

## Biomarkers for endocrine disruptors in three species of Mediterranean large pelagic fish

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### Abstract

The hypothesis that Mediterranean top predator species, such as large pelagic fish, are potentially at risk due to endocrine disrupting chemicals (EDCs), is investigated. The potential estrogenic effects of PHAHs in three fish species of commercial interest, the top predators bluefin tuna (*Thunnus thynnus thynnus*), swordfish (*Xiphias gladius*), and Mediterranean spearfish (*Tetrapturus belone*), were investigated using vitellogenin (Vtg), zona radiata proteins (Zrp) and mixed function oxidases (EROD, BPMO) as diagnostic tools. High induction of Vtg and Zrp was detected by western blot and ELISA techniques in adult males of *X. gladius* and *T. thynnus thynnus*, suggesting that these species are at high toxicological risk in the Mediterranean sea. Comparison of BPMO and EROD activities in the three species indicated, both in male and female, much higher MFO activity in bluefin tuna. This data suggests high exposure of this species to lipophilic xenobiotic contaminants in the Mediterranean environment. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Thunnus thynnus thynnus*; *Xiphias gladius*; *Tetrapturus belone*; EDCs; Vtg; Zrp; Erod; BPMO

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In the Mediterranean marine environment, top predators accumulate high concentrations of polyhalogenated aromatic hydrocarbons (PHAHs) and toxic metals, incurring high toxicological risk (Marsili & Focardi, 1996). Many of these contaminants are recognised as endocrine disrupting chemicals (EDCs). EDCs include chemicals used heavily in the past in industry and agriculture, such as

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polychlorinated biphenyls and organochlorine pesticides, and chemicals currently used, such as plasticizers and surfactants. Because of the lipophilic and persistent nature of most xenobiotic estrogens and their metabolites, many bioaccumulate and biomagnify.

There is thus a need to develop sensitive diagnostic monitoring tools for the evaluation of toxicological risk and potential effects on the reproductive activity and population dynamics of top Mediterranean marine predators of commercial interest, such as the large pelagic bluefin tuna (*Thunnus thynnus thynnus*), swordfish (*Xiphias gladius*) and Mediterranean spearfish (*Tetrapturus belone*). In the Mediterranean basin, high consumption of bluefin tuna and swordfish is typical of several areas. The market for this fish is of high commercial interest in Europe and Asia (Japan).

The potential estrogenic effects of some endocrine disruptors such as PHAHs were investigated in *T. thynnus thynnus*, *X. gladius* and *T. belone*. Vitellogenin (Vtg) and zona radiata proteins (Zrp) were used as biomarkers of the effects of EDCs, and mixed function oxidase activity (MFO) (BPMO, EROD) induction as biomarkers of exposure.

Induction of Vtg and Zrp protein transcription and translation are well-known major responses to estrogens in fish (Arukwe, Knudsen, & Goksoyr, 1996). Induction of MFO activities (BPMO, EROD) is a well-documented biomarker of exposure to PHAHs in marine fauna.

Here we report the results obtained with 20 specimens of bluefin tuna, 47 specimens of swordfish, and five specimens of Mediterranean spearfish caught in summer 2000 in the Straits of Messina (Sicily, Italy) during the spawning period. They were caught by harpoon from the traditional Sicilian “Passerella” fishing boats. Twenty specimens of swordfish were also captured in summer 1999 in Azores coastal waters (Portugal).

Bluefin tuna is a large top predator present throughout the Mediterranean and Black sea and also widely distributed in the Atlantic Ocean. It is fast swimming and affects transoceanic migration in schools. Outside the spawning season, it is a voracious predator of all kinds of fish, crustaceans and molluscs (Fischer, 1973). Maximum age is at least 15 years (Arena, Cefali, & Munao, 1980).

The swordfish is a marine predator with worldwide distribution, preying primarily on cephalopods and secondarily on teleosts. Information on swordfish growth is limited and somewhat contradictory. There is good evidence that males and females have different growth patterns, females attaining the larger size. Swordfish live to at least 9 years of age (Stillwell & Kohler, 1995).

