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# Impact assessment of various rearing systems on fish health using multibiomarker response and metal accumulation

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#### Abstract

European sea bass were reared in three different systems: one flow-through (FTS), one recirculating (RAS), and one recirculating with a high-rate algae pond (RAS + HRAP). After 1 year of rearing, the final fish weight was 15% lower in the RAS compared to the FTS. The accumulation of a growth-inhibiting substance in the RAS is the main hypothesis explaining this difference. As in environmental risk assessment, fish bioaccumulation markers and biomarkers were used to demonstrate exposure to and effects of the rearing water in the three rearing systems. Thirty fish per system were sacrificed before their condition factor (CF) and liver somatic index (LSI) were calculated. Nine biomarkers, including ethoxyresorufin-*O*-deethylase (EROD) and superoxide dismutase (SOD), were measured in liver and twelve metals including As, Cd, Cu, Pb, Cr, and Zn, for which there are regulations regarding human consumption, were measured in liver and muscle. In all systems, CF and LSI were not significantly different and no correlation was found with biomarker activity or metal concentration. EROD and SOD activities were significantly increased in RAS. Accumulation of seven and four metals in muscle and liver, respectively, was significantly higher in the RAS relative to FTS. The HRAP prevented metal accumulation except for chromium and arsenic. Eight metal concentrations were significantly higher in liver than in muscle. Concentrations of toxic metals were similar to reported values and below FAO/WHO recommended values for human consumption.

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## 1. Introduction

Aquaculture production is increasing due to worldwide food demand and a significant reduction in fisheries stocks. However, its impact on the environment is a cause for concern and new environmental regulations are in preparation. Traditional onshore rearing

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systems are open systems that pump water from the environment and discharge it after use without treatment. Recirculating aquaculture systems (RAS) may become a solution as they require fewer water resources and allow better control on wastes than in open systems (Blancheton et al., 1996). In recirculating systems, the daily water replacement rate is reduced 30–50 times compared to that in an open system. However, some dissolved substances (C, N, P), which are not removed by treatment in the RAS, accumulate in the water more or less rapidly depending on feeding and water renewal

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rates (Leonard et al., 2002; Pagand et al., 2000a). For several years, algae cultures have been tested to remove nutrients from fish effluent and to produce valuable seaweed of reliable quality (Cohen and Neori, 1991; Jiménez del Rio et al., 1996; Pagand et al., 2000b). Recently, algae ponds have been tested successfully in integrated rearing systems, where treated water is reused in fish tanks allowing reductions in replacement water (Neori et al., 1996; Schuenhoff et al., 2003). These recirculating and integrated systems offer a promising future because of their potential environmental and economical benefits.

At the Ifremer Palavas station, European sea bass were reared in three different systems: one flowthrough (FTS), one recirculating (RAS) and one recirculating with a high-rate algae pond (RAS+H-RAP). After 1 year of rearing, Deviller et al. (2004) showed that the water quality in the three systems was satisfactory for the fish, as indicated by their low mortality rates and their growth performances. However, the final fish weight was 15% lower in the RAS than in the FTS. The accumulation of a growthinhibiting substance in the RAS is the main hypothesis for explaining this difference. In environmental risk assessment, fish bioaccumulation markers and biomarkers are used to demonstrate exposure to and effects of environmental contaminants. Fish bioaccumulation markers may be used to elucidate the aquatic behavior of environmental contaminants, to identify certain substances present at low concentrations, and to assess exposure of aquatic organisms (Van der Oost et al., 2003). Among pollutants that could accumulate in fish, metals are of great interest because it has been shown that they could trigger oxidative stress in fish and affect their growth (Baker et al., 1997, 1998). Also, metals are ubiquitous in marine waters, they are accurately measurable in trace quantities (Pérez Cid et al., 2001), they correlate well with previous exposure (Kraal et al., 1995; Odzak and Zvonaric, 1995), and some are controlled for human consumption (As, Cd, Cu, Pb, Cr, Zn).

Among the biomarkers described in the literature, the following are reliable and easy assays currently used in environmental risk assessment: phase I and II biotransformation enzyme, oxidative stress indicators, and fish indices (Van der Oost et al., 2003). Also, the stress proteins (HSPs) involved in the protection of the cell in response to stress conditions are promising nonspecific biomarkers, especially HSP70 forms, which have been proposed to detect the toxicity of various chemicals (Aït-Aïssa et al., 2000).

Our hypothesis is that the recirculating loop and the algae treatment can eliminate, concentrate, and/or release various compounds, some of which may be toxic to fish. In this study, biomarkers were measured in the liver because it is the main detoxification organ in fish. For sea bass reared in three different systems, we compared liver multibiomarker levels (liver proteins (LP), ethoxyresorufin-O-Deethylase (EROD), glutathione-S-transferase (GST), total (GSH) and disulfide (GSSG) glutathione, superoxide dismutase (SOD), glutathione peroxidase (GPOX), catalase (CAT), and stress protein (HSP70)) and liver trace metal levels (Cr, Mn, Co, Ni, Cu, Zn, As, Ag, Cd, Sn, Tl, and Pb). The same metals were measured in muscle to define the quality of food for human consumption.

### 2. Materials and methods

#### 2.1. Rearing systems characteristics

Sea bass (Dicentrarchus labrax) were reared over one year in three different systems, FTS, RAS, and RAS+HRAP, in an experimental research institute based in the south of France (IFREMER, Palavas). The systems and their operational characteristics are described in Deviller et al. (2004). In each system, two rearing tanks were used as replicates and fish were fed on demand by operating the tactile trigger of the selffeeders containing commercial sea bass feed (44-52%) proteins, 1.5% phosphorus, 22% fat, 10% ash). In both recirculating systems, the replacement water flow rate was adjusted twice a week according to the ingested food quantity, in order to maintain a constant ratio  $R=2\pm 1 \text{ m}^3 \text{ kg}^{-1}$ . In the FTS, the replacement water flow rate was constant, resulting in 79–41 m<sup>3</sup> kg<sup>-1</sup> values of R. Annual averages (+ standard deviation) were measured in the FTS, RAS, and RAS + HRAP for water temperature  $(22\pm2; 23\pm2; 23\pm2^{\circ}C)$ , salinity  $(38\pm3;$  $32\pm3$ ;  $31\pm6\,\mathrm{g\,L^{-1}}$ ), pH (7.8 $\pm0.2$ ; 7.0 $\pm0.3$ ; 7.1 $\pm0.3$ ), oxygen concentration  $(7.7 \pm 1.7;$ and  $7.3 \pm 1.2;$  $7.8 \pm 1.4 \text{ mg L}^{-1}$ ). Those variations are not correlated with fish-growth variations in the systems as explained in Deviller et al. (2004).

#### 2.2. Fish sampling

Thirty fish per system were caught at random in each of the two replicated tanks and sacrificed by a blow to the head. After capture, total fish length ( $L_{\text{fish}}$ ; mm) and weight ( $W_{\text{fish}}$ ; g) were measured before the livers were dissected and weighed ( $W_{\text{liver}}$ ; g). For an average of 10 fish per circuit, a muscle sample was dissected and weighed for metal assays.

# 2.3. Calculation of growth indices

The condition factor (CF) was calculated according to Bagnenal and Tesch (1978),

 $CF = (W_{fish}/(L_{fish}/10)^3) \times 100$ 

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