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Association of spinal deformities with heavy metal bioaccumulation in natural populations of grass goby, *Zosterisessor ophiocephalus* Pallas, 1811 from the Gulf of Gabès (Tunisia)

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Abstract The present study illustrates an analysis of spinal deformities associated with metal accumulation in natural populations of *Zosterisessor ophiocephalus* derived from polluted (S1) and unpolluted (S2) areas in the Gulf of Gabès in Tunisia. Three basic types of spinal deformities were detected: kyphosis, scoliosis and lordosis. These basic deformities frequently co-occur. Spinal deformities were observed in 10.72% of the total examined fish (n = 494). Deformed fish were 3.85 times more frequent in S1 than in S2. In both sexes, the highest occurrence of deformities was observed in the 111–120 mm class decreasing thereafter with fish length. Hepatic concentrations of cadmium (Cd), copper (Cu) and zinc (Zn) were significantly higher in *Z. ophiocephalus* from S1 than those from S2. Comparisons between fish in each site showed that liver concentrations of Cd and Zn were significantly higher in deformed fish than in normal fish. The relationship between metals accumulation and observed spinal deformities as well as the suitability of this kind of studies for environmental monitoring are discussed.

Keywords Gulf of Gabès · Heavy metals · Liver · Spinal deformities · Tunisia · *Zosterisessor ophiocephalus*

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Introduction

Spinal deformities, including scoliosis, lordosis and kyphosis, have been well described in many species of cultured fish (Poynton 1987; Mair 1992; Chatain 1994; Andrades et al. 1996). Presently, this is a significant problem in hatchery-produced fish, as it influences the economical success of rearing. In general, they develop due to the insufficient knowledge of the optimum environmental preferences of fish at the different stages of their life. However, spinal deformities are rare in natural populations from undisturbed ecosystems (Dahlberg 1970; Daoulas et al. 1991; Boglione et al. 2001; Antunes and Lopes Da Cunha 2002). According to previous reports, high frequencies of spinal deformities in wild specimens of teleost fish have been observed only in polluted waters (Slooff 1982; Bengtsson et al. 1985; Whittle et al. 1992; Antunes and Lopes Da Cunha 2002) or in freshwater which subjected to significant variations of environmental parameters such as temperature (Hubbs 1959).

A relation between various sorts of pollutants and spinal deformities is well established both from laboratory and field studies. A strong relationship has been found between prevalence of spinal curvature and body burden of organochlorine compounds in striped bass (Mehrle et al. 1982). Most of the evidence on heavy metals influence on induction of spinal deformities in fish is from laboratory studies. An increase in spinal deformities rate induced by exposures of fish embryos and larvae to various heavy metals were reported by many authors (Weiss and Weiss 1977, 1989; Kapur and Yadav 1982; Cheng et al. 2000). *Myoxocephalus quadricornis* exposed for one year to an artificial heavy metal-containing effluent displayed spinal curvature and increased frequency of vertebral deformities (Begtsson and Larsson 1986). However, the actions of heavy metals in wild fish leading to abnormal development of skeletal structures remain uncertain.

The Gulf of Gabès is located in the southeastern coast of Tunisia. It is characterized by shallow waters, high temperature and salinity. This area is considered a great Tunisian aquatic resource, contributing to about 65% of the national production (CGP 1996). Grass goby, *Zosterisessor ophiocephalus* (Teleostei, Gobiidae) is a species that lives preferably in coastal brackish-water of estuaries and lagoons, through the Mediterranean Sea (Fredj and Mourim 1987). This species is very common in the Gulf of Gabès (Menif 2000) and is abundant and easy to sampling all year round. In the gulf of Gabès, this species showed a preference for organically enriched sediments where pollutants are, often, more concentrated. It is exposed to many physical and chemical variations, from temperature and salinity changes to pollution, in these most threatened sea ecosystems.

The present study illustrates an analysis of spinal deformities associated with heavy metals accumulation in natural populations of *Z. ophiocephalus* derived from polluted and unpolluted areas in the Gulf of Gabès in Tunisia.

