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Size and Mercury Concentration Relationship as Contamination Index

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Heavy metals contamination of aquatic ecosystems has long been recognized as a serious pollution problem (Benson et al. 1983). Some authors have demonstrated the positive use of aquatic organisms as biological indicators of the environmental contamination by mercury (Levitan et al. 1974; Koli et al. 1977; Gardner et al. 1978; Capelli et al. 1978; Teherani et al. 1979; Sorentino 1979; Bull et al. 1981; Pena and Alberto 1984) due to the fact that high mercury levels in organisms in inland waters have usually been attributed to pollution. Elevated levels of mercury in sediments, fish and fish-eating birds have been previously found in areas of mercury contamination (Bothner and Carpenter 1974).

Moreover, the possible relationship which exists between size (as weight or length) and mercury concentration in muscle is used by some authors as evidence of the degree of mercury contamination present in aquatic environments (Koli et al. 1977; Bull et al. 1981).

In this study, the level of mercury contamination present in populations of red crayfish (Procambarus clarkii) and grey mullet (Liza ramada) of the Marshes of the River Guadalquivir, both of great interest as food in the Marshes surroundings, has been shown. At the same time, the validity of using the relationship between size (as weight or length) and mercury concentration in muscle, as evidence of the mercury contamination present in the aquatic environment investigated, is discussed.

MATERIALS AND METHODS

All the specimens were caught in different samplings made during the summer of 1985 in the fishing area marked in Figure 1. Red crayfish samples were collected with unbaited Holland-type eel nets ("nasas"). Following their collection individual samples

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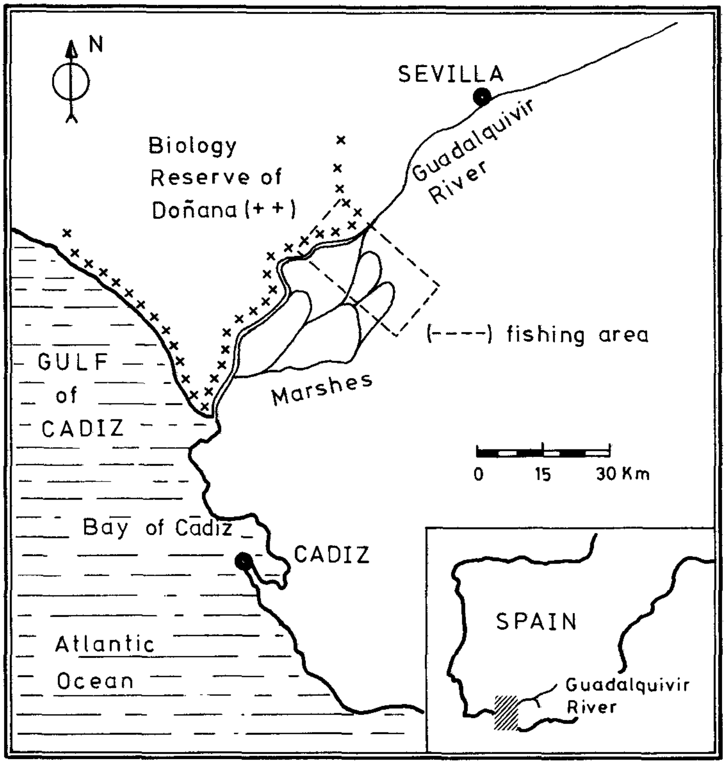

Figure 1. Geographical location of the Marshes of the Guadalquivir River.

were weighed and measured. Since 17.8 % of the samples had only one cheliped, two weighings were made; without chelipeds (W_s) for which the first pair of periopeds were severed at the protoischiopod articulation; and with chelipeds or total weight (W_t). In the samples in which there was only one cheliped W_t was estimated by adding the weight of the specimen available, multiplied by two, to W_s. Two measurements of length were also taken: cephalothoracic (L_c) and total (L_t). Samples of grey mullet were collected with a dragnet and each sample was weighed and measured individually. All samples were then stored at -20 ºC until required for analysis.