The Mediterranean spearfish is a common species in the central basin of the Mediterranean and completes its life cycle in this area (Klawe, 1980). It feeds on pelagic fishes (garfish, cupleoids) and pelagic crustaceans. It inhabits inshore and offshore surface waters and does not generally form schools.

Vtg and Zrp were detected in serum samples of fish by western blot and indirect ELISA analysis. Detection for western blot was as outlined in the Amersham ECL kit booklet. Results of ELISA (Goksoyr, 1991) were expressed as Absorbance at 492 nm. Primary polyclonal rabbit antibodies purchased from BioSense, Bergen, Norway were PO-2 anti-sea bream Vtg and O-173 anti-salmon Zrp antibodies.

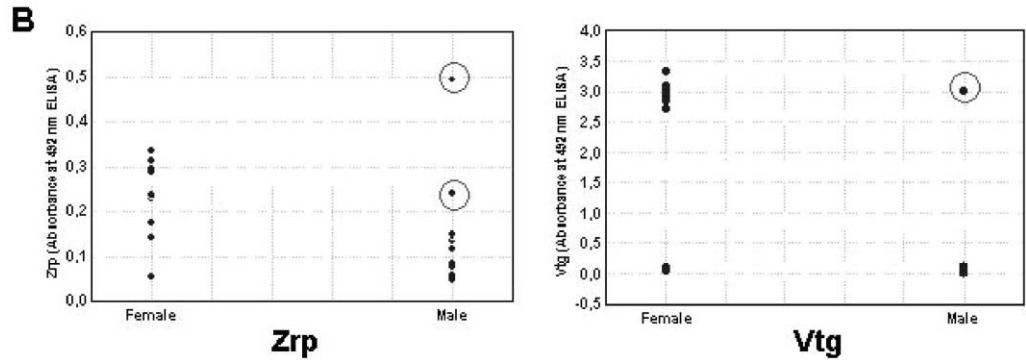
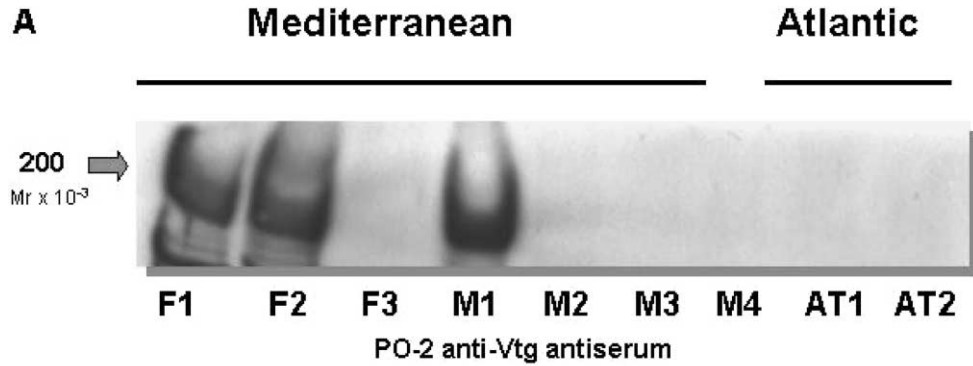


Fig. 1. (A) Western blot analysis of vitellogenin (Vtg) of swordfish (*Xiphias gladius*) captured in the Mediterranean Sea (Straits of Messina, Sicily, Italy) during the spawning period (F1–3, M1–4), and in Azores coastal waters (Portugal) (AT1, AT2). (B) Zona radiata proteins (Zrp) and vitellogenin (Vtg) of male and female bluefin tuna (*Thunnus thynnus thynnus*) captured in the Mediterranean Sea (Straits of Messina, Sicily, Italy) in summer 2000, during the spawning period. Circles indicate male samples, the values of which were nevertheless in the same range as those of reproductive females.

