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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.

Keywords: Biomarkers; Bioaccumulation; Metals; Fish health; Recirculation; Algae; Food safety; Aquaculture

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 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 flow-through (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 growth-inhibiting 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 self-feeders 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 $R = 2 \pm 1\,m^3\,kg^{-1}$. In the FTS, the replacement water flow rate was constant, resulting in 79–41 $m^3\,kg^{-1}$ values of R. Annual averages ($\pm$ standard deviation) were measured in the FTS, RAS, and RAS + HRAP for water temperature (22 ± 2; 23 ± 2; $23 \pm 2\,^{\circ}C$), salinity (38 ± 3; 32 ± 3; $31 \pm 6\,g\,L^{-1}$), pH (7.8 ± 0.2; 7.0 ± 0.3; 7.1 ± 0.3), and oxygen concentration (7.7 ± 1.7; 7.3 ± 1.2; $7.8 \pm 1.4\,mg\,L^{-1}$). 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_{fish}; mm) and weight (W_{fish}; g) were measured before the livers were dissected and weighed (W_{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),

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

and the liver somatic index (LSI) was calculated according to Slooff et al. (1983),

$$LSI = (W_{liver}/W_{fish}) \times 100.$$

2.4. Biomarker analysis

Liver samples were weighed, rinsed with 150 mM KCl, poured into cryotubes, frozen in liquid nitrogen and kept at −80 °C during transport to the laboratory. There, the enzymatic assays were performed at under +4 °C. Livers were homogenized with a Teflon-glass Potter-Elvehjerm in 2.5 mL of phosphate buffer (100 mM phosphate, pH 7.8, with 20% glycerol and 0.2 mM phenyl methyl sulfonide fluorure) and centrifuged at 10,000*g* for 15 min. The supernatants were collected for biomarker measurements. The reference methods used for LP, EROD, GST, GSH and GSSG, SOD, GPOX, and CAT assays are given in Table 1. The LP is not a biomarker according to the Lagadic et al. (1997) definition, but it provides an initial indication of liver status and is used to standardize the other assays. HSP70 were analyzed by western blotting (Lewis, 1999) after electrophoresis separation of total proteins according to the Laemmli (1970) method. Blot densitometric analysis of the bands was performed using Image Master 1D software (Pharmacia). Results are expressed as normalized arbitrary units of HSP70 using normalization with a HSP70 standard (Sigma).

2.5. Trace metals analysis

Liver and muscle pieces were weighed, wrapped in aluminum foil, and stored at −24 °C prior to determination of metal contents. The samples were oven-dried at 100 °C to a constant weight and homogenized using a mortar and pestle. The milled samples were placed in ziplock polythene bags and kept in a dessicator until digestion. Samples were transferred into the flasks of a microwave digester for two-step digestion. Nitric acid (10 mL) and hydrogen peroxide (5 mL) were added successively for a 20-min (60-W) and a 15-min (80-W) digestion. The digests were diluted up to 50 mL using ultra pure water (Millipore MilliQ), filtered (Whatman GF/C), and conserved at 4 °C in plastic vials until analysis. Metal concentrations were measured using an inductively coupled plasma mass spectrophotometer (ICP-MS). The instrument was calibrated using two standard solutions containing all the metals considered at 5 and 10 ppb. An internal standard solution containing Bi 209 and In 115 was added to each sample at 10 ppb.

Table 1
Methods of references used for biochemical biomarker assays

Enzymatic assay	Reference publications
Liver protein (LP)	Bradford (1976)
Ethoxyresorufin-*O*-Deethylase (EROD)	Flammarion and Migeon (1998)
Glutathione-*S*-transferase (GST)	Habig et al. (1974)
Total (GSH) and disulfide (GSSG) glutathione	Vandeputte et al. (1994)
Superoxide dismutase (SOD)	Paoletti et al. (1986)
Glutathione peroxidase (GPOX)	Paglia and Valentine (1967)
Catalase (CAT)	Babo and Vasseur (1992)

