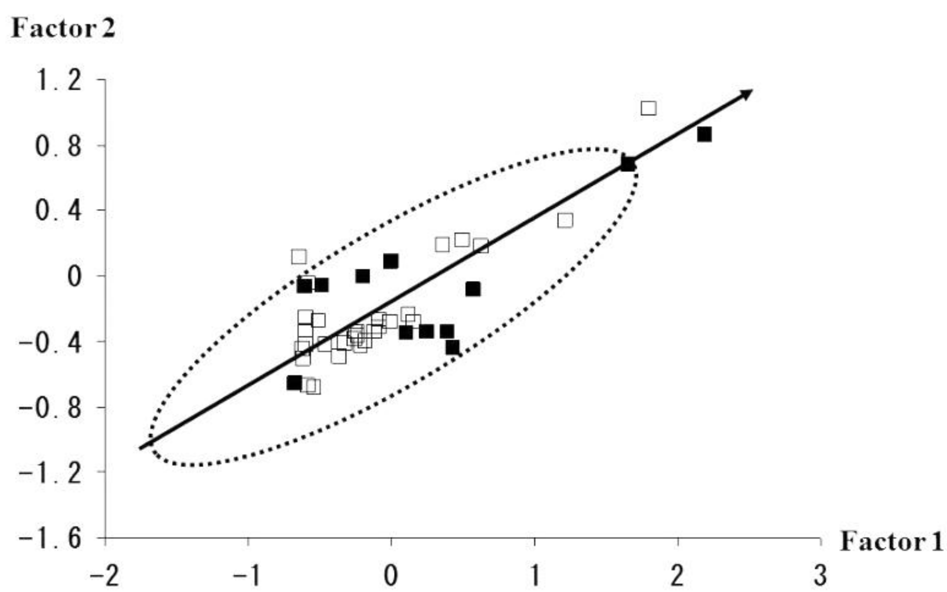

Fig. 6. The relation between the factor score of factor 1 and that of factor 2 in the data used in Figs 4 and 5. Filled squares; birds collected in Chiba Prefecture (Fig. 5), empty squares; common cormorants (Fig. 4). Dotted line; 95% equal probability ellipse, solid line; regression line obtained from wild birds.

3.2.2 Comparison of the degree of contamination of diving and dabbling ducks

The factor scores of diving ducks and dabbling ducks were compared with the index obtained. As shown in Fig. 7, the factor score obtained from diving ducks was observed to fall within the MEPE, except for one data point. Similarly, two of nine data points were observed to fall outside the MEPE when the data from wild birds collected in Chiba Prefecture were compared with the index (Fig. 8).

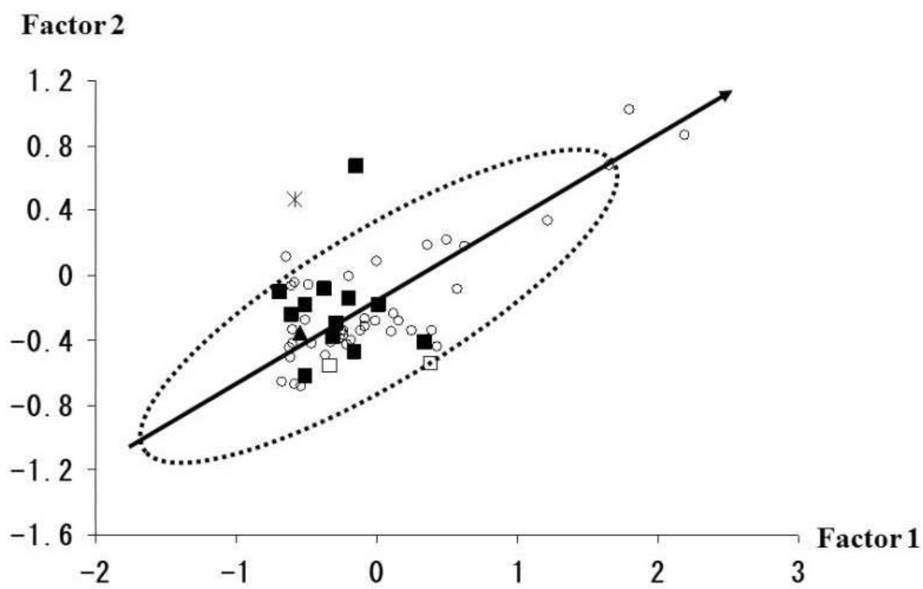

Fig. 7. The comparison between the index and the data obtained from seabirds. Filled squares: diving ducks collected in Chiba Prefecture, empty squares; diving ducks collected in Tokyo, asterisk; seabirds collected in Tokyo, filled triangles; diving ducks collected in Ishikawa Prefecture. Dotted line; 95% equal probability ellipse, solid line; regression line obtained from wild birds (empty circles) used in Fig. 6.