Materials and methods

Study sites and sampling

Two sampling sites were selected in the Gulf of Gabès, in southeastern coast of Tunisia, (Fig. 1). The industrialized coast of Sfax (S1), whish was surrounded by important industrial activities, mainly crude phosphate treatments and chemical industries, was chosen as a polluted site. This site

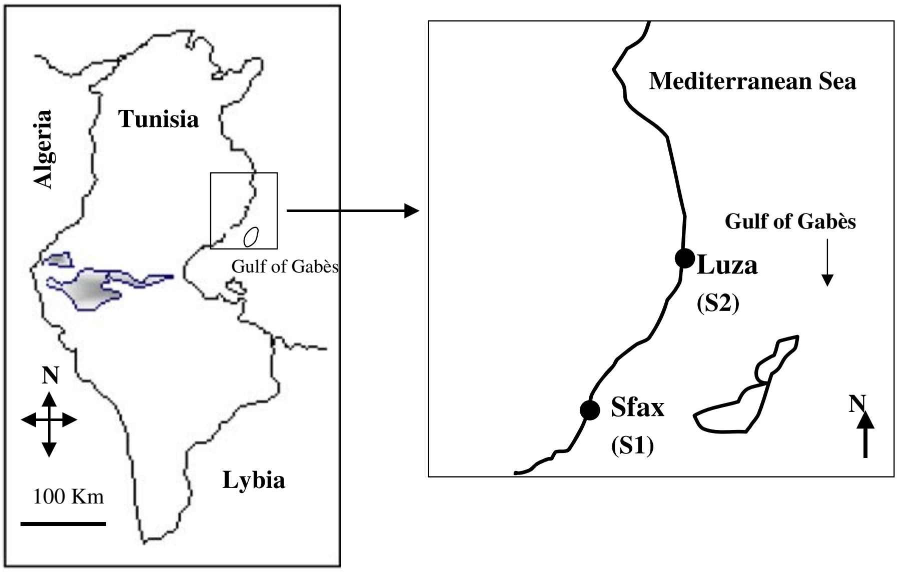

Fig. 1 Localization of the sites sampling in the Gulf of Gabès (Tunisia)

Fig. 2 Normal (**a**) and deformed (**b**) *Zosterisessor ophiocephalus* (females) caught in the industrialized coast of Sfax in the Gulf of Gabès (Tunisia). Scale bar = 1 cm

is highly contaminated with heavy metals, essentially cadmium (Cd; Smaoui-Damak et al. 2003). The coast of Luza (S2), located ∼50 km north of S1 and appeared to be unaffected by human activities, was used as a reference sample site.

Fish samples were collected by professional fishermen with a 4 m beam trawl during January 2006 from polluted and unpolluted sites. Water samples were collected at a depth of 0.5 m in clean bottles from the same area. Sampling bottles were previously cleaned by soaking with 10% nitric acid and rinsed with ultra pure water. Fish and water samples were transported to the laboratory in a thermos flask with ice on the same day.

Analytical procedures

Incidences of spinal deformities determination

Caught fish, from each sampling site, were counted and sexed. Since spinal deformities were visible on the fish body immediately upon catching (Fig. 2), the percentage of deformed fish was calculated. Total length, from the tip of the snout to the end of the caudal fin of all specimens was measured. From the total examined, 265 were females ranging between 105 and 161 mm total length and 229 males between 98 and 164 mm.

Specimens were grouped in 10 mm total length classes in order to study the possibility of any age related trend with the incidence of deformed fish. A fish showing a representative deformity were radiographed using a medical X-ray system and the radiographs were used for an examination of the skeleton. A sample of normal fish, based on external morphology, was also radiographed in order to validate the results of the macroscopic evaluation.

Metal analysis

Cadmium (Cd), zinc (Zn) and copper (Cu) are selected for metal analyses in water and fish liver. In fact, these metals are potential source from industrial activities located in the Gulf of Gabès (Hamza-Chaffai et al. 1995; Serbaji 2000; Smaoui-Damak et al. 2003). Metal analyses were carried out only in females to eliminate any possible interference of sex with metals bioaccumulation. The size and weight of normal and deformed fish samples destined for metal analysis were measured and given in Table 1. The entire liver from each sample was dissected, washed with distilled water and dried in filter paper. Liver samples were dried to constant weight for 48 h at 60°C in Pyrex test tubes. Dried tissues were weighed and digested with concentrated nitric acid (Merck, 65%) at 120°C. When fumes were white and the solution was completely clear, the samples were cooled to room temperature and the tubes were filled to 10 ml with ultra pure water (Warchałowska-Śliwa et al. 2005). Sea water samples were stabilized at pH 2 with 1 M nitric acid prior to direct determi-