2.6. Statistic analysis

A one-way analysis of variance (ANOVA) was used for comparison of body weight, length, LSI, CF, liver biomarkers, and muscle and liver metals in the three rearing systems (FTS, RAS, RAS + HRAP). When the variances were not homogeneous and/or the residuals deviated from the norm, the data were Ln transformed prior to statistical analysis. If the ANOVA showed a significant difference ($P < 0.05$), a Tukey test was carried out to compare the different systems. If the variances were still not homogeneous and/or the residuals deviated from the norm in spite of transformation, a Kruskal–Wallis nonparametric test was carried out. Spearman correlation analyses were used to examine the associations of biomarkers and metals with fish size and of biomarkers with metals.

3. Results

3.1. Growth indices

Table 2 shows length and weight ranges of sampled fish for each circuit and their corresponding condition factor (CF) and liver somatic index (LSI). In the FTS, the mean weight and length of the sampled fish are significantly higher than in the two recirculating systems. However, the CF and the LSI are not significantly different in the three systems and no significant correlation ($P > 0.05$) is found between biomarkers and fish size (length or weight).

3.2. Biomarker analysis

The results for LP, EROD, GST, GSH, GSSG, SOD, GPOX, CAT, and HSP70 are given in Table 3. There is no significant difference between systems for LP, GPOX, CAT, GST, GSH, GSSG, and HSP70. On the other hand, SOD activity is significantly higher in the RAS, and EROD activity is significantly increased in the two recirculating systems, with or without algae treatment.

Table 2
Size ranges of sea bass sampled in the three rearing systems (means ± SD)

Rearing system	N	Length (mm)	Weight (g)	Condition factor (CF)	Liver somatic index (LSI)
FTS	30	317±25[a]	440±117[a]	1.36±0.26[a]	2.19±0.43[a]
RAS	30	297±26[b]	364±81[b]	1.41±0.34[a]	1.94±0.46[a]
RAS+HRAP	30	295±34[b]	363±124[b]	1.38±0.31[a]	2.12±0.43[a]

Note: Values with different superscripts are significantly different ($P<0.05$).

Table 3
Biochemical biomarkers in livers of sea bass after 1 yr of rearing in three different systems (means ± SD)

	FTS	RAS	RAS+HRAP
LP[a]	34.4±8.8	37.5±10.6	38.7±9.0
SOD[b]	15,724±9576	19,424±7402*	15,096±4933
GPOX[b]	31.5±17.3	31.0±12.8	26.5±18.4
CAT[b]	2008±689	2028±556	1974±588
GST[b]	1183±298	1235±412	1297±361
GSH[c]	18.9±8.9	18.3±4.8	17.7±5.8
GSSG[c]	0.51±0.61	0.55±0.69	0.42±0.44
EROD[d]	8.1±5.7	12.2±6.7*	13.1±6.6*
HSP70[e]	0.17±0.13	0.16±0.14	0.19±0.20

*Significantly different for FTS ($P<0.05$).
[a]mg g^{-1} liver.
[b]nmol min^{-1} mg^{-1} protein.
[c]μmol g^{-1} protein.
[d]pmol min^{-1} mg^{-1} protein.
[e]Normalized arbitrary unit.

3.3. Metals analysis

The weight and length averages of sampled fish for metal assays are not significantly different in the three systems. No significant correlation ($P<0.05$) was found between metal concentrations (muscle or liver) and fish size (length or weight).

The mean metal concentrations in the muscle and liver of fish are presented in Tables 4 and 5, respectively. Of the 12 metals measured in muscle, only zinc and arsenic met the normality assumption. The other metals were compared using the nonparametric test. Chromium, manganese, cobalt, nickel, copper, arsenic, and thallium are significantly higher in the muscle of fish from the RAS compared to the FTS. In the RAS+HRAP, only chromium and arsenic are significantly increased in fish muscle compared to the FTS.