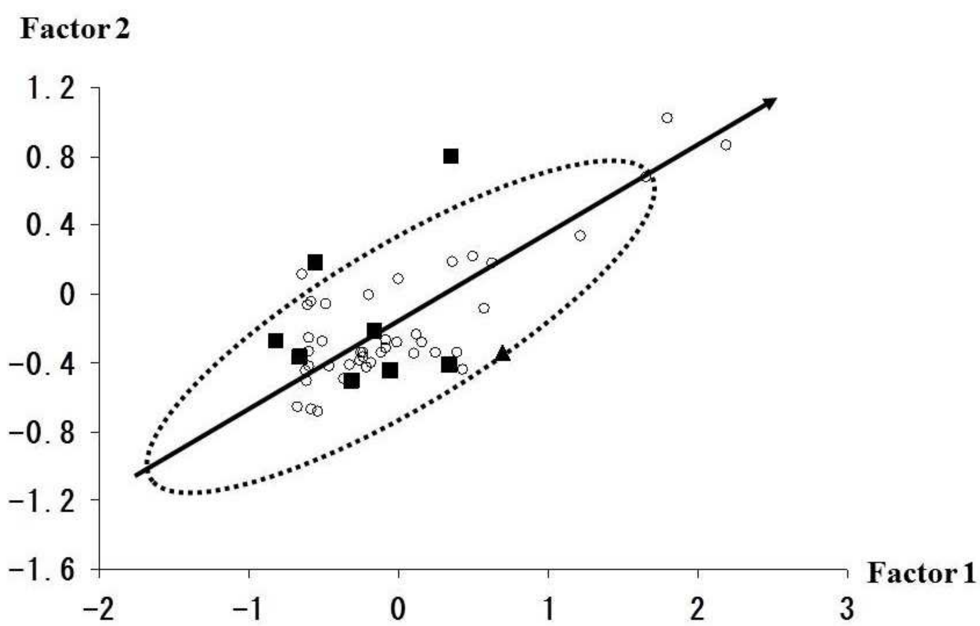

Fig. 8. Comparison between the index and the data obtained from dabbling ducks collected in Chiba Prefecture. Filled squares; wigeon, filled triangles; teal. Dotted line; 95% equal probability ellipse, solid line; regression line obtained from wild birds (empty circles) used in Fig. 6.

On the other hand, the data from dabbling ducks showed a marked tendency to deviate from the MEPE (Figs 9 and 10). Dabbling ducks inhabit inland water environments such as lakes and marshes. Thus, it is thought that the degree of contamination with multiple elements may be more serious in dabbling than in diving ducks. However, two of eight data points from wigeon collected in Chiba Prefecture were observed to fall outside the MEPE, as did seven of nine results from wigeon collected in Ibaraki Prefecture. As mentioned above, the area from which the birds were collected was thought to influence the level of contamination.

