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## Determination of organochlorine compounds in the starry sturgeon (*Acipenser stellatus*) in different areas of the Caspian Sea

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**ABSTRACT:** Nowadays, the presence of organochlorine pesticides in aquatic ecosystems is one of the main environmental concerns, due to the persistent, bioaccumulative and toxic nature of these compounds. In the Caspian Sea, the largest inland body of water in the world, there is a unique ecosystem, which is inhabited by extremely valuable species, like sturgeons. In view of that preoccupation for the aforementioned pesticides, and the critical state of the sturgeon population, our aim in this work was to evaluate their concentration in those animals throughout the Caspian Sea. For this purpose, a total of 40 adult starry sturgeons was obtained within a repopulation programme in the northern and southern coastal waters of this sea. First, the marginal pectoral fin was extracted from them, and then they were set free. The samples from each area were analysed together, and the concentration of several pesticides was ascertained. The latter were lower than the quantification limit for most of the organochlorine compounds evaluated in the different regions investigated. However, it was possible to quantify aldrin, epoxyheptachlor, heptachlor, D.D.E and dieldrin. Given the scant fat content in the study matrix, the detection of some organochlorine substances above the quantification limit in the samples obtained in this work was a reason for concern. That is why we recommend going on evaluating and adopting measures to alleviate organochlorine pollution in this region.

**Keyword:** sturgeon, Caspian Sea, pesticides, pollution.

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## INTRODUCTION

Pesticides comprise a wide group of toxic substances related to adverse effects on ecosystems and humans (El-Sheikh et al. 2021; Islam et al. 2022). Within this group, organochlorines are the compounds that are currently presenting a problem. For decades, these substances have been extensively employed to eradicate pests and disease vectors in the agricultural sector or in homes (Taiwo, 2019). Over the years, different regulations in various countries and organizations have imposed strict constraints on them, or even banned their use (Potapowicz et al. 2020). These chemical compounds are hydrophobic, lipophilic and resistant to degradation, properties which make them degrade very slowly and persist in the environment, as well as facilitating their absorption and bioaccumulation in living organisms (Ashraf, 2015; El-Sheikh et al. 2021).

The Caspian Sea is the world's largest inland body of water, with a surface of approximately 370.000 km<sup>2</sup> and a volume of water of 78.000 km<sup>3</sup>. This sea is located between Europe and Asia and is surrounded by five countries: Russia, Azerbaijan, Iran, Turkmenistan and Kazakhstan. It is generally divided into three areas, taking into account its geophysical characteristics: the northern area with a maximum depth of 30 m and salinity of below 10 g/L; the middle and southern areas that are much deeper, approximately 800 m and 1000 m, respectively, and whose water is saltier (Kosarev, 2005; Lattuada et al. 2019). In addition to rainfall, the river Volga, in the north, is the main source of filling this sea (233 km<sup>3</sup>/año) (Ramazanov et al. 2020). The fact of being inland makes its ecosystem more vulnerable to pollutants (Ghayebzadeh et al. 2020).

The Caspian Sea ichthyofauna totals 76 species and 47 subspecies, which comprise 17 families and 53 genera (Leroy et al., 2020). Among these species, five are sturgeons, which are of great ecological importance in the region and are as follows: the persian sturgeon (*Acipenser persicus*), the russian sturgeon (*Acipenser gueldenstaedti*), the starry sturgeon (*Acipenser stellatus*), the boat sturgeon (*Acipenser nudi-ventris*) and the beluga sturgeon (*Huso huso*) (Wang et al., 2008). Currently, pollution, excessive fishing, and the entry of invasive species have reduced the sturgeon populations in this sea (FAO, 2022). In fact, the five species inhabiting it are now classified as being "critically endangered" by the International Union for the Conservation of Nature (IUCN) on their Red List of Threatened Species (IUCN, 2023).

In view of the ecological value of sturgeons, and the problem of organochlorine pesticides, our study aimed to determine the concentration of several of the latter in the pectoral fin of starry sturgeons in differ-

ent regions of the Caspian Sea, in order to assess the degree of pollution from these chemical substances.

## MATERIAL AND METHODS

### Sampling area and preparation of the samples

A total of 40 adult starry sturgeons (*Acipenser stellatus*) of a mean weight of  $6.38 \pm 1.55$  kg and a mean length of  $134.57 \pm 13.57$  cm were caught in a repopulation programme in the southern and northern coastal waters of the Caspian Sea, as shown in Figure 1. To be specific, three sampling zones were established in the north (N-1, N-2 and N-3), where 8, 4, and 8 fish, respectively, were caught. In the south, sturgeons in two areas were sampled (S-1 and S-2), 10 fish being caught in each. In sum, samples of 20 fish were taken in the north and the same number in the south.

Once caught, the fish were weighed and measured. The marginal pectoral fin was extracted from each, cutting it off at the articulation point. It is important to clarify that this procedure is not lethal, and that the fish were not sacrificed for this study but were released, once the samples had been taken. This work received prior approval from the Ethics Committee of the institution involved. All the instruments used for the sampling were rinsed in 1% nitric acid prior to taking each sample. The samples were frozen at -80 °C up to their analysis.

### Organochlorine analysis

The following organochloride substances were identified: alpha-lindane, lindane, delta-lindane, heptachlor, aldrin, epoxyheptachlor, D.D.E, dieldrin, D.D.D, endrin-aldehyde, endrin-ketone, metoxychlor, endrin and endosulfan. To do so, the individual samples from each zone were mixed and homogenized into a single sample (n= 5). For the extraction, 15 grams of fin from each sample were weighed. They were ground in a clean pestle together with 10 g of anhydrous sodium sulfate until they were completely dry and homogenized. This sample was extracted with a solution of 50 mL of dichloromethane-hexane (1:1). Ten grams of the homogenized sample was placed in a 50 mL extraction bottle that was kept for 60 min. in an ultrasonic bath. The extract was carefully decanted and concentrated to 2 mL, using a rotary evaporator maintained at 20°C. The organochlorine concentrations were determined by gas chromatography using an Agilent-7890 equipped with a quadrupole type Split/Splitless inlet mass spectrometer (Agilent-5975). The quantification limit established for this test was of 0.5 µg/kg.

