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Bioaccumulation markers and biochemical responses in European sea bass (*Dicentrarchus labrax*) raised under different environmental conditions

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A B S T R A C T

Site- and season-specific biochemical responses and bioaccumulations of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) were analysed in tissues from sea bass raised under four different environmental conditions and sampled in April and July. Samples were analysed for condition factor (CF), liver somatic index (LSI), glutathione S-transferase (GST) activity, and glycogen and lactate contents. Results showed the presence of PCBs and DDTs, with site- and season-specific variations in its concentrations. CFs did not differ significantly, while LSIs in samples from two of the four sites decreased between April and July. GST activities were lower in samples with higher concentrations of PCBs and DDTs. Lactate and glycogen contents were influenced to a greater extent by the season than by levels of contamination. The study demonstrated that farming methods could play a crucial role in both health status and bioaccumulation of OC compounds in farmed sea bass.

1. Introduction

Fish are an excellent source of lipids in the human diet, due to their high content of the long chain n-3 polyunsaturated fatty acids, which are of benefit to cardiovascular system. Nevertheless, in recent years these have been a great concern about the fish consumption risk for human health due to the presence of persistent organic pollutants as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), specially in farmed fish (Hites et al., 2004; Serrano et al., 2008).

Aquaculture activities in the Mediterranean Sea are mainly focused on two species: gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*; Grigorakis, 2007). European sea bass is a euryhaline fish species, adaptable to a wide range of water temperatures and salinity. Due to this adaptive ability, and because it is one of the most popular fish consumed in Italy, this species is farmed all along the Italian coast.

In Italy, sea bass is raised using several culture methods including 'vallicolture' in brackish waters (called 'valli'), in ponds or in lagoons (22% of total fish produced) and cage culture in floating cages (32% of total fish produced; FAO, 2007).

An increasing volume of evidence suggests that fish are able to accumulate pollutants in their bodies. This represents a source of chemicals dangerous to consumers. Organochlorine (OC) compounds such as polychlorinated biphenyls (PCBs) and organochlorinated pesticides (OCPs) are persistent, accumulate in the lipid part of the animal and thus bioaccumulate in the food chain. As a consequence, they are often detected in fish (Berg et al., 1997; Roche et al., 2000), birds (Cleemann et al., 2000) and marine mammals (Hernández et al., 2000; Cleemann et al., 2000). Because of the accumulative nature of these compounds, they have been detected in human adipose tissues and fluids (Hernández et al., 2002; Pitarch et al., 2003; De Saeger et al., 2005; Withcomb et al., 2005). Dietary intake, especially of marine organisms, is considered to be one of the most important sources of OCs for the human population (Bjerregaard et al., 2001; Johansen et al., 2004; Naso et al., 2005; Bordajandi et al., 2006). Fish can therefore contribute significantly to human dietary exposure to these contaminants. A report collating all the available data concerning contaminant levels in Italian fish and fishery products highlighted a consistent heterogeneity in data regarding the concentrations of halogenated compounds (especially PCBs) present in marine organisms (Ferrara and Funari, 2004).

Species, season, diet, location, life stage and age all have a major impact on both nutrient and contaminant levels in fish. Contaminants present in fish are derived predominantly from their diet, and levels of bioaccumulative contaminants are higher in fish that appear high up in the food chain. Recently, concerns have been raised about the safety assessment of cultivated fish. Farmed fish are raised on diets of fish meal and fish-oil-based feed, which can be an important source of lipophilic

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contaminants. A study carried out by Hites et al. (2004) on OC levels in farmed and wild salmon showed that concentrations of OC pollutants were significantly higher in farmed salmon than in wild salmon. This high load of pollutants in farmed salmon was also highlighted in a study performed by Easton et al. (2002). However, as regards marine species, Serrano et al. (2008) pointed out that concentrations of OCs are lower in cultured sea bream compared with wild fish. These differences could be attributed to the level of contaminants present in the different diets and different feeding characteristics between salmon and Mediterranean species.

In this study, site- and season-specific biochemical responses and bioaccumulations of OCPs and PCBs were analysed in sea bass specimens raised under different environmental conditions, and using different farming methods. The aim was to study effects of the environment (intended also to represent different farming methods) on both health status of the fish and bioaccumulation of OC contaminants in fish tissues. Bioindicators of the health of individual fish included a general condition factor (CF), the liver somatic index (LSI) and a biomarker of contaminant exposure, glutathione S-transferase (GST) activity (Hamed et al., 2003). Glycogen and lactate contents of the muscle, metabolic indices of stress that are sensitive to a wide variety of stressors, including contamination level, were also assessed. Measures of these indices have been included in this study in order to obtain informative data about general physiological stress possibly induced by pollution.

To our knowledge this is the first time that such an integrated monitoring approach, taking into account seasonal variations in chemical contents and biochemical responses, has been performed on sea bass tissues originating from fish farms on the North West Italian coast.