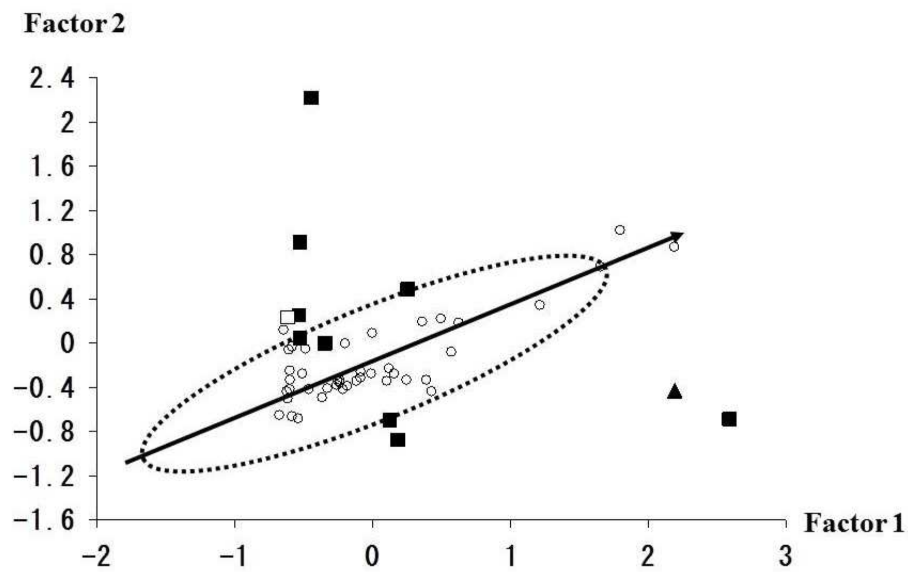

Fig. 9. Comparison between the index and the results from dabbling ducks collected in Ibaraki Prefecture. Filled squares; wigeon, filled triangles; spotbill duck, empty squares; shoveler. Dotted line; 95% equal probability ellipse, solid line; regression line obtained from wild birds (empty circles) used in Fig. 6.

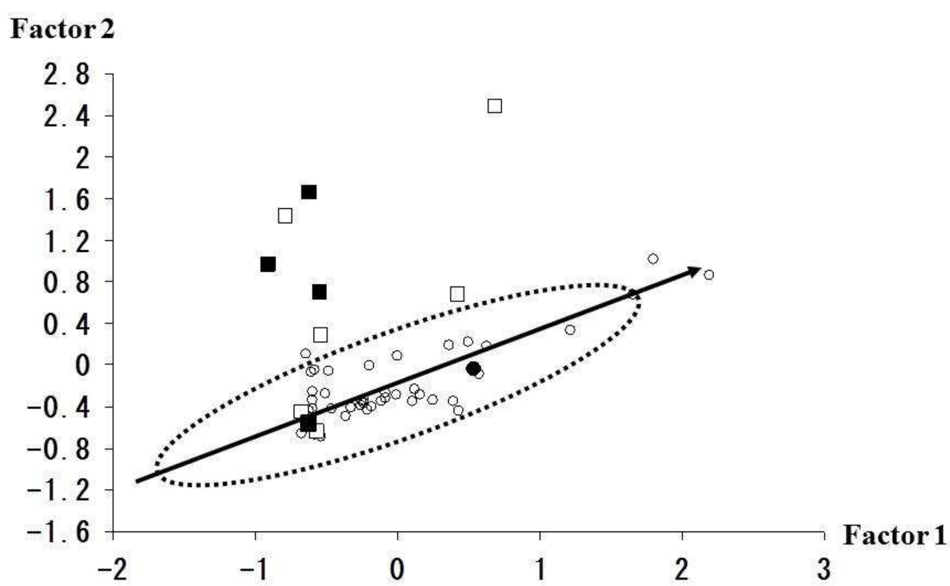

Fig. 10. Comparison between the index and the results from dabbling ducks collected in Akita Prefecture. Filled squares; mallard, empty squares; spotbill duck, filled circles; teal. Dotted line; 95% equal probability ellipse, solid line; regression line obtained from wild birds (empty circles) used in Fig. 6.

4. Conclusion

This study involved the establishment of an index using multiple elements, which is in the early phase of development for use in biological monitoring. Of course, a detailed study using the index is necessary in order to increase our understanding of contamination of wildlife with multiple elements. However, interestingly, the survey revealed that a similar index could be obtained, despite the investigation of multiple elements. Further, the difference between the degree of contamination by multiple elements in dabbling ducks and in diving ducks was clarified using this index. These results suggest that an understanding of the equilibrium among elements in the animal body is important for the investigation of contamination by multiple elements.

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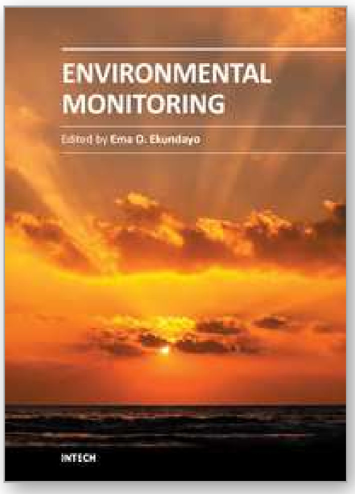

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