PRELIMINARY EVIDENCE OF ENDOCRINE DISRUPTION IN THE RED MULLET, *MULLUS BARBATUS*, FROM THE NW MEDITERRANEAN

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Abstract

Red mullets (*Mullus barbatus*) from the NW Mediterranean were collected to assess potential alterations of the endocrine system as a consequence of pollution exposure. Ovarian P-450 aromatase and liver UDP-glucuronyl transferases, two enzymes that might modulate synthesis and inactivation of endogenous steroids, were determined in field collected specimens. During the spring sampling (before reproduction), low P450 aromatase activity was recorded in females from the most impacted sites (Cortiou and Fos). After reproduction, enhanced glucuronidation of testosterone and estradiol by liver microsomal fractions was detected in specimens from those sites. The study describes for the first time some evidence of endocrine disruption in red mullets from the NW Mediterranean.

Keywords: P450 aromatase, UGTs, red mullet

The Western Mediterranean is the recipient of extensive urban and industrial waste-water discharges from bordering countries, and at the same time is an attractive recreational region; it is thus in need of tools for environmental risk assessment. Some recent studies have determined biochemical and cellular responses to pollutants in coastal fish (1-4) in an attempt to assess the toxicity of chemicals that are currently released into the marine environment. Recently, some evidences of endocrine alterations and intersexuality in pelagic fish predators have been reported (5-6), but the interference of pollutants with the endocrine system of coastal fish is largely unknown.

In this study, we have focussed on the red mullet *Mullus barbatus*, because it is a benthic and territorial fish of commercial interest in the region, which has been used in several studies of coastal pollution monitoring. Specimens were collected in spring and autum 2001 from 4-7 sites along the NW Mediterranean. The impact of coastal pollution was assessed by the combined determination of a battery of biochemical markers, namely 7-ethoxyresorufin *O*-deethylase (EROD), UDP-glucuronyl transferases, catalase, and acetylcholinesterase. Those markers indicated strong differences among sampling sites (Fig. 1), the highest degree of stress detected in red mullets from Cortiou (urban and industrial area) and Fos (oil exposure), but also in specimens from the Ebro Delta (pesticide exposure). Levels of alkylphenols, mainly nonylphenol (NP) and octylphenol (OP) were determined in fish bile as a measure of recent exposure to this type of compounds. High levels of alkylphenols were detected in fish from Cortiou, under the influence of Marseille (0.25 µg OP/g, 28 µg NP/g), that appeared as highly polluted in comparison with nearby areas (0.03-0.08 µg OP/g, 0.2-1.6 µg NP/g). Alkylphenols, and particularly octyl- and nonylphenol, have been shown to elicit estrogenic responses both *in-vivo* and *in-vitro*. Therefore, efforts were addressed to assess potential effects on the endocrine system of exposed fish from those impacted areas (Cortiou and Fos) by comparison with two "cleaner" stations (Portofino and Arenzano in the Italian coast). To this end, ovarian cytochrome P450 aromatase, and rates of conjugation of testosterone and estradiol by liver microsomal fractions were selected as potential markers, and they were determined along a pollution gradient before and after the reproductive period.

