

Review Articles

Endocrine Disruptors in Mediterranean Top Marine Predators

M. Cristina Fossi*, Silvia Casini and Letizia Marsili

Department of Environmental Sciences, University of Siena, Via Mattioli, 4, 53100, Siena, Italy

* Corresponding author (Fossi@unisi.it)

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Abstract

Background, Aims and Scope. Man-made Endocrine Disruptors (EDs) range across all continents and oceans. Some geographic areas are potentially more threatened than others: one of these is the Mediterranean Sea. Levels of some xenobiotics are much higher here than in other seas and oceans. In this paper we review the final results of a project in which the hypothesis that Mediterranean top predator species (such as large pelagic fish and marine mammals) are potentially at risk due to EDs was investigated.

Methods. In a four-year survey on the Mediterranean population of swordfish (*Xiphias gladius*), the potential toxicological effects of organochlorine compounds (OCs) on specimens of swordfish and tuna fish (*Thunnus thynnus thynnus*), caught in the spawning seasons from 1999 to 2002 in the Straits of Messina, Sicily (Italy), were investigated using vitellogenin (Vtg), Zona radiata proteins (Zrp), and cytochrome P4501A (CYP1A) activities (EROD, BPMO).

Subcutaneous tissues (skin and blubber) were obtained from *Stenella coeruleoalba*, *Tursiops truncatus*, *Delphinus delphis* and *Balaenoptera physalus* from the western Ligurian Sea, between Corsica and the French-Italian coast, and Ionic Sea using biopsy darts launched with a crossbow. Benzo(α)pyrene monooxygenase (BPMO) activity was measured in biopsies and chlorinated hydrocarbon levels were detected.

Results and Discussion. We illustrate the need to develop and apply sensitive methodological tools, such as biomarkers (Vitellogenin, Zona Radiata proteins and CYP1A activities) for evaluation of toxicological risk in *Xiphias gladius* and *Thunnus thynnus thynnus*, and nondestructive biomarkers (CYP1A activities and fibroblast cell culture in skin biopsy), for the hazard assessment of threatened marine mammals species (*Stenella coeruleoalba*, *Tursiops truncatus*, *Delphinus delphis* and *Balaenoptera physalus*) exposed to EDs.

Conclusion. The present research shows that: a) Vtg and Zrp can be used as diagnostic tools for fish stocks hazard assessment in the Mediterranean Sea; b) that CYP1A1 (BPMO) induction in cetaceans skin biopsy may be an early sign of exposure to EDs such as OCs and a potential alert for transgenerational effects.

Recommendation and Outlook. This research represents a warning signal of the potential reproductive alterations in marine top predators and suggest the need for continuous monitoring to avoid reductions in population and biodiversity in the Mediterranean Sea.

Keywords: Endocrine disruptors; fibroblast cell culture; marine mammals; Mediterranean Sea; skin biopsy; top predators; Vitellogenin; *Xiphias gladius*; Zona Radiata proteins

Introduction

Recent alarm regarding rapid worldwide depletion of predatory fish communities raised serious concern about the ecological effects of industrialized fishing. Ramsom & Worm (2003) estimate that large predatory fish biomass (including swordfish and tuna) today is only about 10% of pre-industrial levels. In this context, serious concern about Mediterranean pelagic longlines has been recently expressed by both the public and the scientific community. The Mediterranean swordfish population is particularly affected by this industrialized fishing technique. However, there is another unexplored factor that could drastically interfere with the stability of populations of Mediterranean top predators, including large pelagic fish: the toxicological effects of Endocrine Disruptors (EDs).

Endocrine Disruptors are a structurally diverse group of compounds that may adversely affect the health of humans, wildlife and fisheries, or their progenies, by interaction with the endocrine system (Colborn et al. 1993, Colborn et al. 1998, Gillesby & Zacharewski 1998). They include chemicals used heavily in the past, specifically in industry and agriculture, such as polychlorinated biphenyls and organochlorine pesticides. EDs also include chemicals currently used, such as plasticizers and surfactants. Many of the known EDs are estrogenic, affecting particularly reproductive functions. Because of the lipophilic and persistent nature of most xenobiotic estrogens and their metabolites, many bioaccumulate and biomagnify (Colborn 1998, Arukwe et al. 1996).