Because of an analysis error on one liver sample, 9 samples, instead of 10, were considered for the FTS liver range. For the same reason as for the muscle, chromium, nickel, zinc, cadmium, tin, and lead in livers were compared by the nonparametric test. Chromium, cobalt, cadmium, and lead are significantly higher in liver of fish from the RAS compared to fish from the FTS. In the liver of fish from RAS+HRAP only chromium is increased and tin is reduced significantly compared to fish from the FTS. Manganese, cobalt, copper, zinc, silver, cadmium, tin, and thallium are significantly higher in liver compared to muscle, while arsenic in liver is significantly lower than in muscle.

4. Discussion

4.1. Growth indices

Greater length and weight of fish from FTS confirm previous observations and indicate that samples are representative of populations. However, we found the same LSI and CF in all rearing systems. They are not sensitive to specific parameters but they indicate that fish have similar liver conditions and global energy reserves in the three rearing systems (Mayer et al., 1992).

4.2. Biochemical biomarkers in the rearing systems

The same liver protein level in all the fish reinforces the hypothesis of a normal liver condition in all systems. EROD activity is significantly increased in fish of both recirculating systems, suggesting that they are exposed to pollutants. However, the response is slight, as previous studies have shown that most pollutants that interact with cytP450 are responsible for a strong increase in EROD activity (Van der Oost et al., 2003). As we do not have a standard value of EROD activity for this species, it is included in the range of normal physiological values. Nevertheless, the strongest increases were observed in laboratory studies with high concentrations of pollutants and/or exposure by injection (Lemaire et al., 1996; Lemaire-Gony et al., 1995; Viarengo et al., 1997), very different from our experimental conditions. On the other hand, some field studies showed low EROD increase close to our result, in polluted aquatic environments (Flammarion and Garric, 1997; Kosmala et al., 1998; Stien et al., 1998). Therefore, the low response observed in recirculating systems could be due to traces of pollutant contained in commercial fish feed, which could concentrate in such systems. Pollutants could be well-known cytP450

Table 4
Concentrations of heavy metals ($\mu g\,g^{-1}$ dry weight) in the muscle of fish reared in three different circuits (means ± SD)—min and max values are between parentheses

	FTS	RAS	RAS + HRAP
Chromium	0.05 ± 0.09 (0.00–0.32)[a]	0.25 ± 0.14 (0.00–0.38)[b]	0.24 ± 0.13 (0.00–0.09)[b]
Manganese	0.50 ± 0.46 (0.00–1.21)[a]	2.59 ± 2.56 (0.80–8.69)[b]	1.32 ± 0.66 (0.74–2.59)[a,b]
Cobalt	0.004 ± 0.007 (0.000–0.024)[a]	0.032 ± 0.038 (0.000–0.097)[b]	0.012 ± 0.012 (0.002–0.037)[a,b]
Nickel	0.16 ± 0.43 (0.00–1.35)[a]	0.59 ± 1.06 (0.00–0.61)[b]	2.74 ± 5.73 (0.00–14.39)[a,b]
Copper	0.75 ± 2.08 (0.00–6.60)[a]	0.98 ± 0.95 (0.00–3.48)[b]	0.40 ± 0.30 (0.19–0.96)[a,b]
Zinc	13.5 ± 9.9 (1.0–32.8)[a]	19.9 ± 12.7 (1.09–37.9)[a]	20.0 ± 9.7 (11.52–38.39)[a]
Arsenic	6.85 ± 1.38 (4.99–9.22)[a]	9.80 ± 1.18 (7.87–11.38)[b]	8.59 ± 1.33 (6.38–10.06)[b]
Silver	0.17 ± 0.38 (0.00–1.11)[a]	0.44 ± 1.24 (0.00–4.18)[a]	ND
Cadmium	0.003 ± 0.006 (0.000–0.020)[a]	0.014 ± 0.022 (0.000–0.073)[a]	0.005 ± 0.006 (0.000–0.017)[a]
Tin	0.019 ± 0.052 (0.000–0.167)[a]	0.001 ± 0.003 (0.000–0.007)[a]	0.002 ± 0.004 (0.000–0.008)[a]
Thallium	0.001 ± 0.001 (0.000–0.003)[a]	0.005 ± 0.008 (0.000–0.028)[b]	0.002 ± 0.001 (0.001–0.005)[a,b]
Lead	0.049 ± 0.106 (0.000–0.335)[a]	0.120 ± 0.128 (0.000–0.379)[a]	0.044 ± 0.086 (0.000–0.218)[a]

Note: Values with no common letter superscript are significantly different ($P<0.05$). ND: Nondetermined.