## RESULTS AND DISCUSSION

The organochlorine concentrations in the different areas of the Caspian Sea are shown in Table 1. In N-1, S-1 and S-2, they were all below the quanti-



**Figure 1.** Zones where starry sturgeons were caught and sampled in the Caspian Sea.

fication limit. In N-2 and N-3, it was only possible to quantify the following: aldrin ( $0.77 \mu\text{g/kg}$ ) and epoxyheptachlor ( $0.56 \mu\text{g/kg}$ ) in the area N-2, and heptachlor ( $0.68 \mu\text{g/kg}$ ), D.D.E ( $1.01 \mu\text{g/kg}$ ) and dieldrin ( $0.63 \mu\text{g/kg}$ ) in N-3.

The results obtained in this study show that concentrations of different organochlorines are quite low, or even, non existent, as we have been unable to quantify them in the sturgeon pectoral fins sampled in these areas, leading us to presume that there is a low level of these pollutants in those regions. However, certain variables should be taken into account when reaching conclusions on this aspect. The bioaccumulation and biomagnification processes are generally more intense as the trophic position of the species increases (Nejatkhah Manavi et al. 2018). In this respect, sturgeons are situated at a high trophic level, since it is a predatory species that feeds on benthic organisms like crustaceans or aquatic invertebrates (Billard y Lecointre, 2000). Other biotic factors typical of the species that may also affect bioaccumulation or biomagnification are its lifespan or spawning. Regarding the former, sturgeons can live for between 50 and 75 years (Doroshov, 2019), so that they are exposed to pollutants for a long time. With reference to the latter, they are migratory species that can travel long distances and swim through rivers during their spawning (Billard y Lecointre, 2000). They can therefore become exposed in some regions to highly persistent substances like organochlorines.

All these peculiarities, together with the low organochlorine concentrations found, seem to indicate

that, in effect, the pollution level in these regions is low. Nevertheless, the biological matrix analysed in this work, i.e. the fin, should be taken into account, since, being a protected species, access to other tissues is a limiting factor. In this context, the fat content of the tissue is the chief variable in the absorption and distribution of organic pollutants, such as organochlorine pesticides (Nejatkhah Manavi et al. 2018), due to the marked lipophilic nature of these substances (Ashraf, 2015; El-Sheikh et al. 2021). In our study, the matrix employed had a very low fat content, although it was still possible to quantify some of the compounds, thus triggering some concern, as other studies have reported higher concentrations in other species or sediments. For example, on the coasts of Iran, Hoseini et al. (2023), in the Caspian seal (*Puca Caspica*), a species with a high fat percentage, determined mean concentrations of D.D.E. of  $9.06 \mu\text{g/kg}$ , and found D.D.T. in all the samples analysed ( $n=20$ ). Although not directly from the Caspian sea, Shahbazi et al. (2012) sampled some agricultural land near it, in the provinces of Mazandaran and Guilan (Irán), and found large amounts of organochlorine compounds (at a range of between  $8\text{--}151 \mu\text{g/kg}$ ). It should be noted that there are few studies evaluating organochlorine concentrations in the Caspian sea, and that future research would be useful.

## CONCLUSIONS

The organochlorine concentrations found in the marginal pectoral fin of the starry sturgeons (*Acipenser estellatus*) were lower than the limit set for most of the

**Table 1.** Concentrations of several organochlorine substances in the marginal pectoral fin of the starry sturgeon (*Acipenser stellatus*) in different areas in the north and south of the Caspian sea:

Organochlorine (µg/kg)	Zone				
	N-1	N-2	N-3	S-1	S-2
Alpha-lindane	-	-	-	-	-
Lindane	-	-	-	-	-
Delta-lindane	-	-	-	-	-
Heptachlor	-	-	0.68	-	-
Aldrin	-	0.77	-	-	-
Epoxyheptachlor	-	0.56	-	-	-
D.D.E	-	-	1.01	-	-
Dieldrin	-	-	0.63	-	-
D.D.D	-	-	-	-	-
Endrin-aldehyde	-	-	-	-	-
Endrin-ketone	-	-	-	-	-
Metoxychlor	-	-	-	-	-
Endrin	-	-	-	-	-
Endosulfan	-	-	-	-	-

- Means that the concentrations were under the quantification limit (<0.5 µg/kg)

organochlorine compounds evaluated in the different regions. Aldrin and epoxyheptachlor were detected in the zone N-2, and heptachlor, D.D.E and dieldrin in N-3. However, the detection of some organochlorine compounds above the quantification limit in the samples obtained is a reason for disquiet, given the scant fat content in the study matrix. That is why we recommend going on assessing the level of these pollutants in other matrices in the region, in order to investigate the state of pollution that other authors have reported as being high, since their presence could seriously affect the ecosystem of the Caspian sea, that is inhabited by such valuable species as sturgeons.

## STATEMENTS AND DECLARATIONS

**Funding:** No funds, grants, or other support were received.

**Competing Interests:** The authors have no relevant financial or non-financial interests to disclose.

**Ethics approval:** All the applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The use and care of fish in this study were approved by the University of Guilan from the point of Ethical issues (1398.9.16-15/11966).

**Data availability statement:** The data that support the findings of this study are available from the corresponding author.

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