Man-made EDs range across all continents and oceans; some geographic areas, such as the Mediterranean Sea, are potentially more threatened than others. Levels of some xenobiotics are therefore much higher here than in other seas and oceans (Aguilar et al. 2002). Mediterranean marine fauna could therefore be a target for EDs. In this peculiar environment, top predators (such as large pelagic fish and marine mammals) tend to accumulate large quantities of organochlorine contaminants (OCs) and toxic metals (Corsolini et al. 1995, Marsili 2000). This suggests the hypothesis that Mediterranean top predator species are potentially 'at risk' due to EDs contamination.

In this paper we review the final results of a project in which the potential estrogenic effects of organochlorine compounds

(OCs) on Mediterranean top predators is investigated using sensitive biomarkers such as Vitellogenin (Vtg), Zona Radiata proteins (Zrp) and CYP1A activities in order to evaluate the toxicological hazard in *Xiphias gladius* and *Thunnus thynnus thynnus*. Non-lethal techniques, such as non-lethal biomarkers (BPMO-CYP1A) activities in skin biopsy) are also used in order to carry out hazard assessment of threatened species exposed to EDs, such as cetaceans (*Stenella coeruleoalba*, *Tursiops truncatus*, *Delphinus delphis* and *Balaenoptera physalus*).

1 Materials and Methods

1.1 Large pelagic fish

In a four-year survey on the Mediterranean population of swordfish (*Xiphias gladius*) (Fossi et al. 2004) and tuna fish (*Thunnus thynnus thynnus*), the potential toxicological effects of organochlorine compounds were investigated on 192 specimens of swordfish and 20 specimens of tuna fish caught in the spawning seasons from 1999 to 2002 in the Straits of Messina, Sicily (Italy) by a set of biomarker and contaminant analysis. Specimens of swordfish were also sampled in the Atlantic ocean (Azores) and used as a reference population. Vitellogenin (Vtg) and Zona radiata proteins (Zrp) (Goksoyr, 1991) levels were measured in plasma samples, CYP1A activities (Ethoxyresorufin-O-deethylase (EROD), Benzo(α)pyrene monooxygenase (BPMO)) (Kurelec et al. 1977, Lubet et al. 1985) were detected in liver. OCs levels were detected in muscle, liver and gonads by the method of Marsili and Focardi (1996) and Fossi et al. (2004). Recoveries (70–95%) were calculated by adding known quantities of standard to homogeneous replicates of the same sample. The results were expressed in ng/g dry weight (d.w.). The detection limit was 0.1 ppb.

1.2 Marine mammals

Subcutaneous tissues (skin and blubber) were obtained from *Stenella coeruleoalba*, *Tursiops truncatus*, *Delphinus delphis* and *Balaenoptera physalus* from the western Ligurian Sea, between Corsica and the French-Italian coast, and Ionic Sea using biopsy darts launched with a crossbow.

Benzo(α)pyrene monooxygenase (BPMO-CYP1A) activity was measured in biopsies and organochlorine levels were detected. BPMO activity was assessed using the incubation mixture proposed by Fossi et al. (1992) incubating each sample (plus the blanks) in a shaking bath for 2 h at 37°C. The activity was expressed in arbitrary units of fluorescence (A.U.F./h/g tissue). Capillary gas-chromatography revealed the presence of *op'*- and *pp'*- isomers of DDT and its derivatives DDD and DDE, and about 30 PCB congeners (Marsili & Focardi, 1997, Marsili 2000). A mixture of specific isomers was used to calibrate the system, evaluate recovery (80–110%) and confirm the results, which were expressed in ng/g or μ g/g dry weight (d.w.). The detection limit was 0.1 ppb.