Table 5
Concentrations of heavy metals ($\mu g\,g^{-1}$ dry weight) in livers of fish reared in three different circuits (means ± SD): min and max values are between parentheses

	FTS	RAS	RAS + HRAP
Chromium	0.04 ± 0.09 (0.00–0.27)[a]	0.12 ± 0.08 (0.00–0.23)[b]	0.13 ± 0.08 (0.01–0.26)[b]
Manganese	1.88 ± 0.52 (1.11–2.59)[a]	2.44 ± 0.78 (1.47–3.97)[a]	2.15 ± 0.53 (1.58–2.93)[a]
Cobalt	0.029 ± 0.013 (0.000–0.048)[a]	0.045 ± 0.015 (0.028–0.071)[b]	0.048 ± 0.019 (0.024–0.070)[a,b]
Nickel	0.08 ± 0.21 (0.00–0.65)[a]	0.03 ± 0.05 (0.00–0.12)[a]	0.13 ± 0.15 (0.00–0.40)[a]
Copper	233 ± 57 (137–313)[a]	237 ± 82 (87–352)[a]	232 ± 77 (155–327)[a]
Zinc	84.3 ± 9.6 (68.8–103.5)[a]	83.1 ± 25.6 (41.9–133.3)[a]	85.0 ± 16.2 (67.9–112.6)[a]
Arsenic	2.39 ± 0.34 (1.98–2.98)[a]	2.55 ± 0.54 (1.29–3.26)[a]	2.85 ± 0.56 (2.42–3.96)[a]
Silver	0.44 ± 0.15 (0.24–0.63)[a]	0.48 ± 0.30 (0.13–1.05)[a]	0.35 ± 0.19 (0.14–0.63)[a]
Cadmium	0.25 ± 0.13 (0.00–0.46)[a]	0.94 ± 1.67 (0.27–5.94)[b]	0.32 ± 0.05 (0.28–0.40)[a,b]
Tin	0.043 ± 0.078 (0.000–0.249)[a]	0.009 ± 0.009 (0.000–0.025)[a]	0.001 ± 0.002 (0.000–0.005)[b]
Thallium	0.003 ± 0.002 (0.000–0.005)[a]	0.004 ± 0.002 (0.001–0.010)[a]	0.004 ± 0.001 (0.003–0.006)[a]
Lead	0.006 ± 0.012 (0.000–0.036)[a]	0.070 ± 0.078 (0.000–0.214)[b]	0.049 ± 0.092 (0.000–0.237)[a,b]

Note: Values with no common letter superscript are significantly different ($P<0.05$).

inducers, such as PAH and PCBs, or metabolic organic compounds, such as yellow substances, which accumulate in recirculated water (Leonard et al., 2002) and are suspected of having a deleterious effect on fish growth (Hirayama et al., 1988). Further laboratory investigations are required to elucidate the mechanisms for such compounds. In addition, a mixture of inducers and inhibitors is possible, as was observed for sea bass exposed to a secondary treated urban/industrial effluent (Gravato and Santos, 2003a). Another hypothesis is that EROD activity of FTS fish is inhibited by long exposure to low levels of pollutants in replacement water. The replacement water rate was 60 times higher in FTS than in RAS, and previous works showed that liver EROD activity of sea bass was inactivated by a long exposure to a low pollutant concentration (Gravato and Santo, 2003b; Gravato et al., 2000). Further investigations are required to elucidate the origin of EROD activity differences between the rearing circuits and to evaluate fish health, as EROD induction may not only indicate chemical exposure, but also precede effects at various levels of biological organisation (Whyte et al., 2000).