Table 1 Details of fish samples destined for metal analysis

Sites	Phenotype	Number of fish examined	Sex	Total length (mm)±SEM (minimum–maximum)	Body weight (g)±SEM (minimum–maximum)
S1	Normal	7	Female	126.41 ± 2.43 (116–134)	25.97 ± 1.63 (19.86–33.8)
	Deformed	7	Female	126.72 ± 2.62 (114–134)	27.78 ± 4.29 (21.37–34.71)
S2	Normal	7	Female	125.74 ± 1.94 (115–133)	26.22 ± 3.10 (20.63–31.57)
	Deformed	7	Female	122.81 ± 3.18 (118–134)	26.27 ± 2.08 (17.44–34.12)

SEM standard error of the mean; minimum and maximum values are given in parentheses; *S*1 coast of Sfax; *S*2 coast of Luza

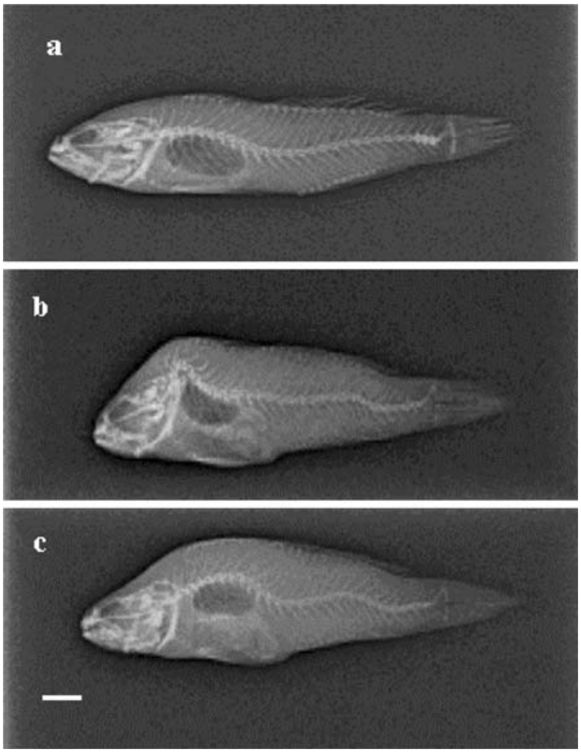

Fig. 3 Lateral view X-ray radiographs of deformed *Zosterisessor ophiocephalus* from the Gulf of Gabès (Tunisia). **a** Normal specimen; **b** specimen with kyphosis–lordosis deformity; **c** specimen with kyphosis–scoliosis–lordosis deformity; scale bar = 1 cm

nation of total metal concentrations (Bervoets and Blust 2003).

All samples were analyzed to determine zinc (Zn) concentration using flame atomic absorption spectrometry (AAS), and Cd and copper (Cu) concentrations were measured using a graphite furnace AAS technique (ZEEnit 700). Samples were analyzed in triplicate. The variation coefficient was usually less than 10%. Concentrations of the metals in the liver were calculated on a dry weight basis and expressed as micrograms per gram.

The transfer factor (Tf) of heavy metals in fish from water was calculated according to the following formula (Rashed 2001):

$$\text{Tf} = \text{concentration of metal in liver}/\text{concentration of metal in water}$$

Statistics

Percentage values comparisons of spinal deformities were tested by way of chi-square. For metal concentrations, data are given as means±SD, and differences were tested using the unpaired t-test. Regression equation and correlation coefficient were applied to the data concerning Zn and Cd accumulation. Differences at $p < 0.05$ were considered as significant.

Results

Types of spinal deformities

Spinal deformities were visible on the fish body immediately upon catching, with all kinds of deformities, vertically and horizontally. According to X-ray radiographs, three basic types of spinal deformities, assorted by the direction of their curvature, were described: kyphosis (dorsal deformity), scoliosis (lateral deformity) and lordosis (ventral deformity). These basic deformities frequently co-occur in varying degrees of severity (Fig. 3).