2 Results and Discussion

2.1 Large pelagic fish

The first warning about toxicological risk to large Mediterranean pelagic fish due to EDs was pinpointed by the results of Fossi et al. (2001a) in swordfish (*Xiphias gladius*) and Fossi et al. (2002) in blue fin tuna (*Thunnus thynnus thynnus*). Dramatic induction of typically female proteins (Vtg and Zrp) was detected by ELISA and western blot in adult males of the two species. Results of the four-year survey on the Mediterranean population of swordfish confirmed the finding of dramatic induction in adult male swordfish of Vtg and Zrp. It is interesting to see that several Mediterranean male specimens show values of Vtg and Zrp (Fig. 1A–B) that are higher than average male values and/or in the same range as those of reproductive females, which suggests that this species is exposed to xenoestrogen in the Mediterranean Sea. This result is particularly evident in 1999 when the age of the swordfish was higher. In general, levels of Vtg and Zrp measured by ELISA test were higher in Mediterranean specimens in comparison with Atlantic. Atlantic swordfish showed a Vtg mean level = 0.17 (Abs at 490 nm) and Zrp mean level = 0.14 (Abs at 490 nm) (Fossi et al. 2001b).

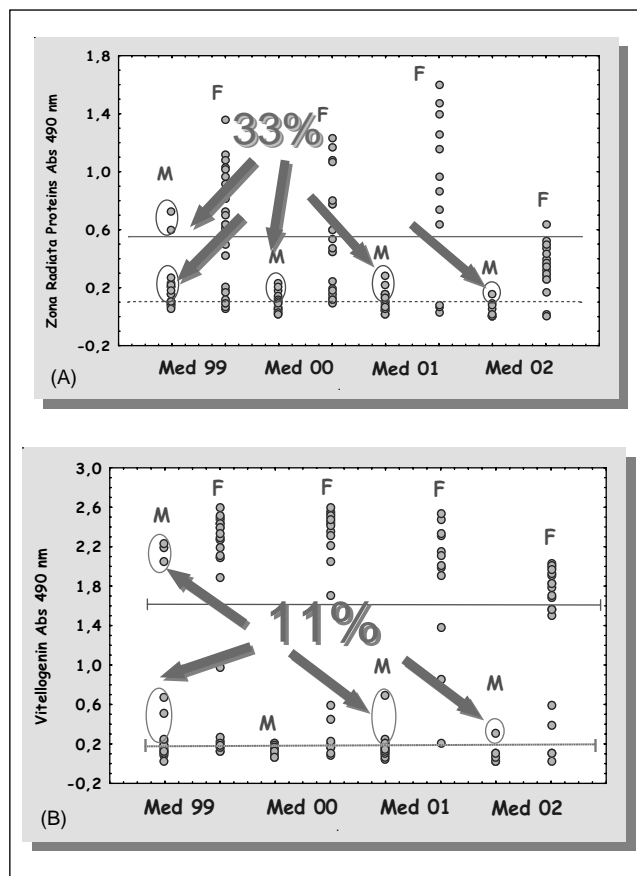


Fig. 1: (A) Zona radiata proteins (Zrp) and (B) vitellogenin (Vtg) of male and female swordfish (*Xiphias gladius*) captured in the Mediterranean Sea (Straits of Messina, Sicily, Italy) in summer 1999, 2000, 2001, 2002, during the spawning period. Circles indicate male specimens showing values higher than average male values (line) and/or in the same range as those of reproductive females. (Fossi et al. 2004, modified)

CYP1A activities (BPMO and EROD) were found to be more than twice higher in Mediterranean specimens in comparison to control population.

A role of organochlorines (PCBs in liver ranged 128–22847 ppb d.w.) in this induction phenomenon in swordfish is suggested by the statistically significant correlations between Zrp levels in plasma and PCB concentrations in muscle (Tau b Kendal = 0.312; $p < 0.032$) and Vtg levels in plasma and PCB concentrations in liver (Tau b Kendal = 0.618, $p < 0.034$) of male specimens. Organochlorine levels (PCBs in liver) were also correlated with total length of male swordfish (Tau b Kendal = 0.377, $p < 0.021$). The present results confirm that induction of Vtg and Zrp can be used as a diagnostic tool for exposure assessment of Mediterranean swordfish stocks exposed to OCs with ED capacity (Fossi et al. 2004). These data, and those recently published by De Metrio (2003) demonstrating a high percentage of intersex in Mediterranean swordfish, sound a warning about potential reproductive alterations in large pelagic fish (Segner et al. 2003, Van der Belt et al. 2003, Hashimoto et al. 2000) and suggest the need for continuous monitoring to avoid reductions in their populations.