GST, GSH, and GSSG are the phase II enzyme and cofactors involved in detoxification and clearance of many xenobiotic compounds. We obtained no significant differences of these parameters between circuits, suggesting the absence of additional pollutants in RAS and RAS + HRAP compared to FTS. Lemaire et al. (1996) found a decrease in GST activity in sea bass 1 and 5 days after 3-methylcholanthrene injection but, in many studies on other species, increased activity or no differences were found between fish collected from polluted vs. reference sites (Fenet et al., 1998; Paris-Palacios et al., 2000). This is also the case for GSH, which can be restored by feedback mechanisms. Nevertheless, in all rearing systems GSH:GSSH ratios are higher than 10:1, which is observed in healthy cells (Stegeman et al., 1992).

Discussion of oxidative stress generated by rearing practices is more controversial because SOD is the only antioxidant enzyme increased in RAS, whereas CAT and GPOX are not different between circuits. However, significant SOD induction was described in most of the studies on polluted sites, when both induction and inhibition occurred for CAT, and GPOX was found to be slightly responsive (Van der Oost et al., 2003). Therefore, SOD induction could indicate additional oxidative stress generated by the recirculating loop and scavenged by HRAP treatment. Cadmium in liver, which was found to be positively correlated with SOD in our circuits (Spearman, $\rho = 0.40$; $P = 0.04$), could be responsible for the SOD induction in RAS fish, as was previously demonstrated in Nile tilapia by Almeida et al. (2002). Cadmium is known for its ability to generate reactive oxygen species (ROS) and create damage to cells (Livingstone, 2001).

HSP70 are involved in the protection and repair of cells in response to chemical or physical stress. Our results showed that the different rearing systems tested do not generate such stress on fish assuming that water parameter levels in our circuit (especially pH and salinity) did not affect HSP responses. However, this biomarker has been described only recently and mainly in laboratory studies; further research is needed to determine its significance in field situations.

4.3. Bioaccumulation of metals in the rearing systems

Mean concentrations of metals in muscle and liver showed great variation in fish inside the same system. This can be explained partially by the fact that metal concentrations are close to the detection limit (Co, Sn, Tl, Pb), and by intraindividual variability (Ni, Cu, Ag). These variations are higher in muscle than in liver, where metal concentrations are more homogeneous. Mean comparison between systems should be interpreted carefully for these metals and more fish should be analyzed in a further survey. However, statistical comparisons revealed that seven and four metal concentrations were significantly higher in muscle and liver, respectively, for RAS fish compared to FTS fish. This result cannot be related to fish size, as demonstrated by Canli and Atli (2003) for muscle concentrations and by Liang et al. (1999) for viscera concentrations. Considering that fish were fed with the same commercial feed, fish metal accumulation is related to water contamination. The water replacement rate in fish tanks was, on average, 60 times lower in recirculating systems than in FTS. Therefore trace metals contained in the food and eliminated by fish metabolism could accumulate in recirculating rearing water, as for nitrate and dissolved organic carbon (Leonard et al., 2002). Another hypothesis for metal origin could be erosion of materials of the recirculation loop. However, this probability is small, as all of them are made of PVC or 316L stainless steel to avoid deterioration of the system.

Differences of metal bioaccumulation in fish depend on the bioavailability of metals, their metabolism and their elimination in the target organism. The bioavailability is defined by the fraction in water that can be absorbed and concentrated by an organism. This fraction depends on metal concentration but also on its specificity, on dissolved and particulate matter concentrations, and on physical–chemical parameters in the water. For example, Hollis et al. (1996, 1997), showed a reduction in toxicity of copper by the presence of dissolved organic carbon in water. Compared to a FTS, metals must be concentrated in a recirculating system, but theoretically the higher dissolved and particulate matter concentrations should reduce their absorption by fish. To explain the differences in metals bioaccumulation between circuits, it will be necessary to determine their bioavailability in each system in a future study.