Spatial distribution of spinal deformities

The percentage values of spinal deformities, according to sampling site, are shown in Table 2. A

Table 2 Spatial distribution of *Zosterisessor ophiocephalus* with spinal deformities in the Gulf of Gabès

Sites	Females			Males			Total		
	N	*S*	Percentage	*N*	*S*	Percentage	*N*	*S*	Percentage
S1	123	24	19.51	109	17	15.59	232	41	17.67
S2	142	7	4.92 ***	120	5	4.16 ***	262	12	4.58 ***

N number of fish examined; *S* number of fish with spinal deformities; *S*1 coast of Sfax; *S*2 coast of Luza

***Statistically differences between S1 and S2, $p < 0.001$

Table 3 Percentage variation of fish with spinal deformities according to sex

Sex	Number of fish examined	S	Percentage
Females	265	31	11.69
Males	229	22	9.60

S number of fish with spinal deformities

total of 494 specimens of *Z. ophiocephalus* was examined and 53 were deformed, which represents 10.72%. The percentage of spinal deformities in population from the industrialized coast (S1), for males and females alike, was significantly higher ($p < 0.001$) than in the population from the unpolluted coast (S2).

Sex and length-related variation of spinal deformities

The statistical test revealed that percentage of deformed fish was not significantly different between males and females (Table 3). In both sexes, variation of deformities incidence with length was very similar. In females and males with less than 110 mm total length, the percentages of spinal deformities were 11.11% and 10.52% respectively, reaching in the next length class 18.51% in females and 14% in males. Thereafter a percentage decrease of deformed fish was observed, in fact none was observed over 150 mm (Table 4).

Heavy metal concentration in water and in fish liver

Metal concentrations in water and in liver of *Z. ophiocephalus* from S1 and S2 are summarized in Table 5. High concentrations of heavy metals were determined in the water collected from S1. This water contained 204.33, 5.01 and 3.20 times more Cd, Cu and Zn, respectively, than water from S2. When the heavy metal concentrations in liver of specimens from S1 were compared with those in liver of specimens from S2, all differences were also statistically significant ($p < 0.01$). Comparisons between fishes in each site showed that liver concentrations of Cd and Zn were significantly higher in deformed fish than in normal fish ($p < 0.01$).

The Tf of Cd, Cu and Zn from the water to fish were cited in Table 6. The results showed that the Tf of Cd and Zn in deformed fish, from S1 and S2 alike, were significantly higher than in the normal fish ($p < 0.01$).

A highly significant correlation ($r = 0.923$, $p < 0.01$ for normal fish, and $r = 0.871$, $p < 0.01$ for deformed fish) between hepatic-Cd and Zn concentrations was observed only in specimens from S1 (Fig. 4).

Discussion

In this study, three types of spinal deformities consisting of a scoliosis, lordosis and kyphosis, which frequently co-occur in varying degrees of severity, are described, to our knowledge for the first time, in *Z. ophiocephalus* from the Gulf of Gabès (Tunisia). These deformities were similar to those described in the same species from the Karin Sea Eastern Middle Adriatic (Dulcic 2004), in *Atherina boyeri* from the estuary of the Neretva (Tutman et al. 2000) and reared *Sparus aurata* (Afonso et al. 2000).

Spinal deformities in *Z. ophiocephalus* were found to be significantly associated with fish

Table 4 Percentage variation of fish with spinal deformities according to length-classes

Length classes (mm)	Females			Males		
	N	*S*	Percentage	*N*	*S*	Percentage
< 111	9	1	11.11	19	2	10.52
111–120	54	10	18.51	50	7	14.00
121–130	77	12	15.58	70	8	11.42
131–140	71	7	9.86	54	4	7.40
141–150	35	1	2.85	25	1	4.00
> 150	20	0	0	11	0	0

N number of fish examined; *S* number of fish with spinal deformities

Table 5 The heavy metal concentrations in water (μg/l) and in liver (μg/g dry weight) of *Zosterisessor ophiocephalus* from the Gulf of Gabès (Tunisia)

Sites	Samples		Number of sample examined	Cd	Cu	Zn
S1	Water		6	$6.13 \pm 1.57^{a,**}$ (4.23–9.31)[b]	$15.29 \pm 2.55^{**}$ (10.87–19.45)	$38.48 \pm 5.48^{**}$ (31.45–46.05)
	Liver	Normal fish	7	$2.49 \pm 0.48^{**}$ (1.56–3.02)	$9.17 \pm 2.62^{**}$ (5.22–13.46)	$22.03 \pm 2.44^{**}$ (17.84–25.15)
		Deformed fish	7	$7.57 \pm 1.09^{**,c}$ (5.23–9)	$8.60 \pm 2.63^{**}$ (5.44–13.54)	$58.93 \pm 8.42^{**,c}$ (44.69–69.12)
S2	Water		6	0.032 ± 0.008 (0.021–0.044)	3.055 ± 1.46 (1.12–5.88)	12.27 ± 2.17 (8.36–15.33)
	Liver	Normal fish	7	0.020 ± 0.004 (0.014–0.029)	1.27 ± 0.63 (0.83–2.78)	6.56 ± 1.38 (4.76–9.36)
		Deformed fish	7	0.078 ± 0.017^{c} (0.047–0.099)	1.36 ± 0.37 (0.88–2.05)	14.75 ± 1.73^{c} (11.87–17.84)