2.2 Marine mammals

Comparing levels of OCs in swordfish with levels found in free-ranging striped dolphins, we found levels 10 to 20 times higher in cetaceans (Fossi & Marsili 2003). The relative estrogenic power of these chemicals, identified by *in vitro* and *in vivo* screening methods (Safe 1995, Safe 2000) is rather weak (10–3 or less) compared with the reference power of 17-estradiol or DES (Miyamoto & Klein 1998). However, the high levels of organochlorine compounds detected in marine mammals, particularly in pinnipeds and odontocetes, and consequently, the high levels of organochlorines with ED capacity, cannot be ignored.

Some general considerations on potential hazard to these Mediterranean species can be drawn from comparison of the data commonly detected in Mediterranean cetaceans and that of other cetacean species with known reproductive impairment (Fossi & Marsili 2003). Several examples suggest that exposure to OC insecticides and PCBs has affected endocrine function and reproduction in marine mammals. It is worth noting that levels of PCBs found in Mediterranean free ranging odontocetes (Fossi et al. 2003) are similar to those detected in the population of beluga whales of the St. Lawrence estuary in which was detected a hermaphrodite specimen (PCBs mean value = 78900 ng/g l.w.) (Muir et al. 1996). This observation suggests the potential hazard that these species are exposed to in the Mediterranean Sea.

Benzo(α)pyrene monooxygenase (CYP1A1) activity in skin biopsies (Fossi et al. 1992) of Mediterranean cetaceans (*Stenella coeruleoalba*, *Delphinus delphis*, *Tursiops truncatus* and *Balaenoptera physalus*) was used as a potential indicator of exposure to organochlorines, with special reference to the compounds with endocrine disrupting capacity.

Organochlorine concentrations (HCB, DDTs and PCBs) and BPMO activities, confirmed literature data and results obtained in our lab before 1994 (Fossi et al. 1992, Marsili 2000), indicated that marked differences in levels of all contaminants exist between fin whales and odontocete species. The same was found for BPMO activity induction in which some odontocete species, such as the striped dolphin, having CYP1A1 activities four times higher than the mysticetes, binded with levels of OCs one order of magnitude higher in odontocetes (Fossi et al. 2003). The difference in organochlorine bioaccumulation and consequently in BPMO induction between the two groups is obviously related to their different positions in the marine food chain with odontocetes as terminal consumers and fin whales as macroplanktophages.

A linear correlation was found between OCs known as endocrine disruptors and CYP1A1 (BPMO) activity (Pearson test) in striped dolphins and common dolphins (Fossi et al. 2003) suggesting the exposure of these species to a potential hazard of OC-EDs. Gender differences in BPMO induction was also investigated. In striped dolphins a linear correlation was found between op'DDT/BPMO and PCB153/BPMO. In the common dolphin there were identified five linear correlations with the BPMO activity: DDTs, pp'DDE, op'DDT, PCBs and PCB153. The main result in this species was non induction of BPMO in females with increasing levels of contaminants (Fossi et al. 2003).

As a new methodological model (cell culture) we explored interspecies and gender susceptibility to OC-EDs and PBDEs using qualitative and semi-quantitative evaluation of target proteins, such as CYP450 1A1 and CYP450 2B in cultured cetacean fibroblasts, by western blot and immunofluorescence technique.

3 Conclusion

In conclusion the present research shows that: a) Vtg and Zrp can be used as diagnostic tools for fish stocks hazard assessment in the Mediterranean Sea; b) that CYP1A1 (BPMO) induction in cetaceans skin biopsy may be an early sign of exposure to EDs such as OCs and a potential alert for transgenerational effects. This research represents a warning signal of the potential reproductive alterations in marine top predators and suggest the need for continuous monitoring to avoid reductions in population and biodiversity in the Mediterranean Sea.

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