Except for chromium and arsenic, HRAP treatment in RAS removed metal accumulation in fish. This is probably due to the ability of seaweed to absorb metals during growth, a property which has been used previously in waste treatment (Aderhold et al., 1996; Davis et al., 2000) and in assessment of polluted sites (Caliceti et al., 2002; Villares et al., 2002). Reduced metal accumulation could also be related to reduced bioavailability. Actually, the pH often increased above 9 in the HRAP (Pagand et al., 2000b) and metals may have precipitated with substrates (hydroxides, carbonates, organic matters, etc.), as has been shown for phosphates (Mesplé et al., 1996).

Regardless of the system, 8 metals out of 12 were more concentrated in liver than in muscle of fish. Higher levels of accumulation were found for zinc (5 times), cadmium (80–90 times), and copper (310 times). Liver accumulation is often mentioned in the literature for many fish species and is related to their metabolism (Kljakovic Gašpic et al., 2002, for Pb and Cd; Eastwood and Couture, 2002, for Cu, Zn, and Ni). Roméo et al. (2000) found similar levels of zinc and cadmium in livers of farmed sea bass, but copper concentrations were 2–10 times lower than in our fish. However, similar copper concentrations were found in liver of other seawater (gray mullet in Canli and Atli, 2003) or freshwater (rainbow trout in Grosell et al., 1998; yellow perch in Eastwood and Couture, 2002) fish species. Arsenic is the only metal with a higher concentration in muscle than in liver. This result is in contrast to a study on mullet caught in a Spanish estuary (Suner et al., 1999). However, comparison with our study is difficult because food and water contamination are not dissociated and target organs for metals vary strongly between fish species (Kraal et al., 1995).

4.4. Flesh quality

Among the elements that accumulated, some are toxic (copper, nickel) and carcinogenic (arsenic, cadmium, lead) for humans. However, after 1 yr of rearing, their concentrations in the flesh are lower than those reported in the literature for caught and farmed fish from the Mediterranean sea (Roméo et al., 2000; Pérez Cid et al., 2001; Canli and Atli, 2003). They are also much lower than the maximum limits recommended for human food by international organization (200–250, 0.25–10, 50–150, and 2.5–30 $\mu g\,g^{-1}$ dry weight for Zn, Cd, Cu, and Pb, respectively) (Food Chemical Codex, 1996) and the median international standards (20, 45, 0.3, 1, and 2 $\mu g\,g^{-1}$ wet weight for Cu, Zn, Cd, Cr, and Pb, respectively) (Philips, 1993). To compare our values to those in the literature, we assumed that dry weight is 10% of wet weight. For arsenic, the FAO/WHO (1989) recommended a provisional tolerable week intake (PTWI) of 15 $\mu g\,kg^{-1}$ body weight. If we consider the highest arsenic concentration found in the fish from the RAS circuit and if we assume that all the arsenic is in the inorganic toxic form, this would result in a maximal authorized consumption of 1.3 kg of fish (fresh weight) per kg of body weight per week.

5. Conclusion

After 1 yr, mean weight was found to be 15% lower in the recirculating system, than in FTS. All biomarker levels were similar in the different systems, except EROD and SOD activities, which were slightly increased in the RAS. SOD induction is correlated with cadmium accumulation in liver and further investigations are needed to determine the significance of the EROD results.

Seven and four metal concentrations, in muscle and liver respectively, were found to be significantly higher in RAS. HRAP treatment avoided metal accumulation in RAS fish, except for chromium and arsenic. Among elements that accumulated, some are toxic, but the highest muscle concentrations were still lower than international standards and FAO/WHO recommended values.

Biomarker and bioaccumulation responses are powerful because they integrate a wide range of toxicological factors that may be modulated by environmental factors. In aquaculture, most confounding factors like sex, age, nutritional status, reproductive and developmental status, season, and population density are thoroughly controlled and/or known. Therefore, in such systems normal standardized values for biomarker and bioaccumulation status could be established, in order to assess the rearing water quality and to use them as sentinel parameters for an early diagnostic of fish health. However, the same factors may reduce the significance of the response in environmental studies.

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