2. Materials and methods

2.1. Enzymes, standards and others chemicals

The chemicals used in this study for biochemical analysis were obtained from Sigma Chemicals Co. (St. Louis, MO, USA) and Merck (Germany). Bradford reagent was from Bio-Rad (Germany). For pesticide and PCB analysis, reagents and solvents were of Pesticide Residue Analysis Quality (Scharlab, Barcelona, Spain); pp′-DDE-D8, used as an internal standard, PCB congeners, DDTs (p,p′-DDT, p,p′-DDE, p,p′-DDD), HCB, heptachlor, metoxychlor and mirex were purchased from Dr. Ehrenstorfer (Augsburg, Germany). All other reagents were of analytical grade.

2.2. Study areas and sampling

Sea bass (*D. labrax*) specimens were obtained from four different fish farms located along the North West Italian coast: three in Tuscany and one in Ligury (Fig. 1). The first Tuscan farm (site 1) is located in the wetland *Diaccia Botrona*, 40 km north from Orbetello Lagoon. The farm, which produces about 400 tons of sea bass per year in brackish water, operates in a semi-closed circuit. The inflow and outflow waters are stabilised in a shallow water lagoon system. The fish are raised in concrete ponds in a semi-intensive culture but with a very low stocking density (5–7 kg/m^3). Fish reach a weight of 1 kg over a period of up to 48 months, at which stage they are ready to be sold. Computed daily feed rations are given between once and three times per day, depending on the season.

The second Tuscan farm (site 2) is located in a steel mill and uses the warm marine water resulting from the cooling of the blast furnace to heat the fish tanks. This allows the water to be maintained at a constant temperature of 22 °C throughout the year. The fish, raised in an intensive culture (35 kg/m^3), grow quickly, reaching a body weight of 0.7–0.8 kg in 18 months. Fish are fed at regular intervals throughout the day using automatic distributors.

The third Tuscan sampling area (site 3) is situated in the Orbetello lagoon, a 2600 ha area on the southern coast of Tuscany, bordered by two sandbars. Water from the Albenga river flows into the western basin to the north, whilst the Nassa canal connects it to the sea in the south. The eastern basin is connected to the sea by the Ansedonia canal, a narrow inlet, which limits the exchange with seawater. In the fish farm the water from the lagoon flows through artificial channels into the fish tanks. The outflow water is stabilised in a shallow water lagoon system before being returned to the lagoon. The fish are raised in an intensive culture (30 kg/m^3) in soil ponds lined with PVC. Fish reach a weight of 0.7–1 kg over a period of up to 36 months. Computed daily feed rations are given between once and three times per day, depending on the season.

The Ligurian fish farm (site 4) comprises marine cages situated 2 km from Lavagna, in the Genoa province. Fish are raised in a semi-intensive culture (20 kg/m^3) and reached a commercial weight of 0.4 kg over a period of up to 21 months. Computed daily feed rations are given once or twice per day, depending on the season. All the fish farms studied use an extruded commercial feed based on fish meal and oil (produced by Skretting®, Verona, Italy; composition: proteins 46%; lipids 20%; 10% ashes; 1% fibres).

To study seasonal effects on the measured parameters, sampling was performed during April and July. In compliance with Council Directive 86/609/EEC (24 November 1986) on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes, fish were maintained in water with ice before sacrifice in order to avoid suffering. At each sampling time, six fish were sacrificed by anoxia with dry ice and kept at −40 °C until analysis. At the time of analysis, fish were weighed (April weight: 519 ± 189 g; July weight: 487 ± 125 g),

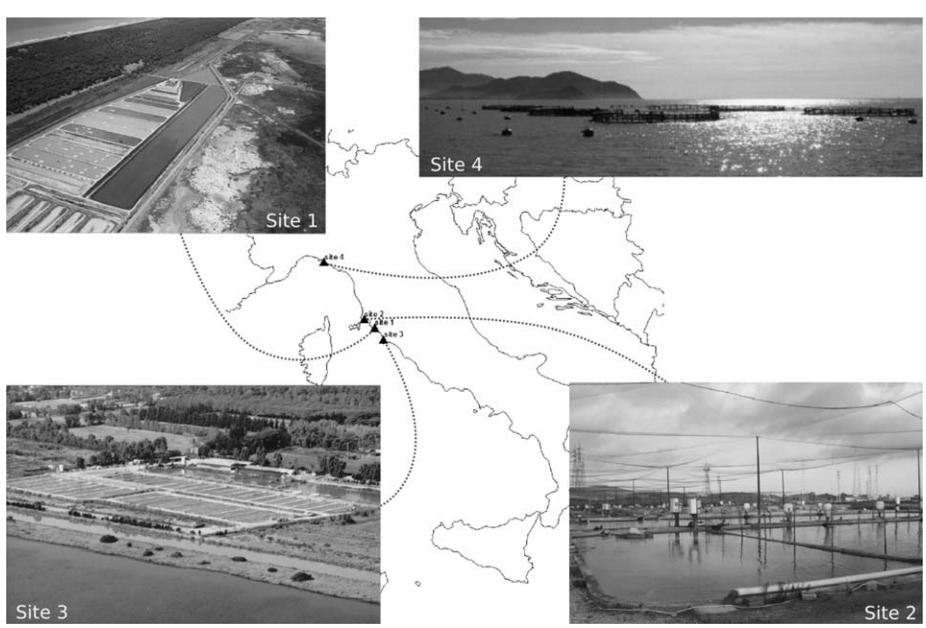

Fig. 1. Site map.