Mercury analyses were carried out using a technique previously described (Rincón et al. 1986). A mineralization using a concentrate "Merck Suprapur" nitric/sulphuric acid mixture (1/4) and a subsequent oxidation with a solution of $KMnO_4$ at 6 % (w/v) were made. As a reducing solution at the moment of determination, a solution of $NaBH_4$ at 3 % (w/v) in NaOH at 1 % (w/v) was used, with a reaction volume of 10 mL. Determinations were carried out with a Perkin-Elmer Mercury/Hydride System-10 connected to Perkin-Elmer 2380 atomic absorption spectrophotometer. The detection limit for 1 g of sample (concentration limit) was 13 ng g^{-1}. The recovery factors were 96 % for 100 ng g^{-1} level and 97.6 % for 500 ng g^{-1} level.

RESULTS AND DISCUSSION

A summary of the results is given in Table 1. It was shown that there are no statistically significant differences between the content of mercury in sexes (F = 3.84 for Procambarus clarkii and F = 0.007 for Liza ramada). These results coincide with those obtained in Mugil cephalus and Mugil capito by Levitan et al. (1974) and in Pacifasticus leniusculus by Stinson and Eaton (1983).

Table 1. Results obtained in the two species studied. The levels of mercury (X $\pm$ SD) are expressed as ng g^{-1}

Procambarus clarkii			Liza ramada		
Sampling	n	mercury	Sampling	n	mercury
1	29	86 $\pm$ 34	1	15	63 $\pm$ 16
2	19	84 $\pm$ 42	2	20	68 $\pm$ 24
3	25	134 $\pm$ 68	3	18	89 $\pm$ 24
4	30	83 $\pm$ 33	4	26	42 $\pm$ 20
5	44	104 $\pm$ 30	Total	79	63 $\pm$ 28
6	48	102 $\pm$ 38			
Total	195	100 $\pm$ 29			

The carrying out of a t-test reveals the existence of statistically significant differences between the concentrations of mercury found in both species (p = 0.001) which can doubtless be attributed to their different feeding habits since the loach are basically herbivorous (Hernando 1978) and crayfish are omnivorous with a strong animal component (Auvergne 1982). The differences in feeding habits also give rise to different levels of contamination between Merlucius merlucius and Mugil auratus (Yannai and Sachs 1978), between Esox lucius and Rutilus rutilus (Bull et al. 1981) and between Dicentrarchus labrax and Mugil auratus (Cordon 1985).

According to results obtained by Pozo et al. (1985) the Marshes of the Guadalquivir River cannot be considered as being a highly contaminated ecosystem since they found a mean value of 0.124 ug g^{-1} in sediments which is very much lower than the level of 1.20 ug g^{-1} found by Mudroch and Capobianco (1980) in an environment which is greatly contaminated by mining activity or the level of

1.83 ug g^{-1} found by Kudo et al. (1980) in an environment which is highly polluted by industries.

Caviglia and Cugurra (1978) obtained a mean value of 45 ng g^{-1} for mullet caught on the Italian coast in an area highly contaminated by a fulminate of mercury factory. However these authors do not specify the size of the samples which could explain the relatively low level found since the area of origin has serious mercury pollution. Ciusa and Giaccio (1972) reveal values of up to 1 ug g^{-1} in *Mugil auratus* caught on the coast of the Adriatic Sea, which is highly polluted; these are very much higher than those obtained by us in this study. Pena and Alberto (1984) found a mean value of 40 ng g^{-1} in *Liza ramada* taken from a scarcely contaminated aquatic environment as is the case of the Delta of the Ebro River. This is lower than that found by us in this species. Balkas et al. (1982) found a value of 25 ng g^{-1} in *Mugil auratus* taken from scarcely contaminated water.

From these references it can be concluded that in spite of the scant contamination present in the Marshes of the River Guadalquivir (Pozo et al. 1985), the values obtained by us in *Liza ramada* are high and coincide with those obtained by Establier (1975) from mullet coming from the coastal area of Cadiz (Spain) which is geographically very near the area tested by us (Figure 1). This fact can be explained if one considers the cathadromic habit of the species (Lotina and Hormaechea 1975) simultaneously with the elevated mercury contamination present in the Bay of Cadiz (Figure 1) with concentration values of up to 2.01 ug g^{-1} being found in *Xiphias gladius* (Establier 1972).

We can therefore consider the levels of contamination found in *Liza ramada* as being an example not only of the mercury contamination present in the Marshes but also that present on the Spanish Atlantic seaboard.