*S*1 coast of Sfax; *S*2 coast of Luza

**Significantly higher compared to sample from S2 $p < 0.01$

[a]Mean values±SD

[b]Values in parentheses indicate the minimum and maximum levels

[c]Significantly higher compared to the normal fish from the same site $p < 0.01$

length. In both sexes, the highest occurrence of deformities was observed in the 111–120 mm class decreasing thereafter with fish length. The same result was obtained by Antunes and Lopes Da Cunha (2002), who showed, in *Gobius niger* from Sado estuary in Portugal, that vertebral deformities were more frequent in the 60–69 mm class decreasing thereafter with fish length. These results also agree with those reported by Tutman et al. (2000), who noticed a decrease in spinal deformities with age in *Atherina boyeri*. The absence of older deformed fish could be explained by higher mortality of deformed fish as compared to normal fish. In fact, different possible effects on the individual due to spinal deformities are described in the literature: impaired swimming performance (Weiss and Weiss 1976), decreased ability to escape (Kroger and Guthrie 1971) and hindrance to catching food (Kroger and Guthrie 1971). Further effects were also suggested by Hickey (1972), such as decreased capability for territorial defence and reduced to compete for a sexual partner.

The concentrations of metals in the liver represent the storage of metals from the water where the fish species live (Romeo et al. 1999; Karadede et al. 2004). Thus, the liver is more often recommended as environmental indicator organ of water pollution than any other fish organs. We have found that Cd, Cu and Zn concentrations in the water and in the liver of *Z. ophiocephalus* from the industrialized coast of Sfax were significantly higher than those from the coast of Luza. These results are consistent with previous studies conducted in this region. In fact, the pollution

Table 6 Transfer factor (Tf) of heavy metals in liver fish from the water

Sites	Liver	Number of fish examined	Cd	Cu	Zn
S1	Normal fish	7	0.40 ± 0.07^{a}	0.60 ± 0.17	0.57 ± 0.06
	Deformed fish	7	1.23 ± 0.17^{b}	0.56 ± 0.17	1.53 ± 0.21^{b}
S2	Normal fish	7	0.68 ± 0.15	0.41 ± 0.20	0.53 ± 0.11
	Deformed fish	7	2.62 ± 0.57^{b}	0.44 ± 0.12	1.20 ± 0.14^{b}

*S*1 coast of Sfax; *S*2 coast of Luza

[a]Mean values±SD

[b]Significantly higher compared to the normal fish from the same site $p < 0.01$

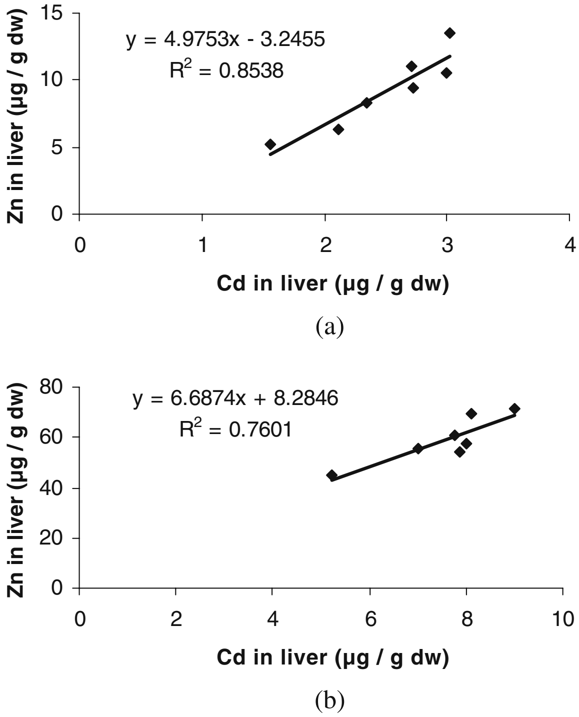

Fig. 4 Cadmium vs. zinc relationship in liver of normal (**a**) and deformed (**b**) *Z. ophiocephalus* from the coast of Sfax (Tunisia)

in this coast is due essentially to the presence of continuous discharge of heavy metals from local industrial activities (Hamza-Chaffai et al. 1997, 1998) and from the phosphogypsum stock (Illou 1999; Serbaji 2000). Several reports have also indicated high metal concentrations in soft tissues of *Reditapes decussatus* (Smaoui-Damak et al. 2003; Hamza-Chaffai and Pellerin 2003) and in the fish *Scorpaena porcus* (Hamza-Chaffai et al. 1995) from this coast.