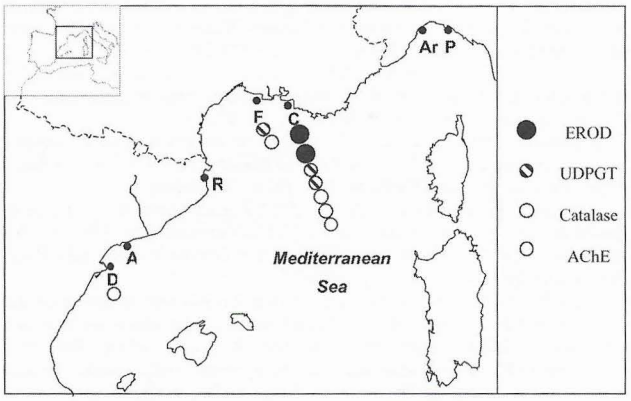

Fig. 1. Map of the NW Mediterranean showing the sites where red mullets were sampled in spring/autumn 2001, together with different biomarkers of exposure, namelly liver microsomal 7-ethoxyresorufin *O*-dethylase (EROD) activity, liver microsomal UDP-glucuronyltransferase using p-nitrophenol as substrate (UDPGT), liver cytosolic catalase activity, and muscle acetylcholinesterase (AChE). The spots in the map indicate those stations that had significantly higher activities when compared to reference sites. Letters of stations belong to P-Portofino, Ar-Arenzano, C-Cortiou, F-Fos-sur-Mer, R-Roses, A-Altafulla, D-Ebro Delta.

During the spring sampling (before reproduction), females from Cortiou and Fos had lower ovarian P450 aromatase activity (16-18 pmol/h/mg protein) than those from the Italian sites (35-36 pmol/h/mg protein). These results suggest the existence of compounds that may interfere (inhibit) this enzymatic activity, and that may lead to a delay in maturation. Histological sections of the gonads confirmed this delay in maturation in both males and females from Cortiou, in comparison with the other stations. After reproduction, ovarian P450 aromatase sharply decreased (up to 10-fold), and although females from Cortiou still had the lowest activity, differences between stations were not statistically significant. At that time, gonads of both males and females were at early stages of development, and no alterations nor differences among sampling sites were recorded.

When the activity of UDP-glucuronyl transferase towards testosterone and estradiol was monitored in liver microsomal fractions, we found that the organisms from the most polluted areas had higher activities of hormone glucuronidation. Toxicant induction of hepatic biotransformation enzymes is a mechanism by which endogenous steroid hormone metabolism and elimination may be altered, thus leading to enhanced testosterone and estradiol clearance, and disruption of the steroid metabolism within the organism. Additionally, glucuronidation of hormones in piscine species appears to be important, both in chemcial signalling and in cessation of their activity via excretion.

Overall, the set of biomarkers used indicated different levels of stress in red mullets along the NW Mediterranean, together with some evidences of endocrine disruption in specimens from highly impacted areas.

References

1 - Roméo M., Mathieu A., Gnassia-Barelli M., Romana A., Lafaurie M., 1994. Heavy metal content and biotrasformation enzymes in two fish species from the NW Mediterranean. *Mar. Ecol. Prog. Ser.* 107: 15–22.

2 - Burgeot T., Bocquéné G., Porte C., Dimeet J., Santella R.M., Garcia de la Parra L.M., Píhol-Leszkowicz A., Raoux C., Galgani F., 1996. Bioindicators of pollutant exposure in the North Western Mediterranean Sea. *Mar. Ecol. Prog. Ser.* 131: 125-141.

3 - Porte C., Escartín E., García de la Parra L.M., Biosca X., Albaigés J., 2002. Assessment of coastal pollution by combined determination of chemical and biochemical markers in *Mullus barbatus*. *Mar. Ecol. Prog. Ser.* 235: 205-216.

4 - Regoli F., Pellegrini D., Winston G.W., Gorbi S., Giuliani S., Virno-Lamberti C., Bompadre S., 2002. Application of biomarkers for assessing the biological impact of dredged materials in the Mediterranean: the relationship between antioxidant responses and susceptibility to oxidative stress in the red mullet (*Mullus barbatus*). *Mar. Pollut. Bull.* 44: 912–922.

5 - Fossi M.C., Casini S., Marsili L., Neri G., Mori G., Ancora S., Moscatelli A., Ausili A., Notarbartolo-di-Sciara G., 2002. Biomarkers for endocrine disruptors in three species of Mediterranean large pelagic fish. *Mar. Environ. Res.* 54: 667-671.

6 - De Metrio G., Corriero A., Desantis D., Zubani D., Cirillo F., Deflorio M., Bridges C.R., Eicker J., de la Serna J.M., Megalofonou P., Kime D.E., 2003. Evidence of a high percentage of intersex in the Mediterranean swordfish (*Xiphias gladius* L.). *Mar. Pollut. Bull.* 46: 358-361.