Mixed function oxidase activity was evaluated in the microsomal fraction of fish liver by assaying benzo[*a*]pyrene hydroxylase (BPMO) and ethoxyresorufin-*O*-deethylase (EROD) activities. All assays were carried out at 30 °C. BPMO activity was measured by the method of Kurelec, Britvic, Rijavec, Muller, and Zahn (1977), activity was expressed in A.F.U./mg prot/h. EROD activity was measured by the method of Lubet, Nims, Mayer, Cameron, and Schechtman (1985), activity was expressed in pmol/mg prot/min.

All the data were processed by parametric statistical analysis (ANOVA) using Statistica software. The multiple range test was used to obtain significant differences between the means (level of significance, 0.05).

The first warning on the toxicological risk to Mediterranean large pelagic fish due to EDCs, was sounded by the results of Fossi and collaborators (2001) in swordfish. These authors used Vtg and Zrp as diagnostic and prognostic biomarkers. Dramatic induction of these typically female proteins was detected by ELISA and western blot in adult males of the species. The present results confirmed the finding of dramatic induction in adult male Mediterranean swordfish with respect to Atlantic specimens (Fig. 1A). A polypeptide having a molecular mass of about 190 kDa was recorded in female swordfish (F1, F2) using anti-Vtg antiserum, and a similar reaction was observed in older male (M1) (approximately 9 years old), whereas a reduced reaction was seen in sample M2 and no reaction was observed in the Atlantic samples (AT1, AT2). Four Mediterranean male specimen show values of Vtg in the same range of mature females.

High induction of Vtg and particularly Zrp was also detected in adult Mediterranean males of *T. thynnus thynnus* (by ELISA technique) (Fig. 1B), which suggests

Table 1

Vitellogenin (Vtg) zona radiata proteins (Zrp), EROD and BPMO activities in female (F) and male (M) specimens of bluefin tuna (*Thunnus thynnus thynnus*), swordfish (*Xiphias gladius*), and Mediterranean spearfish (*Tetrapturus belone*), caught in summer 2000 in the Straits of Messina (Sicily, Italy) (means and standard deviation)

	Vtg (absorbance at 492 nm —ELISA)		Zrp (absorbance at 492 nm —ELISA)		EROD (pmol resor./min/mg prot)		BPMO (A.F.U./mg prot/h)	
	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	SD
<i>Bluefin tuna</i>								
F (N=9)	2.62	1.03	0.25	0.68	67.01	56.12	364.30	330.27
M (N=11)	0.30	0.89	0.14	0.13	67.38	29.27	462.38	192.15
<i>Swordfish</i>								
F (N=26)	1.69	0.98	0.58	0.38	18.88	21.57	25.94	35.03
M (N=21)	0.47	0.71	0.19	0.17	17.61	20.42	29.37	45.43
<i>Mediterranean Spearfish</i>								
F (N=3)	0.19	0.07	0.05	0.02	15.33	8.75	33.44	35.62
M (N=2)	0.05		0.14		37.70	26.64	75.40	64.48

that this species is also exposed to EDCs in the Mediterranean Sea. At the present time no data are available of blue fin tuna collected in the reference site.

Table 1 also shows Vtg and Zrp in the three species investigated. No induction of Vtg and Zrp was detected in the Atlantic swordfish specimens (Fossi, personal communication). BPMO and the EROD activities (Table 1) recorded in the three species showed that male and female bluefin tuna had much higher MFO activity (one order of magnitude for BPMO). These data suggest a high exposure of this species to lipophilic xenobiotic contaminants in the Mediterranean environment.

In conclusion, this paper shows that induction of Vtg, Zrp and MFO activities can be used as diagnostic and prognostic tools for hazard assessment of Mediterranean fish stocks. These data also sound a warning of potential reproductive alterations in large pelagic fish and suggest the need for continuous monitoring to avoid reductions in the population of these species of high commercial and ecological interest.

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