Our results showed that Zn concentration increases simultaneously with that of Cd in both normal and deformed fish from polluted site. Although this kind of relationship between Cd and Zn has been described for other fish species (Marcovecchio 2004), terrestrial mammals, like rats, horses, lambs, pigs and humans (Schroeder and Nason 1974; Elinder et al. 1977; Elinder and Piscator 1978) and also for marine mammals (Honda and Tatsukawa 1983). In most of these papers the increase in Zn concentration has been proposed as a compensation for the increase in Cd concentration and this mechanism probably includes the synthesis of metallothioneins (MTs), which would bind both Cd and Zn in a molar ratio of 1:1 (Nordberg 1972; Das et al. 2000). The absence of this relationship in the fishes from S2 could be explained by the absence of Cd-induced MTs synthesis due to the low hepatic-Cd concentrations.

The transfer factor (Tf) indicates whether heavy metal biomagnifications take place. A Tf greater than 1 indicates bioaccumulation (Kalfakakour and Akrida-Demertzy 2000; Rashed 2001). In both sites, the results showed that the Tf of Cd and Zn in deformed fish from water were greater than 1, and this means that the deformed fish undergo bioaccumulation of these elements from the water. Considering the heavy metal concentrations in the liver and the Tf values, the deformed fish exhibited higher accumulated liver concentrations of Cd and Zn indicative of a differential ability to handle the metals.

The results of this field study revealed that *Z. ophiocephalus* from the industrialized coast of Sfax were more frequently deformed. In accordance with the present study, Bengtsson et al. (1985) have found high frequency of vertebral deformities in *Myoxocephalus quadricornis* exposed to heavy metal pollution in the Gulf of Bothnia (Baltic Sea). Bengtsson (1974) suggested that vertebral damage caused by Zn might be attributed to its effect on the muscle action potential and thus to an effect on the neuromuscular system. A similar action of cadmium was also suggested by Bengtsson et al. (1975). Cheng et al. (2000) showed that the frequency of malformations in zebrafish embryos increased with Cd concentration in culture media and demonstrated a reduced myotome formation in Cd-induced spinal deformity. These authors suggested that fish embryonic malformations induced by Cd might be mediated through ectopic expression of developmental regulatory genes.

The study of deformity occurrences in fish seems to be a good and practical way of assessing environment quality. In fact, field surveys (Valentine 1975; Bengtsson 1979, 1988; Slooff 1982; Bengtsson et al. 1985; Boglione et al. 2006) provide evidence of environmental influence on induction of skeletal abnormalities, including spinal and vertebral deformities. A causal relationship between high environmental levels of chlorinated hydrocarbons or heavy metals and skeletal anomalies in fish has been suggested (Valentine 1975; Mehrle et al. 1982; Bengtsson et al. 1985; Bengtsson 1988). Induction of spinal deformities in fish can be confounded by a number of biotic factors (e.g. hereditary defects, parasite infections) and abiotic factors (e.g. vitamin deficiencies, electrical shock…) which are not related to pollution. Nevertheless, the use of this fish disease as a biomarker offers many advantages in field study, in that the symptoms are easy to recognize and quantify, and diagnosis is rapid and cost-effective (Au 2004). It is also important to take into account that this kind of monitoring is feasible also for developing countries, lacking highly trained laboratory personnel and sophisticated equipment.

Studies regarding the presence and effects of contaminants in Tunisian marine environment represent a recent concern, and are at a start point stage. On the basis of the obtained results and the above mentioned requirements we propose to use the incidence of spinal deformities in *Z. Ophiocephalus* as a biomarker for future monitoring programs, to evaluate the evolution of heavy metal pollution in the Gulf of Gabès. However, this study must be regarded as a preliminary approach to this problem, due to the low number of sites examined.

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