In the case of *Procambarus clarkii* the results obtained coincide with those recorded by Fernández-Aceytuno et al. (1984) finding a value of 115 ng g^{-1} as a mean concentration in specimens of this species coming from the Biology Reserve of Doñana which is part of the Marshes (Figure 1). Vermeer (1972) shows values of mercury concentration of up to 1.103 ug g^{-1} in crayfish caught in Lake Clay (Canada), which is highly contaminated. In *Orconectes viriles* coming from the Winsconsin River (U.S.A.), industrially contaminated by paper and pulp mills and a chlorine-caustic soda plant, industries which historically have been a major source of mercury emission to the environment, Sheffy (1978) obtained a concentration range of 70 to 560 ng g^{-1}, much higher than that recorded by us of 30 to 265 ng g^{-1}. According to this author, due to restricted movement of crayfish, these can be used to pinpoint more precisely those areas contaminated by mercury. Mercury concentrations in muscle from crayfish at the uncontaminated site were from 0.05 to 0.180 ug/g (Stinson and Eaton 1983).

We can sum up by saying that the loach showed a moderate level of contamination and that red crayfish showed a level of contamination that can be termed as low.

For Bull et al. (1981) the increases of mercury content with size are usually associated with elevated concentrations of mercury in the environment. In a previous study (Rincón et al. 1986) we have verified that in the Marshes this hypothesis is fulfilled when the population of samarugo (Valencia hispanica) is used as a biological indicator.

In the case of Procambarus clarkii it can be observed that there is no statistically significant correlation (Table 2) in 5 of 6 samplings made. On considering the totality of the samplings made (n = 195) the correlation obtained is significant (p = 0.05) as well as in sampling nº 6 (p = 0.001). It is obvious that in these 2 cases the Bull et al. (1981) hypothesis is not fulfilled as the environment cannot be regarded as being contaminated and the relationship between size and mercury contamination is statistically significant.

Table 2. Coefficients of correlation obtained and their statistical significance in the relationships size (as length or weight)/mercury concentrations, were L_c = cephalothoracic length, L_t = total length, W_s = weight without chelipeds and W_t = total weight, L = length and W = weight. The lengths are expressed in cms and the weights in g. a and b denote statistical significance for p = 0.05 and 0.01 respectively, c = not statistically significant.

	Procambarus clarkii				Liza ramada		
Sampling	L_c	L_t	W_s	W_t	Sampling	L	W
1	0.233[c]	0.217[c]	0.118[c]	0.088[c]	1	0.588[a]	0.525[a]
2	0.094[c]	0.052[c]	0.012[c]	0.106[c]	2	0.041[c]	0.153[c]
3	0.304[c]	0.341[c]	0.338[c]	0.266[c]	3	0.532[a]	0.527[a]
4	0.177[c]	0.163[c]	0.252[c]	0.272[c]	4	0.515[b]	0.564[b]
5	0.119[c]	0.178[c]	0.294[c]	0.099[c]	Total	0.324[b]	0.265[a]
6	0.393[b]	0.396[b]	0.479[b]	0.436[b]			
Total	0.153[a]	0.162[a]	0.173[a]	0.141[a]			

In the case of the samplings made of Liza ramada it is seen (Table 2) that in sampling nº 2 the relationship was not significant in spite of the elevated contamination present in the

Bay of Cadiz (Establier 1972) so that the hypothesis previously mentioned is not fulfilled in this case either.

These results make it clear that the theory of the existence of a significant relationship between size and mercury concentration in muscle associated with an elevated mercury contamination in aquatic environment is, in general, accepted when Procambarus clarkii and Liza ramada are used as biological indicators. However, it is necessary to allow for the fact that the special characteristics of a sampling can erroneously disguise the level of contamination present in the environment when this is estimated as a function of the size/mercury concentration relationship.

An additional problem which arises is whether the sample which is used to study the characteristics of the size/mercury relationship is representative enough. To this effect, in spite of the mathematical logic we disagree with Koli et al. (1977) who consider that by obtaining statistically significant values of r (test of significance against a null hypothesis Ho: $r = 0$) between size/mercury concentration in only 2 to 4 samples, it is possible to conclude that larger fish had higher mercury levels.

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